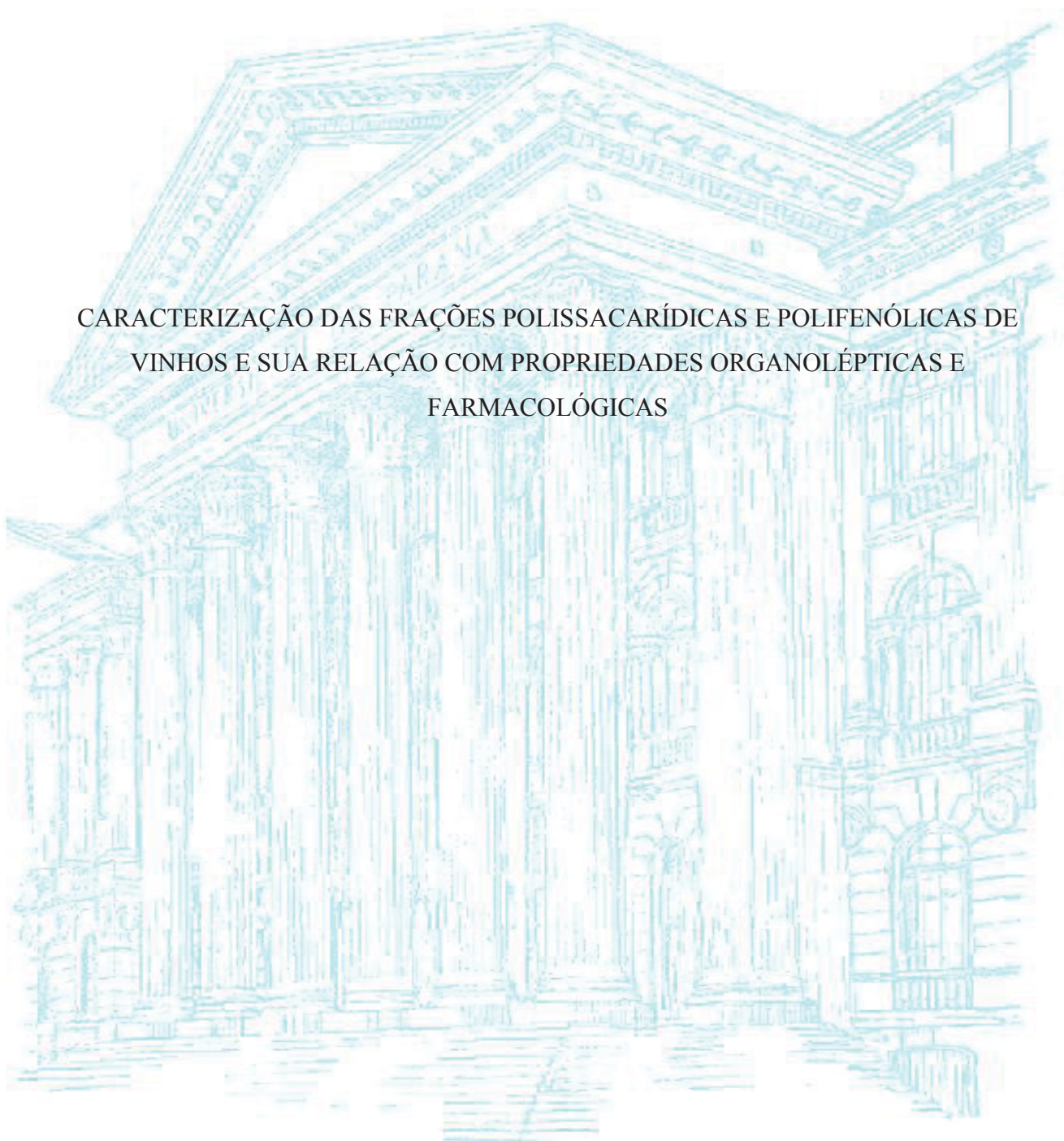


UNIVERSIDADE FEDERAL DO PARANÁ
UNIVERSIDAD DE SALAMANCA

IGLESIAS DE LACERDA BEZERRA

CARACTERIZAÇÃO DAS FRAÇÕES POLISSACARÍDICAS E POLIFENÓLICAS DE
VINHOS E SUA RELAÇÃO COM PROPRIEDADES ORGANOLÉPTICAS E
FARMACOLÓGICAS



CURITIBA

2020



IGLESIAS DE LACERDA BEZERRA



CARACTERIZAÇÃO DAS FRAÇÕES POLISSACARÍDICAS E POLIFENÓLICAS DE VINHOS E SUA RELAÇÃO COM PROPRIEDADES ORGANOLÉPTICAS E FARMACOLÓGICAS

Tese de doutorado apresentada ao Programa de Pós-graduação em Ciências (Bioquímica), Setor de Ciências Biológicas, Universidade Federal do Paraná, sob cotutela com o Programa de Pós-graduação em Enologia, Viticultura e Sustentabilidade, Faculdade de Farmácia, Universidad de Salamanca.

Orientador (Brasil):

Guilherme Lanzi Sassaki

Orientadores (Espanha):

María Teresa Escribano Bailón

Ignacio García Estévez

CURITIBA

2020

Universidade Federal do Paraná
Sistema de Bibliotecas
(Giana Mara Seniski Silva – CRB/9 1406)

Bezerra, Iglesias de Lacerda

Caracterização das frações polissacarídicas e polifenólicas de vinhos e sua relação com propriedades organolépticas e farmacológicas. / Iglesias de Lacerda Bezerra. – Curitiba, 2020.

204 p.: il.

Orientador (Brasil): Guilherme Lanzi Sassaki

Orientadores (Espanha): María Teresa Escribano Bailón

Ignacio García Estévez

Tese (doutorado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências – Bioquímica. sob cotutela da Universidad de Salamanca. Programa de Pós-graduação em Enología, Viticultura e Sustentabilidade. Faculdade de Farmácia.

1. Vinho. 2. Polissacarídeos. 3. Compostos fenólicos. 4. Inflamação. 5. Sepse. I. Título II. Sassaki, Guilherme Lanzi, 1975-. III. Escribano-Bailón, María Teresa. IV. García-Estévez, Ignacio. V. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências – Bioquímica. VI. Universidad de Salamanca. Programa de Pós-graduação em Enología, Viticultura e Sustentabilidade. Faculdade de Farmácia.

CDD (22. ed.) 641.22



MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS BIOLÓGICAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS
(BIOQUÍMICA) - 40001016003P2

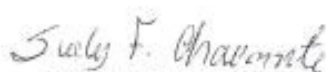
TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS (BIOQUÍMICA) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **IGLESIAS DE LACERDA BEZERRA** intitulada: **CARACTERIZAÇÃO DAS FRAÇÕES POLISSACARÍDICAS E POLIFENÓLICAS DE VINHOS E SUA RELAÇÃO COM PROPRIEDADES ORGANOLÉPTICAS E FARMACOLÓGICAS**, sob orientação do Prof. Dr. GUILHERME LANZI SASSAKI, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

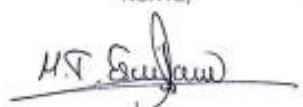
A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 06 de Março de 2020.


GUILHERME LANZI SASSAKI
Presidente da Banca Examinadora


SUELY FERREIRA CHAVANTE
Avaliador Externo (UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE)


YANNA DANTAS RATTMANN
Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANÁ)


MARIA TERESA ESCRIBANO BAILÓN


THALES RICARDO CIPRIANI

Com amor:
À minha família,
Ao meu marido,
Dedico

AGRADECIMENTOS

Ao meu amado Deus por me guiar e me dar força para seguir. Ao Programa de Pós-graduação em Bioquímica-UFPR pela oportunidade de estudo e pela dedicação pelo crescimento do curso. Ao meu querido orientador Prof. Guilherme Lanzi Sassaki, que é minha referência cientista, por toda sua genialidade e dedicação à ciência, serei grata por ter acreditado em mim, me apoiado e aconselhado em todas minhas decisões acadêmicas, pela paciência, um grande professor. À banca interna, Prof. Thales Ricardo Cipriani e Prof. Alexandra Acco pelas correções importantes do meu projeto e relatórios. À Rosane, Elis e Flavia pelas análises de GC-MS e HPSEC-MALLS. Aos técnicos do Centro de Tecnologias Avançadas em Fluorescência, pelas análises histológicas. Ao Centro de RMN por todas as análises. À Prof. Suely Ferreira Chavante, Lais Palhares e Rômulo Cavalcante pela parceira sempre valiosa, tê-los como parceiros foi muito especial. Ao prof. Edvaldo da Silva Trindade e seus alunos pelo auxílio, por serem sempre tão gentis e prestativos. À prof. Yanna Dantas Rattmann, sempre tão doce e querida, por me socorrer com seu conhecimento quando eu não sabia o que fazer nos experimentos de sepsis. Ao Arquimedes e Leociley pela colaboração com as análises de RMN e por todo apoio dado durante a execução do meu trabalho. À Adriana e Aninha, pela ajuda com meus experimentos, por todo apoio, pelos conselhos, amizade, carinho, paciência, risadas e aventuras que marcaram meu doutorado. A todos os amigos do laboratório, pelo apoio, pela amizade e convivência agradável, em especial ao grupo Sassaketes, pelos dias tão alegres e pelo companheirismo. Ao grupo Carbocats pelo carinho, companheirismo, tantas risadas e almoço às 11 h, em especial a Giuliana, minha artista cientista, pela amizade, pelo carinho, por toda ajuda durante meu sanduíche e pelas caronas na volta para casa. A Shayane e Philippe, por serem tão prestativos e me socorrerem tantas vezes durante minha estadia fora do país. A todos do grupo Química de Carboidratos, as melhores pessoas do mundo estão aqui. A todos os professores do Departamento de Bioquímica e Biologia Molecular (UFPR), pelas disciplinas ministradas, pela contribuição para a minha formação. A todos os pós-graduandos e funcionários do Departamento de Bioquímica e Biologia Molecular, pela colaboração e amizade. À CAPES e CNPq pelo auxílio financeiro e pela oportunidade de estudo fora do país.

À minha mãezinha Socorro por ser meu apoio, minha base e minha fortaleza, por toda a vida ter se dedicado a minha educação. Aos meus paidrinhos, Francisco e Francinaldo, e ao meu paidrasto, José, pelo apoio, pelos conselhos e incentivo, por terem batalhado tanto pelo

meu sucesso. Aos meus irmãos, Sara e Lucas, pelo companheirismo, por dar leveza a minha vida. A toda minha família pelo apoio e por me tratarem com tanto carinho e zelo.

Ao meu querido e amado marido Marcelo, meu companheiro, por ser tão incentivador, por me ajudar sempre que preciso e cuidar de mim. Obrigada pelo amor, por acreditar que eu sou capaz e me fazer acreditar. A toda Família Ramos, pelo carinho, apoio e amizade.

À minha querida orientadora Prof. María Teresa Escibano Bailón, tão doce, a voz mais acalmadora da Espanha. Obrigada por ter me aceitado em seu grupo, por ser tão paciente e atenciosa comigo e não ter desistido da minha cotutela, por todo apoio e pelos ensinamentos. Ao meu orientador Prof. Ignacio Estévez García, excelentíssimo Nacho, por ser tão prestativo, amável e acolhedor, por todo apoio, por ser a calma quando tudo parece ser caos. Obrigada pela força, por ser a base do laboratório e pelas risadas que deixavam os dias mais leves.

Ao programa em Enologia, Vicultura e Sustentabilidade pela oportunidade de estudo e por terem aceitado minha cotutela. À María José e Joaquín, por serem sempre prestativos e pelo carinho. Joaquin, obrigada por me salvar com vidrarias e me chamar sempre para o café e dizer que vai embora antes de mim. À Montse, o olhar mais confortante, por ser tão atenciosa, pelo companheirismo e carinho, por todo apoio, por me socorrer sempre, por torcer por mim. À Rebeca, tão doce e prestativa, obrigada pelo apoio, por toda a ajuda nos experimentos e pela atenção. À Cristina, a gênio dos antocianos, pelos ensinamentos, pela paciência e carinho, minha grande admiração. À minha querida amiga, dona da alegria contagiante, Bárbara, que tornou minha estadia mais feliz, por todo companheirismo e apoio psicológico tão importante, pela ajuda com trâmites espanhóis tão difíceis, por estar sempre ao meu lado. À Eva, por ser tão atenciosa, pela amizade e por me chamar para tomar café, almoçar e tomar vermut. À Elvira, um poço de corações, pelo apoio, pela ajuda nos experimentos, por ser tão atenciosa. À Alba, por todo apoio, por toda ajuda durante minha estadia. À Siyu Li pelo toque chinês, pela amizade, pelos dias divertidos que passei tentando dar um abraço. A todos do grupo Polifenois pelo carinho, pela atenção e pelo apoio, fui muito feliz nesse grupo maravilhoso, fui bem acolhida, todos foram importantes para o meu crescimento acadêmico e pessoal. Meus queridos espanhóis! ¡Muchas gracias!

A todas as pessoas que me ajudaram de alguma forma na minha formação e na realização desse trabalho, e que torceram pelo meu sucesso, muito obrigada!

RESUMO

Polissacarídeos e compostos fenólicos são compostos altamente relacionados à qualidade do vinho, devido ao seu impacto não apenas em suas propriedades organolépticas (cor, sabor, adstringência), mas também com seus efeitos benéficos a saúde humana. Portanto, este trabalho teve como objetivo identificar, caracterizar e quantificar os polissacarídeos e compostos fenólicos encontrados nos vinhos e avaliar a interação entre eles. Além disso, avaliar a semelhança entre os polissacarídeos extraídos de vinhos comerciais de diferentes safras e estudar as propriedades anti-inflamatórias dos polissacarídeos *in vitro* e *in vivo*, bem como a biodistribuição desses polissacarídeos durante o processo inflamatório. Para isso, foram escolhidos vinhos comerciais feitos com uvas *Vitis vinífera* de diferentes variedades. Os polissacarídeos foram extraídos, caracterizados e quantificados por GC-MS e RMN. Foi identificada a presença de manana, arabinogalactana tipo II e ramannogalactonana tipo I e II. Também foi demonstrado que os vinhos produzidos a partir da mesma variedade de uva e da mesma vinícola têm padrões de polissacarídeos semelhantes, embora tenham sido produzidos em anos diferentes. Os compostos fenólicos foram identificados e quantificados por HPLC-DAD-MS. A análise mostrou uma prevalência de conteúdo de procianidina (entre 58 e 275 mg/L) versus a de prodelphinidinas (entre 4 e 8 mg/L). A análise de antocianina mostrou a maioria dos derivados monoglicósidos e seus derivados acetilados. As análises estatísticas revelaram a existência de relações entre a estrutura dos polissacarídeos e sua capacidade de precipitar procianidinas, o que explicaria a relação observada nos vinhos entre a composição dos polissacarídeos e a concentração de flavanóis. As frações de polissacarídeos inibiram a produção de mediadores inflamatórios em células RAW 264.7. A administração subcutânea e oral de polissacarídeos reduziu a taxa de mortalidade dos camundongos. O tratamento reduziu a migração de leucócitos, inibiu citocinas pró-inflamatórias e aumentou a produção da citocina anti-inflamatória IL-10. Eles também reduziram os níveis plasmáticos de AST, ALT, bilirrubina, uréia e creatinina, com a possível proteção contra danos nos tecidos. Além disso, a biodistribuição de polissacarídeos pelo organismo, quando administrada via subcutânea, parece ocorrer através do sistema imunológico. Os polissacarídeos demonstraram um potente efeito anti-inflamatório *in vitro* e *in vivo*, o que pode indicar efeitos benéficos do consumo moderado de vinho na saúde humana.

Palavras-chave: vinhos, polissacarídeos, compostos fenólicos, RMN, HPLC, inflamação, seps.

ABSTRACT

Polysaccharides and phenolic compounds are compounds highly related to wine quality, due to their impact not only on their organoleptic properties (color, flavor, astringency), but also on their healthy properties. Therefore, this work aimed to identify, characterize and quantify the polysaccharides and phenolic compounds found in wines and to evaluate the interaction between them. In addition, to evaluate the similarity between the polysaccharides extracted from commercial wines from different vintages and to study the anti-inflammatory properties of the polysaccharides *in vitro* and *in vivo* by, as well as the biodistribution of these polysaccharides during the inflammatory process. For this, commercial wines made with *Vitis vinifera* grapes of different varieties were chosen. Polysaccharides were extracted, characterized, and quantified by GC-MS and NMR. The presence of mannan, type II arabinogalactans and type I and II rhamnogalactonans were identified. It has also been shown that wines produced from the same grape variety and from the same winery have similar polysaccharide patterns, although they have been produced in different years. The phenolic compounds were identified and quantified by HPLC-DAD-MS. The analysis showed a prevalence of procyanidin content (between 58 and 275 mg/L) versus that of prodelphinidin (between 4 and 8 mg/L). The anthocyanin analysis showed the majority of monoglycoside derivatives and their acetylated derivatives. Statistical analyzes revealed the existence of relationships between the structure of polysaccharides and their ability to precipitate procyanidins, which would explain the relationship observed in wines between the composition of polysaccharides and the concentration of flavanols. The polysaccharide fractions inhibited the production of inflammatory cytokines (TNF- α and IL-1 β) and mediator (NO) in RAW 264.7 cells. Subcutaneous and oral administration of polysaccharides reduced the mortality rate of mice. The treatment reduced leukocyte migration, inhibited pro-inflammatory cytokines and increased production of the anti-inflammatory cytokine IL-10. They also reduced plasma levels of AST, ALT, bilirubin, urea and creatinine, with consequent protection against tissue damage. In addition, the polysaccharide biodistribution by the body, when administered subcutaneously, seems to occur through the immune system. Polysaccharides have demonstrated a potent anti-inflammatory effect *in vitro* and *in vivo*, which may indicate beneficial effects of moderate wine consumption on human health.

Keywords: wines, polysaccharides, phenolic compounds, NMR, HPLC, inflammation, sepsis

RESUMEN

Los polisacáridos y los compuestos fenólicos son compuestos altamente relacionados con la calidad del vino, debido a su impacto no sólo en sus propiedades organolépticas (color, sabor, astringencia) sino también en sus propiedades saludables. Por lo tanto, este trabajo tuvo como objetivo identificar, caracterizar y cuantificar los polisacáridos y compuestos fenólicos encontrados en los vinos y evaluar la interacción entre ellos. Además, pretende evaluar la similitud entre los polisacáridos extraídos de vinos comerciales de diferentes añadas y estudiar las propiedades antiinflamatorias de los polisacáridos *in vitro* e *in vivo*, así como la biodistribución de estos polisacáridos durante el proceso inflamatorio. Para este propósito, se eligieron vinos comerciales elaborados con uvas *Vitis vinífera* de diferentes variedades. Los polisacáridos fueron extraídos, caracterizados y cuantificados por GC-MS y RMN. Se identificó la presencia de manano, arabinogalactano tipo II y ramannogalactonano tipo I y II. También se ha demostrado que los vinos producidos a partir de la misma variedad de uva y de la misma bodega tienen patrones de polisacáridos similares, aunque hayan sido producidos en años diferentes. Los compuestos fenólicos fueron identificados y cuantificados por HPLC-DAD-MS. El análisis mostró una prevalencia del contenido de procianidinas (entre 58 y 275 mg/L) frente al de las prodelphinidinas (entre 4 y 8 mg/L). El análisis de antocianinas mostró la mayoría de los derivados de monoglucósidos y sus derivados acetilados. Los análisis estadísticos revelaron la existencia de relaciones entre la estructura de los polisacáridos y su capacidad para precipitar procianidinas, lo que explicaría la relación observada en los vinos entre la composición de polisacáridos y la concentración de flavanoles. Las fracciones de polisacárido inhibieron la producción de citoquinas inflamatorias (TNF- α e IL-1 β) y mediador (NO) en células RAW 264.7. La administración subcutánea y oral de polisacáridos redujo la tasa de mortalidad de los ratones. El tratamiento redujo la migración de leucocitos, inhibió las citocinas proinflamatorias y aumentó la producción de la citocina antiinflamatoria IL-10. También redujeron los niveles plasmáticos de AST, ALT, bilirrubina, urea y creatinina, con la consiguiente protección contra el daño tisular. Además, la biodistribución de polisacáridos por el cuerpo cuando se administra por vía subcutánea parece ocurrir a través del sistema inmune. Los polisacáridos han demostrado un potente efecto antiinflamatorio *in vitro* e *in vivo*, lo que puede indicar efectos beneficiosos del consumo moderado de vino en la salud humana.

Palabras clave: vinos, polisacáridos, compuestos fenólicos, RMN, HPLC, inflamación, sepsis.

“Desconfie do destino e acredite em você. Gaste mais horas realizando que sonhando, fazendo que planejando, vivendo que esperando porque, embora quem quase morre esteja vivo, quem quase vive já morreu.”

Luiz Fernando Veríssimo

LISTA DE FIGURAS

CAPITULO I	CONSIDERAÇÕES GERAIS.....	26
FIGURA 1	Estrutura da baya da uva.....	31
FIGURA 2	Produção mundial de vinho em 2018	33
FIGURA 3	Fluxograma da produção do vinho	35
FIGURA 4	Modelo da estrutura da parede celular primária de vegetal.....	37
FIGURA 5	Recrutamento de leucócitos e processo inflamatório.....	44
CAPITULO II	Structural Characterization of Polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: anti-inflammatory activity in LPS stimulated RAW 264.7 cells.....	59
FIGURE 1	(Scheme of extraction and purification of polysaccharides; Elution profile of (A) PCF and (B) FCF fractions determined by HPSEC using light scattering and refractive index detectors.....	66
FIGURE 2	¹³ C-NMR spectra of (A) WCF, (B) WCS and (C) WSB in D ₂ O at 30 °C (chemical shifts are expressed in δ ppm).....	67
FIGURE 3	¹ H/ ¹³ C HSQC NMR spectrum of PCF. Solvent D ₂ O at 30 °C; numerical values are in δ ppm. E (Manp); NRT (non-reducing end units); The letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed, COSY, TOCSY and HMBC experiments.....	69
FIGURE 4	¹ H/ ¹³ C HSQC NMR spectrum of FCF. Solvent D ₂ O at 30 °C; numerical values are in δ ppm. A (α-L-Araf); B (β-D-Galp); C (α-L-Rhap); D (α-D-GalpA); D' (6-OMe-β-D-GalpA). The letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed, COSY, TOCSY and HMBC experiments.....	70
FIGURE 5	Effect of WCF, WCS and WSB mixed fractions and the isolated fractions FCF and PCF on the viability of RAW 264.7 cells. The viability was measured by MTT assay. The	

	values for each concentration tested (0.1, 1, 10 e 100 µg/mL) represent the average (mean ± S.D.); Ctrl (control).....	72
FIGURE 6	Anti-inflammatory activities of WCF, WCS, WSB, PCF and on RAW 264.7 cells. (A-E) Expression levels of TNF-α. Expression levels of IL-1β. (K-O) NO production levels. (control).....	75
FIGURE S1	Suggested structure for mannan isolated.....	85
FIGURE S2	Suggested structure for AGII.....	86
FIGURE S3	Suggested structure for RGI.....	86
FIGURE S4	Chromatogram and mass spectra containing retention fragmentation time from (A) 2- <i>O</i> -methyl-xylitol and those (B) 2- <i>O</i> -methyl-fucitol, the identification of fragments at m/z.	87
CAPITULO III	Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice.....	89
FIGURE 1	¹ H-NMR spectra of (A) WCS-2014, (B) WCS-2015 and (C) WCS-2016 in D ₂ O at 30 °C (chemical shifts are expressed in δ ppm).....	97
FIGURE 2	Effects of CSO (A), CSS (B), FCS (C) and PCS (D) fractions on survival rate. Mice (10 animals/group) were orally administered various doses of CSO (3, 7 or 10 mg/kg) or subcutaneous administered various doses of CSS, FCS and PCS (0.1, 1, 10 mg/kg), vehicle (saline s.c.) and dexamethasone (0.5 mg/kg s.c.). Values represent means ± SEM. ***p < 0.001 when compared with CLP plus vehicle group; ANOVA followed by the Tukey's test.....	101
FIGURE 3	Expression levels of TNF-α. (A), IL-1β (B), IL-10 (C), and NO (D) production levels. Dex (dexamethasone).....	104
FIGURE 4	Representative histological sections from mice in groups: sham, vehicle, CSS, CSO, FCS, PCS and DEX (dexamethasone). Liver (A-G), lung (H-N) and kidney (O- U). Original magnification × 50. H&E. Scale bar = 50µm.....	108
FIGURE S1	¹ H/ ¹³ C HSQC NMR spectrum of WCS. Solvent D ₂ O at 30	

	°C; numerical values are in δ ppm. A (α -L-Araf); B (β -D-Galp); C (α -L-Rhap); D (α -D-GalpA); D' (6-OMe- β -D-GalpA), E (Manp); F (α -D-Glc). Letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed experiment.....	118
FIGURE S2	Chromatogram and mass spectra containing retention and fragmentation times from (A) 2- <i>O</i> -methyl-xylitol and those from (B) 2- <i>O</i> -methyl-fucitol. Identification of fragments at m/z.....	119
FIGURE S3	Representative histological sections of kidney from mice in groups: sham (A), vehicle (B), CSS (C), CSO (D), FCS (E), PCS (F) and dexamethasone (G). Original magnification $\times$ 200. H&E. Scale bar = 20 μ m.....	120
CAPITULO IV	Biodistribution and anti-inflammatory activity of red wine polysaccharides: a sweet fluorescent trip in the mice body.....	122
FIGURE 1	Structural characterization of fluorescence-labeled WCF-F. (a) Reaction of WCF with fluoresceinamine. (b) WCF-F and WCF (c) fractions determined by HPSEC using refractive index detector, UV at 495 nm (green). STD spectrum of WCF-F at 303 K (d) and 343 K (e). (f) 2D DOSY spectrum with 2% and 98% of gradient of WCF-F in D ₂ O at 30 °C (chemical shifts are expressed in δ ppm). (f) ¹ H/ ¹³ C HSQC NMR spectrum of WCF-F. Solvent D ₂ O at 30 °C; numerical values are in δ ppm. A (α -L-Araf); B (β -D-Galp); C (α -L-Rhap); D (α -D-GalpA); E (Manp); F (β -D-Glcp); G (fluoresceinamine). The letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed, COSY, TOCSY and HMBC experiments.....	132
FIGURE 2	Expression levels of TNF- α . (a), IL-1 β (b), IL-10 (c), and NO (d) production levels. Dex (dexamethasone).....	135
FIGURE 3	Representative histological sections from mice in groups:	

	sham, vehicle, FLN, WCF-F and Dex (dexamethasone). Liver (a-e), lung (f-j) and kidney (k-o). Original magnification $\times 50$. H&E. Scale bar = $50\mu\text{m}$	139
FIGURE 4	Fluorescence microscopy images of macrophage cell lines after incubation with $100\mu\text{g/mL}$ of WCF-F or FLN fraction after 0 h 6 h, 24 h and 48 h. Average fluorescence intensity is plotted for WCF-F and FLN fractions according to time of analysis. ANOVA followed by the Tukey's test. *** $p < 0.001$	141
FIGURE 5	Imaging of Multi-wavelength fluorescence of WCF-F (a-d) and FLN (e-h) after 0 h 1 h, 3 h and 6 h of treatment with live animals. Computer command center running Windows 7 Pro with Carestream MI acquisition and analysis software.....	142
FIGURE 6	Representative histological sections from mice in groups: sham, WCF-F and FLN. Liver (a-c), lung (d-f) and kidney (g-i). Original magnification $\times 20$. Scale bar = $100\mu\text{m}$. Average fluorescence intensity is plotted for WCF-F and FLN fractions of the liver, lung and kidney. ANOVA followed by the Tukey's test. *** $p < 0.001$	143
FIGURE S1.	Effects of WCF-F and FLN fractions on survival rate. Mice (10 animals/group) were subcutaneous administered various doses (0.1, 1, 10 mg/kg), vehicle (saline s.c.) and dexamethasone (0.5 mg/kg s.c.). Values represent means $\pm$ SEM. *** $p < 0.001$ when compared with CLP plus vehicle group; ANOVA followed by the Tukey's test.....	149
FIGURE S2	Effect of WCF-F and FLN fractions on the viability of RAW 264.7 cells. The viability was measured by MTT assay. The values for each concentration tested (0.1, 1, 10 e $100\mu\text{g/mL}$) represent the average (mean $\pm$ S.D.); Ctrl (control).....	149
CAPITULO V	Influence of red wine soluble polysaccharides profile on the flavanol composition and precipitation.....	151
FIGURE 1	The score plot of the main component analysis relating (A) polysaccharide families percentage, flavanols percentage and	

	molecular weight percentage (B) samples profile CSCM, CSJA, CSVA, CSFA and TPHO. PC: procyanidin; PD: Prodelphinidin.....	161
FIGURE 2	The score plot of the main component analysis relating (A) polysaccharide families percentage, molecular weight percentage, and percentage of procynidins precipitated after interaction test (B) samples profile ICSCM, ICSJA, ICSVA, ICSFA and ITPHO.....	164
FIGURE S1	¹ H-NMR spectra of (A) CSCM. (B) CSJA. (C) CSVA. (D) CSFA and (E) TPHO in D ₂ O at 30 °C (chemical shifts are expressed in δ ppm).....	170

LISTA DE TABELAS

CAPITULO II	Structural Characterization of Polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: anti-inflammatory activity in LPS stimulated RAW 264.7 cells.....	59
TABELA S1	Monosaccharide composition of fractions WCF, WCS, WSB, PCF and FCF.....	84
TABELA S2	Profile of partially <i>O</i> -methylated alditol acetates obtained by methylation analysis of PCF.....	84
TABELA S3	Profile of partially <i>O</i> -methylated alditol acetates obtained by methylation analysis of FCF.....	85
CAPÍTULO III	Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice.....	89
TABELA 1	Composition Monosaccharide composition of the red wine polysaccharides of different vintages.....	99
TABELA 2	Quantification of each polysaccharide in relation to the total yield.....	99
TABELA 3	Hematological parameters evaluated during sepsis in mice..	102
TABELA 4	Plasmatic parameters evaluate during sepsis in mice.....	106
CAPITULO IV	Biodistribution and anti-inflammatory activity of red wine polysaccharides: a sweet fluorescent trip in the mice body.....	122
TABELA 1	Hematological parameters evaluated during sepsis in mice..	134
TABELA 2	Plasmatic parameters evaluate during sepsis in mice.....	137
CAPITULO V	Influence of red wine soluble polysaccharides profile on the flavanol composition and precipitation.....	151
TABELA 1	Percentage of polysaccharides determined in Cabernet Sauvignon and Tempranillo wines.....	158
TABELA 2	Molecular weights of the polysaccharides determined in Cabernet Sauvignon and Tempranillo wines.....	159

TABELA 3	Flavanols determined in Cabernet Sauvignon and Tempranillo wines.....	160
TABELA 4	Percentage of procyanidins precipitated after polysaccharide interaction assay.....	163
TABELA S1	Description of the samples used in the experiments.....	172
TABELA S2	Procyanidins determined in Cabernet Sauvignon seed grapes.....	172
TABELA S3	Pearson's Coefficients Correlation between polysaccharidic composition and molecular weight.....	173
CAPITULO VI	Resultados Complementares.....	175
TABELA 1	Antocianinas determinadas nos vinhos Cabernet Sauvignon e Tempranillo.....	175
TABELA 2	Parâmetros CIELAB (L , Cab e Hab) dos vinhos Cabernet Sauvignon e Tempranillo.....	176

LISTA DE SIGLAS E ABREVIATURAS

AcOEt – Acetado de Etila

AG I – Arabinogalactana do Tipo I

AG II – Arabinogalactana do Tipo II

Ara – Arabinose

CP – Polissacarideo Bruto

CuSO₄ – Sulfato de Cobre

D₂O – Óxido de deutério

DEPT - Intensificação sem distorção por transferência de polarização

ELISA – Enzyme Linked Immuno Sorbent Assay

Gal – Galactose

GalA – Ácido galacturônico

GC-MS – Cromatografia gasosa acoplada à espectrometria de massas

Glc – Glucose

HG – Homogalacturonana

HPLC-SEC - Cromatografia de exclusão por tamanho de alta eficiência

HSQC – Espectroscopia de correlação heteronuclear Single-Quantum

i.p. – Intraperitoneal

IL-10 – Interleucina 10

IL-1 β – Interleucina -1 β

LPS – Lipopolissacarídeo

mDP – Grau médio de polimerização

MeOH – Metanol

MTT – (3,4,5-dimethyl-2-thiazolyl-2,5-diphenyl-2H-tetrazolium)

NaBH₄ – Boridreto de Sódio

NaNO₂ – Nitrito de Sódio

PBS – Phosphate buffered saline

PMN: Polymorphonuclear leukocytes

PC – Procianidina

PCA – Análise de componentes principais

PD – Prodelfinidina

RG I – Ramnogalacturonana do Tipo I

RG II – Ramnogalacturonana do Tipo II

Rha – Ramnose

RMN – Ressonância magnética nuclear

s.c. - Subcutânea

TFA – Trifluoroacetic acid

TNF- α – Fator de Necrose Tumoral

WBC – White blood cell count

δ – Deslocamento químico

NOTA EXPLICATIVA

Esta tese está estruturada na forma de capítulos segundo as normas do Programa de Pós-Graduação em Ciências – Bioquímica e do Sistema de Bibliotecas (SiBi) da Universidade Federal do Paraná (UFPR). A tese contém seis capítulos - Capítulo I: Considerações Gerais: Introdução geral, revisão bibliográfica, objetivo e referencias - Capítulo II ao V: artigos científicos (incluindo revisão bibliográfica, materiais, metodologias, resultados, discussão de resultados, conclusão e referências) - Capítulo VI: Resultados complementares, referências, conclusões gerais e lista geral de referências.

SUMÁRIO

CAPITULO I	26
1. INTRODUÇÃO GERAL.....	27
2. OBJETIVOS.....	30
2.1. OBJETIVO GERAL.....	30
2.2. OBJETIVOS ESPECÍFICOS	30
3. REVISÃO BIBLIOGRÁFICA.....	31
3.1. UVA	31
3.1.1. Tipos de videira	32
3.2. VINHO	32
3.2.1. Processo de produção do vinho	33
3.2.2. Benefícios do vinho	35
3.2.3. Carboidratos dos vinhos	36
3.2.3.1. Parede celular vegetal e polissacarídeos	37
3.2.3.1.1. Arabinogalactanas.....	38
3.2.3.1.2. Ramnogalacturonanas.....	39
3.2.3.2. Parede celular de leveduras e polissacarídeos	39
3.2.3.2.1. Mananas	40
3.2.4. Polifenóis do vinho	41
3.2.4.1. Flavanóis.....	42
3.2.4.2. Pigmentos antociânicos	42
3.2.4.3. Compostos fenólicos e índice de cor	43
3.3. PROCESSO INFLAMATÓRIO E POLISSACARÍDEOS.....	43
3.3.1. Seps.....	45
CAPITULO II.....	58

1. Introduction	60
2. Materials and methods.....	62
2.1. Materials.....	62
2.2. Polysaccharides extraction and purification.....	62
2.3. Monosaccharide analysis.....	63
2.4. Methylation analysis.....	63
2.5. Nuclear magnetic resonance (NMR) spectroscopy	64
2.6. Determination of homogeneity and molar mass.....	64
2.7. Cell Culture	65
2.8. Analysis of cell viability.....	65
2.9. Measurement of cytokines production and NO production	65
2.10. Statistical analysis.....	66
3. Results and Discussion	66
3.1. Isolation and structural analysis of the polysaccharides.....	66
3.2. Biological experiments.....	72
3.2.1. Analysis of cell viability	72
3.2.2. Anti-inflammatory activity	73
4. Conclusions	76
References	76
CAPITULO III	88
1. Introduction	90
2. Materials and methods.....	92
2.1. Materials.....	92
2.2. Polysaccharides extraction and purification.....	92
2.3. Monosaccharide analysis.....	93
2.4. Structural identification by NMR.....	93

2.5. <i>In vivo</i> experiments	93
2.6. Sepsis induction by cecal ligation and puncture (CLP)	94
2.7. Hematological assays	95
2.8. Biochemical assays.....	95
2.9. Histopathological analysis.....	96
2.10. Statistical analysis.....	96
3. Results and Discussion	96
3.1. Analysis of the polysaccharides of Cabernet Sauvignon wine.....	96
3.2. Biological experiments.....	99
4. Conclusions	109
References	109
CAPITULO IV.....	121
1. Introduction	123
2. Materials and methods.....	124
2.1. Materials.....	124
2.2. Polysaccharides extraction	125
2.3. Labeling of WCF with fluoresceinamine	125
2.4. Structural characterization.....	125
2.5. Sepsis induction by cecal ligation and puncture (CLP)	126
2.6. Hematological assays	127
2.7. Biochemical assays.....	128
2.8. Histopathological analysis.....	128
2.9. Cell culture	128
2.10. Analysis of cell viability	128
2.11. Macrophage internalization of WCF-F.....	129
2.12. Imaging of Multi-wavelength fluorescence.....	129

2.13. Statistical analysis.....	130
3. Results	130
3.1. Structural characteristics of fluorescent labeled WCF-F	130
3.2. Anti-inflammatory effect of wine polysaccharides	133
3.3. Analysis of cell viability.....	139
3.4. Macrophage internalization of fluorescent labeled WCF-F	140
3.5. Imaging of Multi-wavelength fluorescence <i>in vivo</i>	141
4. Discussion.....	143
References	145
Supporting Information	149
CAPITULO V	150
1. Introduction	152
2. Materials and methods.....	153
2.1. Materials.....	153
2.2. Polysaccharides extraction	154
2.3. Polysaccharide identification by NMR	154
2.4. Determination of molecular weight distribution of polysaccharides	155
2.5. Analysis of phenolic compounds in wines	155
2.6. Flavanols extraction from grape seeds	155
2.7. Interaction assays	156
2.8. Statistical analysis	156
3. Results and Discussion.....	156
3.1. Analysis of the polysaccharide extracts from wines	156
3.2. Analysis of flavanols extract of wines	159
3.3. Interaction experiments	161
4. Conclusions	165

References	166
Supporting Information	170
CAPITULO VI.....	174
1. RESULTADOS COMPLEMENTARES	175
1.1. Análise dos pigmentos antocianos em vinhos.....	175
1.2. Análises colorimétricas	176
Referências	177
CONCLUSÃO GERAL	178
CONCLUSIONES	180
LISTA DE REFERÊNCIAS	182

CAPITULO I

No capítulo I estão as considerações gerais compostas por Introdução, Justificativa, Objetivos, Revisão Bibliográfica, bem como as referências utilizadas no desenvolvimento do capítulo.

1. INTRODUÇÃO GERAL

O vinho é produzido a partir de uvas das variedades (ou castas) da espécie *Vitis vinifera* utilizada para produção de vinhos finos e da espécie *Vitis labrusca* utilizada para produção de vinhos de mesa (comuns). A constituição química das uvas permite que elas fermentem durante a vinificação sem que lhes sejam adicionados açúcares, ácidos, enzimas ou outros nutrientes. Essa fermentação é feita por leveduras que consomem os açúcares presentes nas uvas transformando-os em álcool (JOHNSON, 1989; (CRUZ, 2006). As principais macromoléculas encontradas na bebida são polissacarídeos (até 15%) e polifenóis (até 35%) (CARPITA *et al.*, 1993).

Os polissacarídeos presentes nos vinhos possuem influência importante em várias etapas do processo de vinificação, incluindo fermentação, filtração, estabilização e são parcialmente responsáveis pelas propriedades organolépticas dos vinhos (GERBAUD *et al.*, 1997; MOINE-LEDOUX & DUBOURDIEU, 1999). No entanto, estudos já mostraram que nem todos os polissacarídeos apresentam o mesmo comportamento em relação aos vinhos, e sua influência no processamento e nas propriedades sensoriais depende da quantidade, classe e características estruturais (RIOU *et al.*, 2002; VIDAL *et al.*, 2003; GUADALUPE & AYESTARÁN, 2007).

Essas macromoléculas são provenientes de bagas da uva, de leveduras, de bactérias e da contaminação por fungos. Do ponto de vista enológico e quantitativo, os polissacarídeos de uvas e de leveduras são os mais importantes. A estrutura dos polissacarídeos e suas concentrações dependem de muitos parâmetros, tais como cultivo, estágio de maturação, técnicas de vinificação e os tratamentos para aumento da solubilização dos componentes macromoleculares das paredes celulares de bagas de uva (DOCO *et al.*, 1999; CABANIS & CABANIS, 2000; AYESTARAN *et al.*, 2004). Arabinose e polissacarídeos ricos em galactose (AGs), como as arabinogalactanas-proteínas tipo II e arabinanas, ramnogalacturonanas tipo I e tipo II, e homogalacturonanas são provenientes de bagas de uva, enquanto as glucanas, mananas e manoproteínas são liberadas da levedura durante a fermentação ou pela atividade enzimática da levedura por autólise durante o envelhecimento em barris (PELLERIN *et al.*, 1995; VIDAL *et al.*, 2003; AYESTARÁN *et al.*, 2004). Esses açúcares parecem ser moléculas interessantes para a enologia devido aos seus efeitos na qualidade final do vinho (FOURNAIRON *et al.*, 2002; DOCO *et al.*, 2003). No entanto, informações detalhadas sobre composição e caracterização estrutural dos polissacarídeos dos vinhos são relativamente escassas, o que torna importante o estudo dessas moléculas.

Os polifenóis presentes no vinho são provenientes das uvas, do metabolismo dos microrganismos e também da madeira dos barris utilizados durante o amadurecimento (JACKSON, 2008). Portanto, sua composição e concentração também dependem da variedade de uva usada na vinificação, do procedimento usado para a vinificação e das reações químicas que ocorrem durante o envelhecimento (RIBÉREAU-GAYON *et al.*, 2006; RECAMALES *et al.*, 2006).

Os compostos fenólicos derivados de uvas, de grande importância para a enologia, são encontrados principalmente em sementes e peles (ESCRIBANO-BAILÓN *et al.*, 1992; 1995 HANLIN *et al.*, 2011). Esses compostos estão envolvidos na estabilidade e nas propriedades organolépticas, como cor e adstringência, ou seja, na qualidade do vinho (RIBÉREAU-GAYON *et al.*, 2006; QUIJADA-MORÍN *et al.*, 2014). Nesse sentido, é amplamente reconhecido que os vinhos tintos de alta qualidade têm um nível equilibrado de adstringência, ou seja, possuem níveis equilibrados de compostos fenólicos e polissacarídeos (BATE-SMITH, 1954; GAWEL *et al.*, 2000; BROSSAUD *et al.*, 2001; PREYS *et al.*, 2006; QUIJADA-MORÍN *et al.*, 2014).

Estudos mostram que os polissacarídeos podem afetar as propriedades organolépticas do vinho (OZAWA *et al.*, 1987; QUIJADA-MORÍN *et al.*, 2014; OSETE-ALCARAZ *et al.*, 2019). Polissacarídeos podem limitar a concentração de proantocianidinas disponíveis e reduzir a adstringência do vinho (ESCOT *et al.*, 2001; MATEUS *et al.*, 2004; CARVALHO *et al.*, 2006). A falta de equilíbrio entre os compostos fenólicos e polissacarídeos e a consequente alteração das propriedades organolépticas leva a uma diminuição na qualidade e estabilidade do vinho (COOMBE, 1973; ORDUÑA, 2010;). Portanto, o estudo desses compostos e a relação entre eles são extremamente importantes para a produção de um vinho de qualidade e competitivo no mercado.

Além disso, há um grande interesse em conhecer os benefícios que o consumo moderado de vinho pode trazer à saúde. Alguns autores descreveram importantes efeitos imunomoduladores, antioxidantes, anti-septicêmicos, anti-inflamatórios, antineoplásicos e gastroprotetores dos polissacarídeos extraídos de plantas, frutas e vinho (MUELLER & ANDERER, 1990; MELLINGER *et al.*, 2008; CIPRIANI *et al.*, 2009; NASCIMENTO *et al.*, 2013; PARK *et al.*, 2013; DARTORA *et al.*, 2013; INNGJERDINGEN *et al.*, 2014; STIPP *et al.*, 2016; CAILLOT *et al.*, 2018). Alguns efeitos benéficos do vinho na saúde têm sido atribuídos aos polifenóis presentes na pele das uvas (DIAZ-GEREVINI *et al.*, 2016; GOJKOVIC-BUKARICA *et al.*, 2018). No entanto, existem poucos estudos sobre atividades

biológicas e modulação de mediadores inflamatórios por polissacarídeos de vinho o que torna importante o estudo dos possíveis benefícios dos polissacarídeos encontrados nos vinhos.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Extração, caracterização, quantificação dos polissacarídeos e polifenóis de vinhos e avaliação da similaridade entre polissacarídeos extraídos de vinhos comerciais de diferentes safras. Como também, investigar interação entre essas moléculas e os benefícios desses polissacarídeos para a saúde, através da avaliação da propriedade anti-inflamatória em ensaios *in vitro* e *in vivo*.

2.2. OBJETIVOS ESPECÍFICOS

- Extrair, caracterizar e quantificar os polissacarídeos de vinhos.
- Avaliar a similaridade entre polissacarídeos extraídos de vinhos comerciais de diferentes safras.
- Caracterizar e quantificar os compostos fenólicos extraídos de vinhos e estabelecer uma possível relação com polissacarídeos.
- Analisar o efeito anti-inflamatório *in vitro* dos polissacarídeos extraídos dos vinhos utilizando células RAW 264.7 estimuladas com lipopolissacarídeos (LPS).
- Avaliar os efeitos anti-inflamatórios das frações polissacarídicas obtidas dos vinhos, através do modelo de septicemia induzida pela cirurgia de ligadura e perfuração do ceco (CLP) e verificar a taxa de sobrevivência dos camundongos tratados com os polissacarídeos.

3. REVISÃO BIBLIOGRÁFICA

3.1. UVA

A baga da uva é composta pela casca, polpa e sementes (Figura 1) e a estrutura do cacho de uva é fornecida pela ráquis, a qual tem a função de sustentar as bagas. As cascas ou películas contêm matéria corante e compostos odoríferos, além de polifenóis. Na superfície externa da casca está a pruína que é uma camada de substância cerosa onde ficam aderidos micro-organismos (exemplo, leveduras alcoolgênicas e bactérias lácticas), os quais conduzem a fermentação espontânea do mosto (KUHN, 2003).

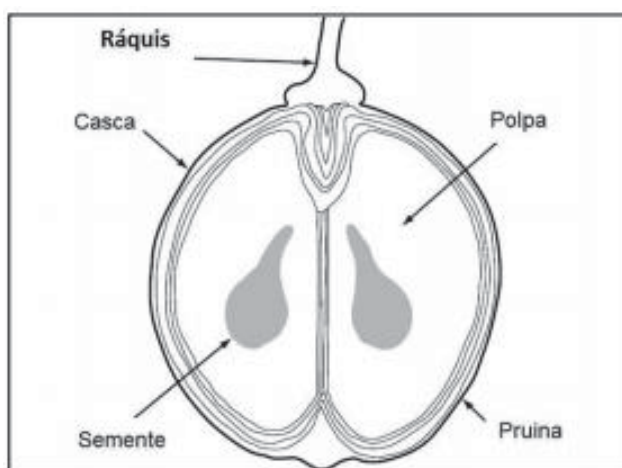


FIGURA 1: Estrutura da baga da uva. FONTE: GRAINGER & TATTERSALL, 2005.

A polpa representa 4/5 do peso da baga e é composta por água, açúcares, ácidos, proteínas e minerais. Os principais açúcares encontrados na uva são a frutose e a glucose. Já os ácidos mais importantes encontrados na baga verde são o ácido tartárico e o málico, sendo que durante o amadurecimento da fruta o ácido tartárico se torna o principal. Quanto aos minerais, o potássio é o que se apresenta em maior quantidade (2.500 mg/kg), já a concentração dos demais (exemplo, cálcio e magnésio) não ultrapassa 200 mg/kg cada (GUERRA, 2003; GRAINGER & TATTERSALL, 2005).

À medida que amadurecem, as uvas passam por mudanças de forma e de composição, como o ganho de açúcar, a queda da acidez, o aumento de volume e de peso, a mudança de coloração e o enriquecimento dos aromas. O tempo da colheita é provavelmente o mais importante aspecto na viticultura, pois a qualidade da uva é indispensável para a qualidade do vinho (GUERRA, 2003).

3.1.1. Tipos de videira

Mundialmente existem milhares de variedades de uva. A maioria delas pertence à espécie *Vitis vinifera*, originária do Cáucaso entre 7.000 e 5.000 a.C., de onde foi difundida por toda a costa mediterrânea. Entre as variedades dessa espécie estão as conhecidas uvas tintas Cabernet Sauvignon, Cabernet Franc, Merlot, Pinot Noir, Tempranillo, Syrah e Tannat e, entre as uvas brancas, destacam-se a Chardonnay, Sauvignon Blanc, Prosecco e Riesling. Algumas delas se difundiram pelo mundo em virtude da sua capacidade de adaptação e pelas características dos vinhos que originam. Outras, de adaptação mais restrita, permaneceram em suas regiões de origem, proporcionando aos seus habitantes a oportunidade de elaboração de produtos típicos e exclusivos (GUERRA *et al.*, 2009).

As uvas oriundas de videiras *Vitis vinifera*, cultivares europeus, são comumente usadas na elaboração de vinho pelo mundo, principalmente na Europa. Nos Estados Unidos, espécies como a *Vitis labrusca*, *Vitis riparia*, *Vitis aestivalis*, *Vitis rupestris* e *Vitis rotundifolia* são as mais usadas para a vinificação (YANG, MARTINSON & LIU, 2009). As frutas destas últimas espécies contêm menos açúcar que as oriundas de espécies *Vitis vinifera*. Desta forma, seus vinhos também não apresentam uma alta qualidade (ESTREICHER, 2006).

3.2. VINHO

O vinho é uma das primeiras criações da humanidade e ocupou um lugar privilegiado em inúmeras civilizações. Há cerca de 60 milhões de anos, surgiram as espécies *Vitis vinifera* e as genericamente chamadas de americanas, como *Vitis labrusca* e *Vitis riparia*. Desde há muito que esta bebida vem acompanhando ascensões e declínios de civilizações e impérios, estando a sua produção fortemente interligada com a agricultura ao longo dos anos. O cultivo de vinha é realizado entre os povos europeus desde os anos 12.000 a.C., no entanto, não há qualquer registro de produção de vinho que remonte a essas datas. Pensa-se ter sido na Ásia há 9000 anos que a utilização de uva fermentada terá tido o seu início e em 4000 a.C. a produção de vinho teria chegado aos povos europeus (ROSADO, 2013; LAROUSSE DO VINHO, 2007).

Com crescimento do comércio do vinho, a ciência passou a ocupar papel importante na vinicultura, e desenvolveram-se programas de pesquisa sobre a vinha, a fermentação e o envelhecimento em adega. Paralelamente, o consumo do vinho disseminou para o mundo

inteiro. Com isso, novos países produtores chegaram ao mercado, ocorrendo a ascensão de países do Novo Mundo, que atualmente competem em qualidade e quantidade com os vinhos europeus (LAROUSSE DO VINHO, 2007).

Dados mostram que o vinho é uma das bebidas mais consumidas atualmente e de maior importância econômica. O nível de produção global em 2018 foi de 292 milhões de hectolitros de vinho, sendo França, Itália e Espanha os três maiores produtores de vinho do mundo, movimentando em torno de 30 bilhões de euros no mercado mundial (figura 2) (dados da Organização Internacional da Vinha e do Vinho - OIV). O Brasil vem se destacando nesse mercado e, produziu 3,1 milhões de litros em 2018, assumindo a 15ª posição no ranking de maiores produtores de vinho segundo a OIV.

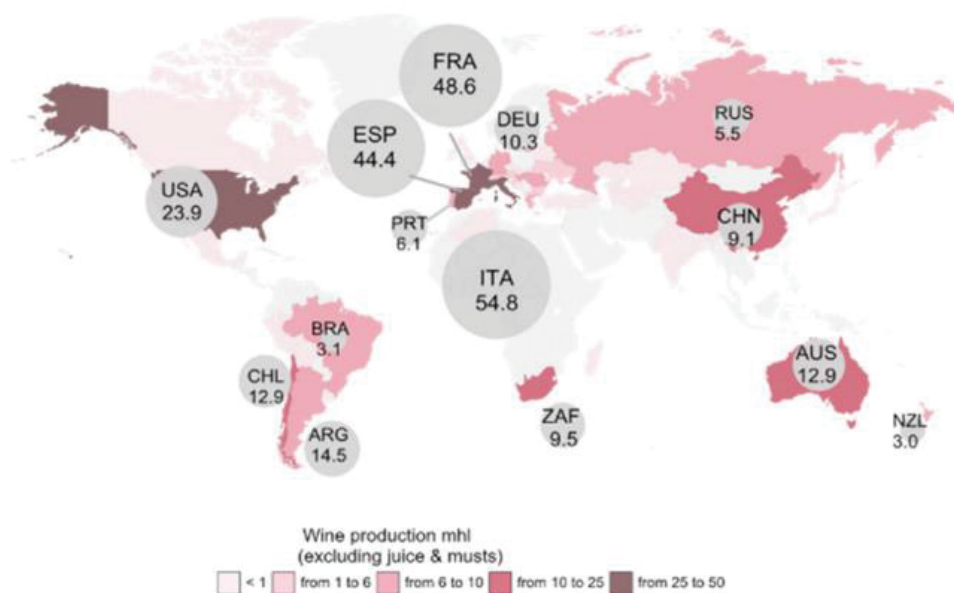


FIGURA 2: Produção mundial de vinho em 2018. FONTE: 2019 Statistical Report on World Vitiviniculture. International Organisation of Vine and Wine.

3.2.1. Processo de produção do vinho

Os vinhos tintos são produzidos a partir de uvas tintas, já os vinhos brancos, são geralmente elaborados a partir de uvas brancas, mas também podem ser produzidos com uvas rosadas e tintas. Para isso, é necessário separar imediatamente a fase líquida da sólida, que contém os pigmentos responsáveis pela cor (CRUZ, 2006; HOFFMAN, 2009).

O processo de produção do vinho inicia-se com a colheita seguida da recepção das uvas, esmagamento e desengace, fermentação, clarificação, filtração, envelhecimento, engarrafamento e rotulagem, análises do vinho e resíduos (figura 3) (CRUZ, 2006; HOFFMAN, 2009). Os vinhos apresentam diferenças ao nível das operações tecnológicas dependendo da vinícola. No processo de fabricação do vinho, as etapas mais importantes descritas detalhadamente são (GRAINGER, *et. al.*, 2005):

- **Recepção e Descarga no Tegão:** Na recepção da uva ocorre o primeiro controle de qualidade para a produção de vinho. Este controle define a ordem de descarga e o tipo de vinho que as uvas irão dar origem e baseia-se no grau alcoólico provável e peso das uvas bem como no resultado da inspeção visual à presença de corpos estranhos e do estado sanitário das uvas.
- **Desengace e Esmagamento:** Após a descarga no tegão, as uvas sofrem um processo de desengace para remoção da parte lenhosa do cacho e um processo de esmagamento para quebrar a película da uva e promover o arejamento que facilita a multiplicação de leveduras. Os vinhos são trasfegados para depósitos onde são mantidos a temperaturas entre 14 e 16°C. Pode ser adicionado SO₂ para proteger de contaminações microbianas e também enzimas de extracção aromática e ácido tartárico.
- **Fermentação alcoólica.** A fermentação é um processo natural de transformação dos açúcares das uvas em etanol. Este processo ocorre a menos de 18 °C, permitindo assim manter os aromas característicos das uvas, e dura 10 a 15 dias. Nesta fase podem ser adicionadas leveduras, de ação conhecida.
- **Prensagem:** Após o esmagamento, o sumo passa pela prensa pneumática para separar a parte sólida da parte líquida.
- **Fermentação malolática:** Nessa etapa o ácido málico é convertido em ácido láctico, menos ácido e menos agressivo, dando sabor e mais agradável ao vinho. Geralmente é feita apenas nos vinhos tintos.
- **Trasfega:** Processo de transferência do vinho para um novo depósito. Após a fermentação alcoólica, o vinho é transferido para um depósito de armazenamento e decantação e mantido entre 15 e 17 °C. Depois, é novamente trasfegado permitindo a remoção das partículas que se encontram no fundo – borras.
- **Escolha e estudo do lote:** Permite a uniformização das características dos vinhos de acordo com as especificações. Nesta fase o vinho pode sofrer vários processos

de alteração do aroma como, por exemplo, a adição de madeiras, mistura de vinhos de castas diferentes, etc.

- **Clarificação.** Este processo tem como propósito a eliminação de impurezas em suspensão por coagulação e formação de partículas mais densas que sedimentam.
- **Envelhecimento:** Essa etapa é realizada apenas com vinho tinto. O envelhecimento do vinho pode ser feito em tanque de inox, em barrica de carvalho e na garrafa. O tempo e a forma de envelhecimento do vinho dependem da variedade da uva, de seu processo de elaboração, do tipo e da estrutura do produto que se deseja, da safra e do valor agregado que terá esse vinho.
- **Filtração e homogeneização:** O vinho passa por uma série de filtrações e homogeneizações de modo a reter a maior quantidade possível de elementos em suspensão e impurezas.
- **Engarrafamento:** o vinho é engarrafado em máquinas especiais que impedem o contato com ar, evitando oxidações e contaminações.

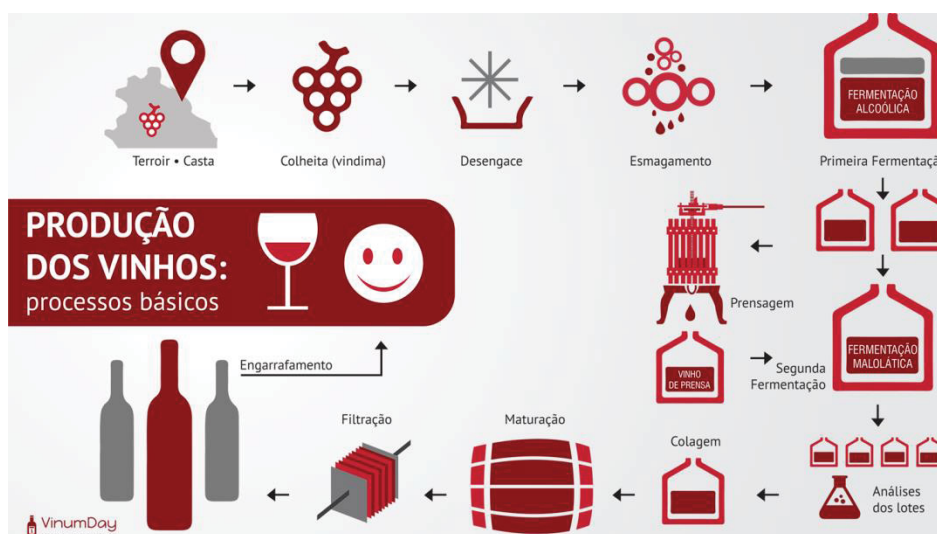


FIGURA 3: Fluxograma da produção do vinho tinto e branco.
<http://blog.vinumday.com.br/como-e-feito-o-vinho/> (acesso em 2 de fevereiro de 2020).

3.2.2. Benefícios do vinho

O vinho é uma bebida obtida exclusivamente a partir da fermentação alcoólica do mosto da uva. Apresenta grande complexidade química devido à natureza dos seus

constituintes. O hábito do consumo moderado de vinho é benéfico à saúde do consumidor, e participa da dieta da população de muitos países (AMERINE *et al.*, 1977; ABAD *et al.*, 1993; NDIAYE *et al.*, 2004).

Entre os efeitos benéficos do vinho destaca-se a capacidade de favorecer a formação em maior quantidade do “bom colesterol” (HDL), o efeito para combater os radicais livres, o efeito antimicrobiano, diurético, anti-inflamatório e antineoplásico (ABAD *et al.*, 1993; STIPP *et al.*, 2017; BEZERRA *et al.*, 2018).

Os taninos, flavonóis e estilbenos são compostos fenólicos presentes em maior quantidade nos vinhos envelhecidos. Os taninos favorecem a digestão e os flavonóis têm sido estudados devido ao seu poder antioxidante e efeito protetor em relação aos radicais livres. No grupo dos estilbenos encontra-se o resveratrol, que é uma fitoalexina. O resveratrol tem sido muito estudado devido ao provável efeito protetor contra doenças cardiovasculares. Na uva, o resveratrol encontra-se na película, e o vinho tinto apresenta maior quantidade, devido ao processo de elaboração. A concentração de resveratrol no vinho varia de 1,3 a 7,0 mg/L (BOURZEIX *et al.*, 1989; ADRIAN *et al.*, 2000; JANNIN *et al.*, 2001). Muitos efeitos benéficos do vinho na saúde têm sido atribuídos ao resveratrol, um polifenol presente na pele das uvas (DIAZ-GEREVINI *et al.*, 2016; GOJKOVIC-BUKARICA *et al.*, 2018). No entanto, existem poucos estudos sobre atividades biológicas e modulação de mediadores inflamatórios por outros componentes do vinho, como os polissacarídeos de vinho.

3.2.3. Carboidratos dos vinhos

Os carboidratos são um dos principais constituintes químicos da maioria dos tecidos e células dos organismos (SIMÕES *et al.*, 2003). Eles existem como monossacarídeos, oligossacarídeos, polissacarídeos e seus derivados (AVIGAD & DEY, 1997). De acordo com a sua função, os carboidratos podem ser classificados em dois grandes grupos: estruturais e de reserva. Os carboidratos estruturais são responsáveis pela formação da parede celular e de outras estruturas, sendo considerados os compostos orgânicos mais abundantes da Terra (REID, 1997).

Os carboidratos presentes no vinho são derivados tanto das paredes das células de micro-organismos quanto das uvas (DOCO *et al.*, 1999; CABANIS & CABANIS, 2000b; VIDAL *et al.*, 2003; AYESTARAN *et al.*, 2004). Os originários das paredes celulares de uva incluem as arabinogalactanas, ramnoglacturonanas e homogalacturonanas, enquanto que

aquelas originadas a partir de paredes das células de levedura são principalmente glucanas, mananas e manoproteínas (WATERS *et al.*, 1994; PELLERIN *et al.*, 1995; MARTÍNEZ-LAPUENTE *et al.*, 2013).

3.2.3.1. Parede celular vegetal e polissacarídeos

A parede celular é dividida em: primária, não lignificada, com células jovens que ainda mantêm a capacidade de divisão e alongamento, apresentam uma parede celular muito fina, com 0,1 a 1 μm de espessura; e secundária, lignificada, com células sem capacidade de crescimento e divisão, que conferem rigidez aos tecidos (REID, 1997).

A parede celular dos vegetais é altamente organizada e constituída de polissacarídeos, proteínas e substâncias aromáticas. Os polissacarídeos são os principais componentes da parede celular e são divididos em pectinas, hemiceluloses e celulose, como pode ser observado na figura 4 (CARPITA & McCANN, 2000). A parede celular primária é composta de 25-40% celulose, 15-25% hemicelulose, 15-40% pectina e 5-10% proteínas e proporções muito pequenas de compostos fenólicos. Já a parede celular secundária, muito mais espessa que a parede primária, possui de 40-45% de celulose, 15-35% de hemicelulose, 15-30% de lignina e quantidade mínima de pectina (DEY *et al.*, 1997).

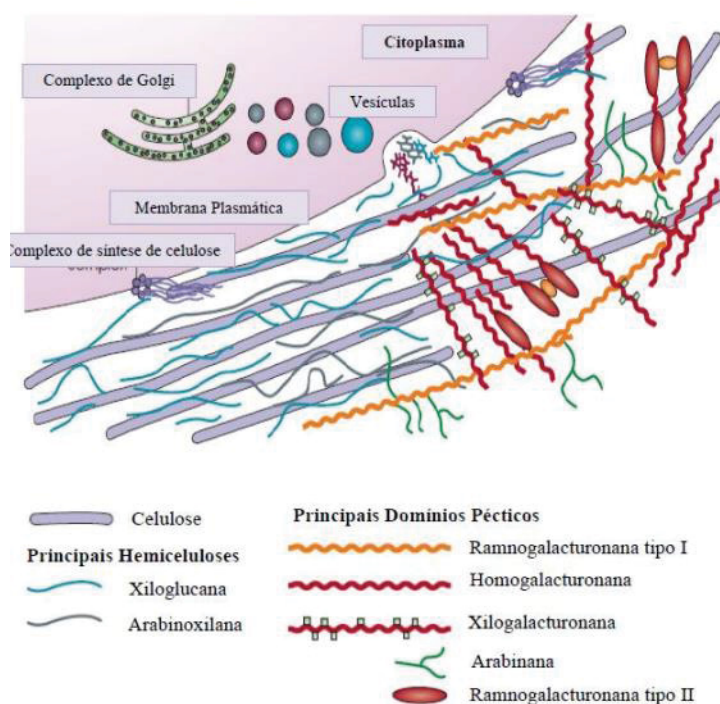


FIGURA 4: Modelo da estrutura da parede celular primária de vegetal. FONTE: Adaptado de COSGROVE, 2005.

As hemiceluloses são polissacarídeos que ocorrem juntamente com a celulose, são constituídas de unidades monossacarídicas de D-manose, D-galactose, D-glucose, L-arabinose, D-xilose, ácido D-galacturônico, ácido D-glucurônico e ácido 4-*O*-metilglucurônico e podem apresentar ainda grupamentos *O*-acetil ligados às unidades pertencentes às cadeias principal e/ou lateral (ASPINALL, 1959; FENGEL & WEGNER, 1989). Esses monossacarídeos formam diferentes polissacarídeos que podem ser incluídos nas classes arabinogalactanas, glucuronoxilanas arabinoxilanas, xilanas, galactoglucomananas, galactomananas, glucuronomananas, glucomananas, mananas e xiloglucanas (ASPINALL, 1980; BRETT & WALDRON, 1990; PULS & SCHUSEIL, 1993; KACURÁCOVÁ *et al.*, 2000). Alguns estudos já relataram a presença de arabinogalactanas e mananas no vinho (PELLERIN *et al.*, 1995; MARTÍNEZ-LAPUENTE *et al.*, 2013).

As pectinas são polissacarídeos ricos em ácido galacturônico, ramnose, arabinose e galactose. O termo pectina refere-se a polissacarídeos complexos que contêm unidades de ácido α -D-galacturônico (GalA). Os principais representantes são: ramnogalacturonas do tipo I (RG I) e ramnogalacturonanas do tipo II (RG II) e homogalacturonanas (ASPINALL, 1980; BRETT & WALDRON, 1990; CARPITA & GIBEAUT, 1993). Dentre elas, as RG I e RG II já foram relatadas em estudos feitos com o vinho (PELLERIN *et al.*, 1995; MARTÍNEZ-LAPUENTE *et al.*, 2013).

3.2.3.1.1. Arabinogalactanas

As arabinogalactanas podem ser classificadas em dois grupos de acordo com as ligações químicas da cadeia principal: arabinogalactanas do tipo I (AG I) e arabinogalactanas do tipo II (AG II) (ASPINAL, 1973).

As arabinogalactanas do tipo I apresentam cadeia principal formada por (1→4)- β -D-galactanas. São pouco ramificadas e, na maioria das vezes, apresentam unidades de arabinose ligadas no *O*-3 das unidades de galactose da cadeia principal (CARPITA & GIBEAUT, 1993).

As arabinogalactanas do tipo II são altamente ramificadas, possuem cadeia principal formada por (1→3) e/ou (1→6)- β -D-galactanas ligadas umas as outras por pontos de

ramificação em *O*-3 e *O*-6 e apresentam a maior parte das posições *O*-3 e *O*-6 restantes ocupadas por unidades de arabinose (CARPITA & GIBEAUT, 1993). Embora apresentem uma estrutura geral semelhante, a estrutura fina das arabinogalactanas do tipo II varia muito entre as espécies (CARPITA & GIBEAUT, 1993; ALBERSHEIM *et al.*, 1996).

3.2.3.1.2. Ramnogalacturonanas

As ramnogalacturonanas são polissacarídeos ricos em ácido galacturônico e ramnose (REID, 1997). Esses polímeros podem ser classificados em dois grupos: ramnogalacturonana do tipo I (RG I) e ramnogalacturonana do tipo II (RG II).

As ramnogalacturonanas do tipo I são heteropolímeros formados por cadeia principal constituída de unidades alternadas de ácido α -D-galacturônico ligadas α -(1 $\rightarrow$ 4) e unidades de α -L-ramnose ligadas α -(1 $\rightarrow$ 2). Esses polímeros podem ser estendidos por ácido poligalacturônico em seus terminais e as unidades ramnosil podem interromper longos períodos de ácido poligalacturônico (CARPITA & GIBEAUT, 1993).

A ramnogalacturonana do tipo II é o polissacarídeo péctico mais complexo, porém o que apresenta a estrutura mais conservada. A característica mais marcante dessa molécula é possuir açúcares raros. São polímeros altamente substituídos que apresentam cadeia principal constituída por unidades de ácido α -D-galacturônico unidas por ligação do tipo (1 $\rightarrow$ 4) (O'NEILL *et al.*, 1990). Esses polissacarídeos apresentam cadeias laterais geralmente substituídas em C-2 das unidades de α -D-GalpA e seus substituintes são formados por oligossacarídeos contendo monossacarídeos raros como apiose, 2-*O*-metil-fucose, 2-*O*-metil-xilose, ácido acérico, Kdo (ácido 2-ceto-3-deoxi-D-mano-octulosônico), Dha (ácido 3-deoxi-D-lixo-2-heotulosárico) entre outros, formando estruturas altamente complexas (CARPITA & GIBEAUT, 1993; REID, 1997; PÉREZ, MAZEAU & PENHOAT, 2000; PAULSEN & BARSETT, 2005; ATMODJO *et al.*, 2013).

3.2.3.2. Parede celular de leveduras e polissacarídeos

Leveduras são fungos que possuem a mesma estrutura subcelular de células animais e vegetais (OSUMI, 1998). São micro-organismos unicelulares, com tamanho que varia de 5 a 10 microns. As espécies variam entre si segundo a morfologia ou forma, metabolismo com

relação a diferentes substratos, modo de reprodução habitat. Enquanto existem aproximadamente 50.000 espécies de fungos, existem apenas 60 gêneros diferentes de leveduras, com aproximadamente 500 espécies diferentes (STONE & MILLS, 2006).

A parede celular da levedura é constituída por aproximadamente 40% de β -glucanas, 40% de α -mananas, 8% de proteínas, 7% de lipídios, 3% de substâncias inorgânicas e 2% de hexosaminas e quitina (HOUGH, 1990). Os principais monossacarídeos presentes nos polímeros são a glucose e a manose, seguidos pela galactose, xilose, N-acetil-D-glucosamina, ácidos urônicos e outros componentes secundários. A composição química qualitativa da parede celular é característica de cada espécie, podendo ser empregada como marcador taxonômico (FARKAS, 1989).

A principal levedura responsável pela fermentação na produção do vinho é a *Saccharomyces cerevisiae*, a qual possui em sua parede celular cerca de 10-20% de proteínas e lipídios e 80-90% de carboidratos, dos quais 50% são glucanas e mananas (NORTHCOTE & HORNE, 1952; BARTNICKI-GARCIA & LIPPMAN, 1982; VUKOVIC *et al.*, 1994). Segundo Vukovic (1994), a *S. cerevisiae* apresenta a parede celular em camadas, sendo as cadeias de mananas externas e as glucanas, internas. Já foi demonstrada a presença de mananas entre os polissacarídeos encontrados no vinho (MARTÍNEZ-LAPUENTE *et al.*, 2013).

3.2.3.2.1. Mananas

As mananas são um dos polissacarídeos de maior importância da parede celular da levedura, responsáveis por eventos de reconhecimento célula-célula, além de limitar o acesso de agentes líticos às porções mais internas da parede celular e à membrana celular (KLIS, *et al.*, 2002).

Há evidências de que as mananas juntamente com os peptídeos complexos constituem a camada mais externa da parede celular das leveduras (BALLOU, 1970; CABIB, 1975). Esses polissacarídeos são altamente ramificado, contendo uma cadeia principal constituída por ligações α -(1 $\rightarrow$ 6) substituída nas posições O-2 por cadeias laterais de unidades de α -D-manopirranose ou oligossacarídeos com unidades de manose (1 $\rightarrow$ 2) e (1 $\rightarrow$ 3) ligadas (STEWART & BALLOU, 1968; BARRETO BERTGER & GORIN, 1983; HALÁSZ & LÁSZTITI, 1991).

3.2.4. Polifenóis do vinho

Os compostos fenólicos constituem um grupo diversificado de metabólitos secundários presentes nas uvas e no vinho. O teor e a composição dos fenólicos nos vinhos são grandemente influenciados pela prática tecnológica à qual as uvas estão expostas. Os polifenóis são componentes intrínsecos das uvas e produtos relacionados, particularmente o vinho (LINSKENS & JACKSON, 1988; SCALBERT, 1993). Os compostos fenólicos desempenham um papel importante nas características sensoriais do vinho, são responsáveis por algumas das propriedades organolépticas como: aroma, cor, sabor e adstringência (LINSKENS & JACKSON, 1988; SCALBERT, 1993).

Existe uma grande diversidade química na composição fenólica das uvas e do vinho. Isto se deve não apenas às diferentes variedades de uvas, como também ao fato de que esse tipo de compostos existe em ambas as formas livre e conjugada, pois podem estar ligadas ao ácido quínico ou a uma ou mais moléculas de açúcar (glicose, galactose, sacarose e manose) produzindo mono-, di-, tri- ou mesmo tetraglicósidos (CHEYNIER *et al.*, 2010; LINSKENS & JACKSON, 1988; SCALBERT, 1993).

Os componentes fenólicos não voláteis das uvas e do vinho compreendem diversas classes: ácidos fenólicos, flavonoides, taninos, estilbenos, cumarinas, derivados de fenilpropanol, lignanas e neolignanas (LINSKENS & JACKSON, 1988; SCALBERT, 1993).. Os polifenóis encontrados no vinho são provenientes da uva e estão localizados principalmente nas partes sólidas: pele, semente e tecido vascular. Os flavonóis e antocianidinas são encontrados na pele e sementes da uva, já as antocianidinas são encontradas apenas na pele da uva tinta, sendo responsável pela cor vermelha característica dos vinhos tintos (KOSIR & KIDRIC, 2002; FLAMINI, 2003).

Os flavonóides pertencem a uma classe química que exibe uma estrutura básica de 15 átomos de carbono compreendendo dois anéis aromáticos ligados através de uma cadeia de 3 carbonos (C6 - C3 - C6), que pode ou não fazer parte de um terceiro anel. Esse esqueleto de carbono é responsável pela diversidade química dessa família de compostos. Os flavonóides são geralmente agrupados em várias classes, que diferem principalmente no grau de oxidação do anel pirano central, exceto no caso das chalconas. Eles compreendem diferentes tipos de compostos tais como flavonas, flavonóis, flavanonas, flavanos, flavanóis, antocianidinas e antocianinas, chalconas e di-hidrochalcones (BADERSCHNEIDER & WINTERHALTER, 2001).

3.2.4.1. Flavanóis

Flavanóis são benzopiranos que possuem carbono saturado cadeia entre C2 e C3, uma função hidroxila em C3 e nenhuma carbonila grupo em C4. Tanto o flavan-3-ols quanto o flavan-3,4-dióis podem ser encontrados na natureza, sendo este último frequentemente presente em madeira e casca de árvore, mas raramente encontrado em frutas. Flavan-3,4-dióis também são freqüentemente chamados de leucoantocianidinas (GARRIDO & BORGES, 2013).

Os flavanois mais abundantes na natureza são a catequina e seu enantiômero epicatequina. Estes compostos estão presentes na casca e sementes de uvas, bem como no vinho. Nos vinhos brancos produzidos em condições especiais, evitando contato prolongado com a pele das uvas, a catequina foi considerada a mais abundante flavonóide, sendo amplamente responsável pelo sabor característico do vinho (LUNTE, BLANKENSHIP, & READ, 1988). Alguns derivados da catequina, nomeados galocatequina, epigalocatequina, e epicatequina galato foram identificados em uvas e vinho (DECENDIT *et al.*, 2002; MATTIVI *et al.*, 2009).

3.2.4.2. Pigmentos antociânicos

Nos últimos anos, várias famílias de derivados de antocianinas têm sido relatadas em uvas e vinho. Pigmentos antociânicos são responsáveis pela cor das uvas e vinho, uma característica determinada pela sua estrutura química, grau de hidroxilação, metilação e/ou glicosilação (HE *et al.*, 2010). Em uvas vermelhas e vinho, seis antocianidinas foram identificadas: cianidina, peonidina, delphinidina, pelargonidina, petunidina e malvidina (HE *et al.*, 2010; KOPONEN *et al.*, 2007). Este último é considerado o composto mais representativo em uvas *Vitis vinifera* (CASTILLO-MUÑOZ *et al.*, 2010). As antocianidinas são geralmente encontradas na natureza nas formas glicosiladas: 3-monoglucósidos 3,5 e 3,7-diglucósidos (KOPONEN *et al.*, 2007). São encontradas apenas na pele da uva, a quantidade de antocianinas e flavonóides extraídos após a vinificação dependem da duração do processo e das condições em que ele ocorre, como a temperatura ou a extensão da ruptura das uvas (ZOECKLEIN *et al.*, 1995).

3.2.4.3. Compostos fenólicos e índice de cor

A coloração dos vinhos está estreitamente relacionada com a sua composição em pigmentos fenólicos (MAZZA *et al.*, 1999). Esses pigmentos apresentam uma variação de cor de acordo com certos parâmetros físico-químicos do meio onde se encontram. Um dos aspectos principais a se ter em conta é o pH do vinho, uma vez que, em meio ácido, as antocianinas apresentam uma coloração vermelha que se dissipa à medida que sobe o pH, apresentando uma cor azul-arroxeadada quando o pH é superior a 4 e uma cor amarela quando o pH é neutro ou básico (RIBÉREAU-GAYON, *et al.* 2006b). Algumas classes de pigmentos antociânicos apresentam maior estabilidade com mudança de pH e com envelhecimento.

Polifenóis são compostos instáveis e suas reações começam assim que a uva é esmagada ou prensada e continuam durante o processo de vinificação e envelhecimento, resultando uma diversidade grande de novos produtos. Tais produtos apresentam propriedades organolépticas específicas, frequentemente diferentes daquelas de seus precursores. A composição fenólica nos vinhos depende da uva utilizada e das condições de vinificação que influenciam na extração de vários compostos da uva e das subsequentes reações (CHEYNIER *et al.* 2006). Durante o armazenamento, os pigmentos antociânicos podem provocar mudança de coloração (CEJUDO-BASTANTE *et al.* 2011). O aumento da concentração dos compostos fenólicos ao longo do tempo é atribuído às reações de oxidação e polimerização, onde também ocorrer um aumento na absorbância de 420 nm, diretamente relacionada com o escurecimento dos vinhos (BARÓN *et al.* 1997). Portanto, os compostos fenólicos são moléculas de grande importância para a enologia, sendo um dos principais responsáveis pelas propriedades organolépticas do vinho.

3.3. PROCESSO INFLAMATÓRIO E POLISSACARÍDEOS

A reação da imunidade natural, ou inflamação, é uma resposta do organismo a estímulos nocivos. Durante uma invasão por patógeno ou danos nos tecidos do hospedeiro, a imunidade natural é a primeira linha de defesa do organismo (WANG *et al.*, 2002). Essa ação protetora é obtida principalmente pelo recrutamento de leucócitos, seguida por uma cascata de reações bioquímicas que se propagam e iniciam a resposta inflamatória (NAKAMURA *et al.*, 1992; VARKI, 1997; SIMON *et al.*, 1999; MULLER, 2003; YADAV *et al.*, 2003). Essa migração leucocitária ocorre do compartimento intravascular, principalmente de vênulas pós-

capilares, para o tecido onde ocorreu o estímulo nocivo, sendo denominado de extravasamento ou diapedese. Esse mecanismo é constituído de várias etapas: marginação, ligação inicial e rolamento dos leucócitos no endotélio inflamado, ativação dos leucócitos, firme aderência à parede vascular, degradação da membrana basal e migração dos leucócitos para o sítio de inflamação (Figura 4) (SEELY *et al.*, 2003; PARISH, 2006; SHERWOOD *et al.*, 2004).

Os indutores inflamatórios no sítio específico levam à produção de citocinas pró-inflamatórias, TNF (Fator de Necrose Tumoral) e IL-1 (Interleucina-1) pelas células locais (BARRINGTON *et al.*, 2001; NAKAMURA *et al.*, 1992; SIEDLE *et al.*, 2003). Essas substâncias irão agir no endotélio vascular, causando alterações intracelulares que culminam na expressão de moléculas de adesão e citocinas, seguida pela modificação do seu citoesqueleto de retração. Isso garantirá a passagem dos leucócitos da circulação para o local da lesão (SIMON *et al.*, 1999; HOGG *et al.*, 1995; LASKY, 1992; VARKY, 1997). A aderência e o rolamento de leucócitos no endotélio ativado pelo estímulo inflamatório envolvem selectinas de linfócitos, plaquetas e células endoteliais (L-, P-, e E-selectinas, respectivamente) e seus ligantes (figura 5) (PARISH, 2006). A L-selectina é expressa constitutivamente por leucócitos e se liga ao endotélio inflamado através das cadeias de heparan sulfato (HS). P- e E-selectina são expressas nas células endoteliais após ativação por citocinas pró-inflamatórias (LOWE, 2003).

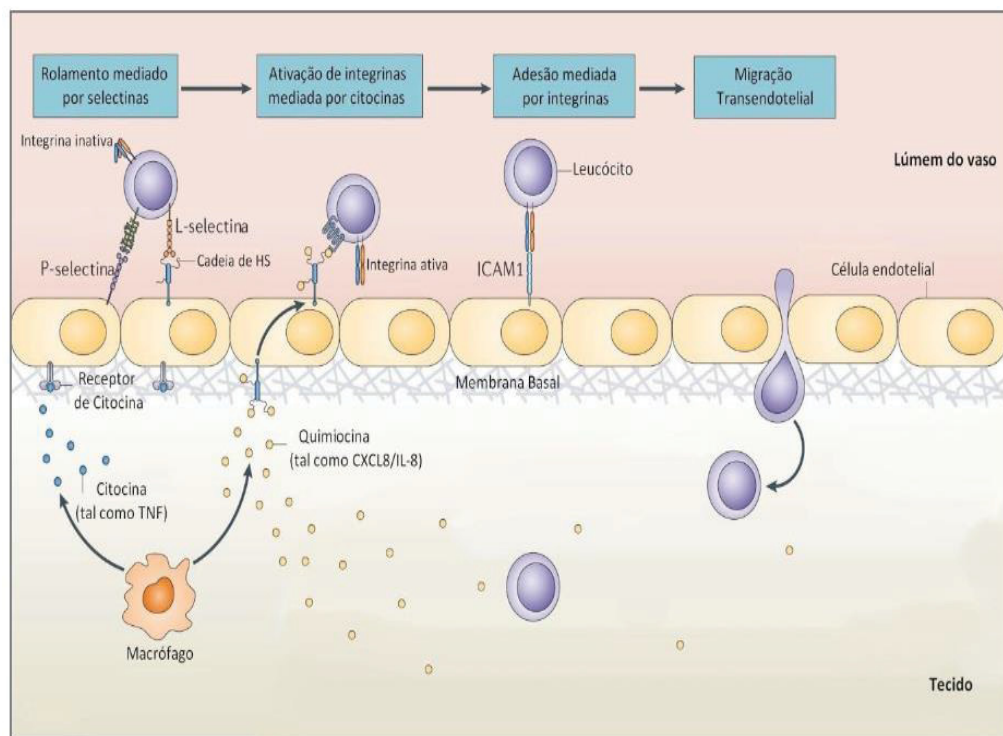


FIGURA 5: Recrutamento de leucócitos e processo inflamatório. FONTE: Adaptada de PARISH, 2006.

Dessa forma, as primeiras células a chegarem ao sítio de injúria são os leucócitos polimorfonucleares (PMNs). Em seguida, aparecem os monócitos, que se transformam em macrófagos nos tecidos e exercem importante papel na fagocitose e eliminação dos antígenos através dos mecanismos microbicidas, inerentes a essas células (ZARBOCK *et al.*, 2008). Em um período mais tardio, migram as células da imunidade adquirida, os linfócitos, que auxiliam de modo direto ou indireto as células da imunidade inata em um mecanismo de amplificação da resposta imune.

Quando a resposta inflamatória torna-se exagerada pode culminar em doenças inflamatórias graves como a sepse (SERHAM *et al.*, 2008), sendo necessária a intervenção com uso de moléculas que possam modular o processo inflamatório.

3.3.1. Sepses

A inflamação é considerada uma resposta normal e essencial do hospedeiro que pode ocorrer na presença de agentes infecciosos. Alguns estudos sugerem que o início e a progressão da sepse se devem a uma “desregulação” da resposta normal, com uma ativação maciça e descontrolada de células inflamatórias (PECK, 2007). Em indivíduos que evoluem para esse quadro, observa-se uma completa ativação da resposta imune e isso ocorre devido à liberação de altos níveis de padrões moleculares associados ao dano celular dos micro-organismos invasores e/ou tecido do hospedeiro lesado levando a estimulação de células do sistema imune.

A fisiopatologia da sepse é extremamente complexa e ainda bastante investigada. Seu principal evento é a resposta inflamatória sistêmica ao agente infeccioso (CINEL *et al.*, 2009) após o reconhecimento dos constituintes expressos pelo micro-organismo invasor e os receptores de reconhecimento padrão (RRP), como os receptores Toll-like (TLR) e o CD14, que reconhecem os patógenos ou seus produtos, identificados como PAMPs (padrões moleculares associados a patógenos). As células hospedeiras sintetizam uma série de proteínas incluindo citocinas pró-inflamatórias que, juntamente com outros mediadores gerados pela cascata inflamatória, agem contra os micro-organismos desencadeando distúrbios hemostáticos e disfunção orgânica (LEVI *et al.*, 2010; SEMERARO *et al.*, 2010).

A inflamação pode também resultar em apoptose ou necrose de células (REETTA *et al.*, 2011). Evidências recentes indicam que produtos liberados a partir de células mortas, tais como as proteínas nucleares, são capazes de propagar ainda mais inflamação, morte celular e falência de órgãos (CINEL *et al.*, 2009; XU *et al.*, 2009).

A falência de órgãos, também conhecida como síndrome da disfunção de múltiplos órgãos (MODS), provocada pela sepse grave representa a principal causa da elevada taxa de mortalidade nessas condições, causada pela hiper-resposta inflamatória generalizada desencadeada pela invasão de um patógeno (WANG *et al.*, 2008). Estudos sugerem que a MODS durante a sepse pode ser causada, também, por espécies reativas de oxigênio e enzimas proteolíticas liberadas pelos neutrófilos recrutados para os tecidos, além disso, a presença de altas concentrações de citocinas no espaço intersticial pode ser tóxica e desempenha importante papel na sepse com leucocitose severa (WANG *et al.*, 2008).

Os pulmões, o fígado e os rins são os primeiros órgãos afetados pela MODS na sepse. Por esse motivo se faz necessária uma avaliação das funções desses órgãos no estágio inicial da doença na prática clínica, além do estudo histopatológico, é realizada também por meio de avaliação de parâmetros bioquímico como análises das enzimas aminotransferases e da bilirrubina para avaliação da disfunção hepática e as análises de creatinina sérica e ureia para avaliação da disfunção renal (PRATT *et al.*, 2010).

Diversos trabalhos foram realizados e as atividades biológicas mais comumente atribuídas aos carboidratos de diversas fontes são: atividade anti-inflamatória, antitumoral, antiviral, antiúlcera gástrica, e anticoagulante (BOHN & MILLER, 1995; CAPEK *et al.*, 2003; RENARD *et al.*, 2005, CIPRIANI *et al.*, 2006, 2009; SIMAS-TOSIN *et al.*, 2012; DARTORA *et al.*, 2013).

Polissacarídeos, como ramnogalactofuranana e arabinogalactana de várias fontes apresentaram atividade anti-inflamatória e redução da letalidade de camundongos quando submetidos à sepse polimicrobiana induzida por ligadura e punção cecal (CLP) (SCOPARO *et al.*, 2013). Esse modelo biológico mimetiza a sepse em humanos, causada por patógenos derivados do trato intestinal e é considerada como uma simulação próxima da situação clínica (RITTIRSCH, HUBER-LANG, FLIERL, & WARD, 2009).

REFERÊNCIAS BIBLIOGRÁFICAS

ABAD, F. B.; PLASENCIA, J. M. B. Consumo moderado de bebidas alcohólicas: salud y civilización. Madrid: Inesiba, 135 p, 1993.

ADRIAN, M.; JEANDET, P.; BREUIL, A. C.; LEVITE, D.; DEBORD, S.; BESSIS, R. Assay of resveratrol and derivative stilbenes in wines by direct injection High Performance Liquid Chromatography. *American Journal of Enology & Viticulture*, Davis, v. 51, n. 1, p. 37-40, 2000.

ALBERSHEIM, P.; DARVILL, A. G.; O'NEILL, M. A.; SCHOLS, H. A.; VORAGEN, A. G. J. An hypothesis: the same six polysaccharides are components of the primary cell walls of all higher plants. *Progress in biotechnology: Pectins and Pectinases*. Amsterdam: Elsevier, v. 14, p. 47-55, 1996.

AMERINE, M. A. Le vin dans l'alimentation humaine. *Bulletin de l'O.I.V.* Paris, número especial, p. 137-151, 1977.

ASPINAL, G. O. In: *Biogenesis of Plant Cell Wall Polysaccharides*. New York: Academic Press, p. 95-115, 1973.

ASPINAL, G. O. Chemistry of cell wall polysaccharides. In: STUMPF, P. K.; CONN, E. E. *The Biochemistry of Plants*. New York: Academic Press. v. 3, p. 473-500, 1980.

ASPINAL, G.O. Structural chemistry of the hemicelluloses. *Advances in Carbohydrate Chemistry*. New York, p. 429-430, 1959.

ATMODJO, M. A.; HAO, Z.; MOHNEN, D. Evolving views of pectin biosynthesis. *Annual Review of Plant Biology*, vol. 64, p. 747-749, 2013.

AVIGAD, G.; DEY, P. M. Carbohydrate metabolism: storage carbohydrates. In: DEY, P. M.; HARBORNE, J. B. *Plant Biochemistry*. Bristol: Academic Press, p.143, 1997.

AYESTARAN, B.; GUADALUPE, Z.; LEON, D. Quantification of major grape polysaccharides (Tremanillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta*, v. 513, p. 29-39, 2004.

BADERSCHNEIDER, B., & WINTERHALTER, P. Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry*, 49(6), 2788–2798. (2001).

BALLOU, C.E. A study of the immunochemistry of three yeast mannans. *Journal of Biological Chemistry*, v.245, n.5, p.1197-1203, 1970.

Barón, R.; Mayén, M.; Mérida, J.; Medina, M. Changes in phenolic compounds and browning during biological aging of SherryType Wine. *J. Agric. Food Chem.* 45: 1682- 1685. (1997).

BARRETO-BERGTER, E.; GORIN, P. A. J.; TRAVASSOS, L. R. Cell constituents of the mycelia and conidia of *A. fumigatus*. *Carbohydrates Letters*, 95 (1): 205-218, 1981.

BARRINGTON, R.; ZHANG, M., FISCHER, M., CARROLL, M.C. The role of complemente in inflammation and adaptative immunity. *Immunolo Rev.* 180, 5-15, 2001.

BARTNICKI-GARCIA, S; LIPPMAN, E. Fungal morphogenesis: Cell wall construction in *Muca rouxii*. *Scienc*, 1659(1): 302-304, 1969.

BEZERRA, I.L.; CAILLOT, A. R. C.; PALHARES, L. C. G. F.; SANTANA-FILHO, A. P.; CHAVANTE, S. F. GUILHERME LANZI SASSAKI, G. L. Structural characterization of polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: Anti-inflammatory activity in LPS stimulated RAW 264.7 cells. *Carbohydrate Polymers*, 186, 91-99. 2018.

BOHN, J. A.; Be MILLER, J. N. 1 $\rightarrow$ 3 β -D-glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers*, v. 28, p. 3-14, 1995.

BOURZEIX, M. Raisin, vin, nutrition et santé. Les catéchines et proanthocyanidols du raisin et du vin. *Progrés Agricole et Viticole*, Montpellier, v. 106, n. 22, p. 487-491, 1989.

BRETT, C.; WALDRON, K. Physiology and biochemistry of plant cell walls. London: Unwin Hyman, 194p, 1990.

CAILLOT, A. R. C.; BEZERRA, I.L.; PALHARES, L. C. G. F.; SANTANA-FILHO, A. P.; CHAVANTE, S.; SASSAKI, G. L. Structural characterization of blackberry wine polysaccharides and immunomodulatory effects on LPS-activated RAW 264.7 macrophages. *Food Chemistry*, 257, 143-159.,2018.

CABANIS, J. C.; CABANIS, M. T. Tablas de composición. Cap.8. In: FLANZY, C. *Enología: Fundamentos científicos y tecnológicos*. Madrid: Ediciones A. Madrid Vicente, Ediciones Mundi-Prensa. 1ª edición, 783p, 2000b.

CABIB, E. Molecular aspects of yeast morphogenesis. *Annual Review of Microbiology*, 29: 191-214, 1975.

CAPEK, P.; HRIBALOV£, V.; SVANDOV£, E.; EBRINGEROV£, A.; SASINKOV£, V., MASAROV£, J. Characterization of immunomodulatory polysaccharides from *Salvia officinalis* L. *International Journal of Biological Macromolecules*, v. 33, p. 113-119, 2003.

CARPITA, N. C.; GIBEAUT, D. M. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, v. 3, p. 1-30, 1993.

CARPITA, N.; McCANN, M. The cell wall. In: BUCHANAN, B. B.; GRUISSEM, W.; JONES, R. L. *Biochemistry & Molecular Biology of Plants*. USA: Courier. p. 52-108, 2000.

CEJUDO-BASTANTE, M.J.; HERMOSÍN-GUTIERREZ, I.; CASTRO-VAZQUEZ, L.I.; PÉREZ-COELLO, M.S. Hydroxylation and bottle storage of Chardonnay white wines: Effects on color-related phenolics, volatile composition, and sensory characteristics. *J. Agric. Food Chem.* 59: 4171- 4182. (2011).

CHEYNIER, V.; DUEÑAS-PATON, M.; SALAS, E.; MAURY, C.; SOUQUET, J. M.; SARNI-MANCHADO, P.; FULCRAND, H. Structure and properties of wine pigments and tannins. *Am. J. Enology Vitic.*, 57: 298-305. (2006).

CINEL I, OPAL SM. Molecular biology of inflammation and sepsis: a primer. *Crit Care Med*;37:291–304. 2009.

CASTILLO-MUÑOZ, N., WINTERHALTER, P., WEBER, F., GÓMEZ, M. V., GÓMEZ-ALONSO, S., GARCÍA-ROMERO, E. Structure elucidation of peonidin 3,7-O- β -diglucoside isolated from Garnacha Tintorera (*Vitis vinifera* L.) grapes. *Journal of Agricultural and Food Chemistry*, 58(20), 11105–11111. (2010).

CHEYNIER, V., SCHNEIDER, R., SALMON, J. -M., & FULCRAND, H. Chemistry of wine. *Comprehensive Natural Products II*, 3, 1119–1172. (2010).

CIPRIANI, T. R.; MELLINGER, C. G.; BERTOLINI, L. C.; BAGGIO, C. H.; FREITAS, C. S.; MARQUES, M. C. A.; GORIN, P. A. J.; SASSAKI, G. L.; IACOMINI, M. Gastroprotective effect of a type I arabinogalactan from soybean meal, *Food Chemistry*. V115. p. 687-690, 2009a.

CIPRIANI, T. R.; MELLINGER, C. G.; SOUZA, L. M., BAGGIO, C. H., FREITAS, C. S. Polysaccharide from a tea (Infusion) of *Maytenus ilicifolia* leaves with anti-ulcer protective effects. *Journal of Natural Products*, v. 69, p. 1018-1021, 2006.

CIPRIANI, T. R.; MELLINGER, C. G.; SOUZA, L. M.; BAGGIO, C. H., FREITAS, C. S.; MARQUES, M. C. A.; GORIN, P. A. J.; SASSAKI, G. L. IACOMINI, M. Polygalacturonic acid: Another anti-ulcer polysaccharide from the medicinal plant *Maytenus ilicifolia*. *Carbohydrate Polymers*, v. 78, p.361-363, 2009b.

CRUZ, INÊZ. A *Vitis vinifera* e as demais *Vitis*. *Saúde de Viver*. p.63-95. 2006.

DARTORA, N.; SOUZA, L. M.; SIMONE M.M. PAIVA, S. M. M.; CAMILA T. SCOPARO, C. T.; MARCELLO IACOMINI, M.; GORIN, P. A. J.; YANNA D. RATTMANN, Y. D.; SASSAKI, G. L. Rhamnogalacturonan from *Ilex paraguariensis*: A potential adjuvant in sepsis treatment. *Carbohydrate Polymers*, 1776–1782, 2013.

DECENDIT, A., WAFFO-TEGUO, P., RICHARD, T., KRISA, S., VERCAUTEREN, J., MONTI, J. -P., et al. Galloylated catechins and stilbene diglucosides in *Vitis vinifera* cell suspensions cultures. *Phytochemistry*, 60(8), 795–798. (2002).

DEY, P.M.; BROWNLEADER, M.D.; HARBORNE, J.B. The plant, the cell and its molecular components. In: DEY, P. M.; HARBORNE, J. B. *Plant Biochemistry*. Bristol: Academic Press, p. 6-9. 1997.

DIAZ-GEREVINI, G. T., REPOSSI, G., DAIN, A., TARRES, M. C., DAS, U. N., EYNARD, A. R. Beneficial action of resveratrol: How and why?. *Nutrition*, 2, 174-178. 2016.

DOCO, T.; VUCHOT, P.; CHEYNIER, V.; MOUTOUNET, M. Structural modification of arabinogalactan-proteins during aging of red wines on lees. *American Journal of Enology and Viticulture*, 54, 150–157. 2003.

DOCO, T.; QUELLEC, N.; MOUTOUNET, M.; PELLERIN, P.; Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of nology and Viticulture*, v. 50, p. 28-32, 1999.

ESTREICHER, S. K., Ed. *Wine: From Neolithic Times to 21st Century*. Nova York: Algora, 1 ed. 2006.

FARKAŠ, V. Polysaccharide Metabolism. In: ROSE, A.H.; HARRISON, J.S. *The yeasts: metabolism and physiology of yeasts*, 2ed. London: Academic Press, Vol.3, cap. 9, p. 317-366, 1989.

FENGEL, D.; WEGNER, G. *Wood: chemistry, ultrastructure, reactions*. Berlin: Gruyter, W. 613p, 1989.

FLAMINI, R. Mass spectrometry in grape and wine chemistry. Part I: Polyphenols. *Mass Spectrometry Reviews*, 22(4), 218–250. (2003).

FOURNAIRON, C.; CAMARASA, C.; MOUTOUNET, M.; SALMON, J. M. New trends on yeast autolysis and wine ageing on lees. *Journal International de la Vigne et du Vin*, 36, 49–69, 2002.

GARRIDO J., BORGES F. Wine and grape polyphenols. A chemical perspective. *Food Research International*; 54: 1844-1858, 2013.

GERBAUD, V.; GABAS, N., BLOUNIN, J.; PELLERIN, P.; MOUTOUNET, M. Influence of wine polysaccharides and polyphenols on the crystallization of potassium hydrogen tartrate. *Journal International Science Vigne et du Vin*, 31, 65–83. 1997.

GOJKOVIC-BUKARICA, L., MARKOVIC-LIPKOVSKI, J., HEINLE, H., CIROVIC, S., JOVANA RAJKOVIC, J., DJOKIC, V., ZIVANOVIC, V., ANA BUKARICA, A., NOVAKOVIC, R. The red wine polyphenol resveratrol induced relaxation of the isolated renal artery of diabetic rats: The role of potassium channels. *Journal of Functional Foods*, 52, 266–275. 2018.

GRAINGER, K.; TATTERSALL, H., Eds. *Wine Production: Vine to Bottle*. Oxford: Blackwell, p.127, 1 ed. 2005.

GUADALUPE, Z.; AYESTARAN, B. Polysaccharide profile and content during the vinification and aging of Trempanillo red wines. *Journal of Agricultural and Food Chemistry*, v. 55, p. 10720-10728, 2007.

GUERRA, C. C. *Conhecendo o Essencial sobre Uvas e Vinhos*. 2009. Disponível em: <<http://www.cnpuv.embrapa.br/publica/documentos/doc048.pdf>>. Acesso em: 20/06/2018.

GUERRA, C. C. *Maturação e Colheita*. In: GUERRA, C. C. *UVA para processamento Pós-colheita*. Brasília: Embrapa, 2003.

HALÁSZ, A., LÁSZTITY, R. *Use of yeast biomass in food production*. New York, CRC press, 312p, 1991.

HE, F., MU, L., YAN, G. -L., LIANG, N. -N., PAN, Q. -H., WANG, J. Biosynthesis of anthocyanins and their regulation in colored grapes. *Molecules*, 15(12), 9057–9091. (2010).

HOFFMAN, ALEXANDRE. *Sistema de produção de destilado de vinho*. EMBRAPA. 2009.

HOGG, J.C.; DORESCHUK, CM. Leukocyte traffic in the lung. *Annu. Rev. Physiol.* 57, 97-114, 1995.

HOUGH, J. S. *Biotechnologia de la cerveza y de la malta*. Zaragoza, Acribia. 194.p, 1990.

INNGJERDINGEN, K. T., THÖLE, C., DIALLO, D., PAULSEN, B. S. HENSEL, A. Inhibition of *Helicobacter pylori* adhesion to human gastric adenocarcinoma epithelial cells by aqueous extracts and pectic polysaccharides from the roots of *Cochlospermum tinctorium* A. Rich. And *Vernonia kotschyana* Sch. Bip. Ex Walp. *Fitoterapia*, 95, 127–132, 2014.

JANNIN, B.; DELMAS, D.; CHERKAOUI MALKI, M.; LATRUFFE, N. Étude du mécanisme d'action du resvératrol, un polyphénol du vin, sur la prolifération cellulaire. *Bulletin de l'O.I.V.*, Paris, v. 74, n. 839-840, p. 54-60, 2001.

JOHNSON, H. *Vintage: The Story of Wine*. Simon & Schuster. p.11-16.1989.

KAKURÁKOVÁ, M.; CAPEK, P.; WELLNER, N.; EBRINGEROVÁ, A. FT-IR study of plant cell model compounds: pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers*, v. 43, p. 195-203, 2000.

KOPONEN, J. M., HAPPONEN, A.M., MATTILA, P. H., & TÖRRÖNEN, A.R. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *Journal of Agricultural and Food Chemistry*, 55(4), 1612–1619. (2007).

KOSIR, I. J., & KIDRIC, J. Use of modern nuclear magnetic resonance spectroscopy in wine analysis: Determination of minor compounds. *Analytica Chimica Acta*, 458(1), 77–84. (2002).

KLIS, F. M.; MOL, P.; HELLINGWERF, K.; BRUL, S. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiology Reviews*, v. 26, p. 239-256, 2002.

LAROUSSE DO VINHO. Edição brasileira atualizada. 2ª edição, 2007.

LASKY, L.A. Selectins: interpreters of cell-specific information during inflammation. *Science*. 258, 964-969, 1992.

LEVI M, SCHULTZ M, VAN DER POLL T. Disseminated intravascular coagulation in infectious disease. *Semin Thromb Hemost.*;36:367–77. 2010.

LINSKENS, H. F., & JACKSON, J. F. Phenolic composition of natural wine types — Wine analysis. Berlin: Springer-Verlag Press. (1988).

LOWE, J.B. Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Curr. Opin. Cell Biol.* 15: 531–538, 2003.

MARTÍNEZ-LAPUENTE, L.; GUADALUPE, Z.; AYESTARAN, B.; ORTEGA-HERAS, M.; PEREZ-MAGARINO, S. Changes in polysaccharide composition during sparkling wine maturing and aging. *Journal Agricultural and Food Chemistry*, v. 61, p. 12362-12373, 2013.

MATTIVI, F., GUZZON, R., VRHOVSEK, U., STEFANINI, M., & VELASCO, R. Metabolite profiling of grape: Flavonols and anthocyanins. *Journal of Agricultural and Food Chemistry*, 54(20), 7692–7702. (2006).

MAZZA, G., FUKUMOTO, L., DELAQUIS, P., GIRARD, B., & EWERT, B. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir Wines from British Columbia. *Journal of Agricultural and Food Chemistry*, 47(10), 4009–4017. (1999).

MELLINGER, C. G., CIPRIANI, T. R., NOLETO, G. R., CARBONERO, E. R., OLIVEIRA, M. B. M., GORIN, P., IACOMINI, M. Chemical and immunological modifications of an arabinogalactan present in tea preparations of *Phyllanthus niruri* after treatment with gastric fluid. *International Journal of Biological Macromolecules*, 43, 115–120, 2008.

MUELLER, E. A., & ANDERER, F. A. Synergistic action of a plant rhamnogalacturonan enhancing antitumor cytotoxicity of human natural killer and lymphokine-activated killer cells: Chemical specificity of target cell recognition. *Cancer Research*, 50(12), 3646–3651. 1990.

MULLER, W.A. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunology*. 24: 327-334, 2003.

NAKAMURA, H. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J. Clin Invest.* 89, 1478-1484, 1992.

NDIAYE, M.; CHATAIGNEAU, M.; LOBYSHEVA, I.; CHATAIGNEAU, T.; SCHINI-KERTH, V.B. Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. *FASEB J.* 2005 Mar;19(3):455-7. Epub 2004.

NORTHCOTE, D. H.; HORNE, R. W. The Chemical composition and structure of the yeast cell wall. *Biochemical Journal* 51: 232-236, 1952.

O'NEILL, M.; ALBERSHEIM, P.; DARVILL, A. The pectic polysaccharides of primary cell walls. In: DEY, D. M. *Methods in Plant Biochemistry*. London: Academic Press, p. 415-441, 1990.

OIV (Office International de la Vigne et du Vin. 2019 Statistical Report on World Vitiviniculture. (2019)

OSUMI, M. The ultrastructure of yeast: Cell wall formation and structure. *Micron*, v.29, n.2/3, p.207-233, 1998.

PARISH, C.R. The role of Heparan sulphate in inflammation. *Nature Reviews Immunology*. 6: 633-643, 2006.

PARK, S. N., NOH, K. T., JEONG, Y.-I., JUNG, I. D., KANG, H. K., CHA, G. S., PARK, Y.-M. Rhamnogalacturonan II is a Toll-like receptor 4 agonist that inhibits tumor growth by activating dendritic cell-mediated CD8⁺ T cells. *Experimental & Molecular Medicine*, 45(2), e8, 2013.

PAULSEN, B. S. Plant polysaccharides with immunostimulatory activities. *Current Organic Chemistry*, vol. 5, p. 939-950, 2001.

PECK, G. M. F. Ativação de macrófagos e sua correlação com o desfecho de sepse. [S.l.]: Dissertação de mestrado. Universidade do Extremo Sul Catarinense, 2007.

PELLERIN, P.; VIDAL, S.; WILLIAMS, P.; BRILLOUET, J. M. Characterization of five type II arabinogalactan-protein fractions from red wine of increasing uronic acid content. *Carbohydrate Research* 277, 135- 143, 1995.

PÉREZ, S.; MAZEAU, K.; PENHOAT, H. The three-dimensional structures of the pectic polysaccharides. *Plant Physiology and Biochemistry*, vol. 32, n. 1/2, p. 37-55, 2000.

PRATT, D S. Liver chemistry and function tests. In: Feldman M, Friedman LS, Brandt LJ, editors. *Sleisenger and Fordtran's gastrointestinal and liver disease*. 9th ed. Philadelphia: Saunders. p. 1227–37. 2010.

PULS, J.; SCHUSEIL, J. Chemistry of hemicelluloses: relationship between hemicellulose structure and enzymes required for hydrolysis. In: *Hemicellulose and hemicellulases.*, London: Portland Press Research Monograph, p. 1-27, 1993.

REETTA HUTTUNEN, JANNE AITTONIEMI. New concepts in the pathogenesis, diagnosis and treatment of bacteremia and sepsis. *British infection association*. 63, 407 e 419. 2011.

REID, J. S. G. Carbohydrate metabolism: structural carbohydrates. In: DEY, P. M.; HARBORNE, J. B. *Plant Biochemistry*. Bristol: Academic Press, p. 205-236, 1997.

RENARD, C. M. G. C.; LAHAYE, M.; MUTTER, M.; VORAGEN, F. G. J.; THIBAULT, J. F. Isolation and structural characterisation of rhamnogalacturonan oligomers generated by controlled acid hydrolysis of sugar-beet pulp. *Carbohydrate Research*, v. 305, p. 271-280, 1998.

RIBÉREAU-GAYON, P.; GLORIES, Y.; MAUJEAN, A.; DUBOURDIEU, D. *Handbook of enology - The chemistry of wine stabilization and treatments*, Wiley, West Sussex. vol 2. 2006.

RITTIRSCH, D., HUBER-LANG, M. S., FLIERL, M. A., WARD, P. A. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nature Protocols*. 4, 31–36. 2009.

ROSADO A., "Evolução de Parâmetros Físicos, Químicos e Controlo Microbiológico em Vinhos Brancos e Tintos da Adega Cooperativa de Palmela", FCT-UNL, 2013.

SCALBERT, A. Phenolics in fruits and fruit products: Progress and prospects, polyphenolic phenomena. Paris: INRA Editions. (1993).

SCOPARO, C. T., SOUZA, L. M., RATTMANN Y. D., DARTORA, N., PAIVA, S. M. M., SASSAKI, G. L., GORIN, P. A. J., IACOMINI, M. Polysaccharides from green and black teas and their protective effect against murine sepsis. *Food Research International*, 53, 780–785. 2013.

SEELY, A. J. E.; PASCUAL, J. L.; CHRISTOU, N. V. Science review: cell membrane expression (connectivity) regulates neutrophil delivery, function and clearance. *Critical Care*, v. 7, p. 291-307, 2003.

SEMERARO N, AMMOLLO CT, SEMERARO F, COLUCCI M. Sepsis-associated disseminated intravascular coagulation and thromboembolic disease. *Mediterr J Hematol Infect Dis*;2(3):e2010024, 2010.

SHERWOOD, E. R.; TOLIVER-KINSKY, T. Mechanisms of the inflammatory response. *Best Pract Res Clin Anaesthesiol*, v. 18, n. 3, p. 385–405, Sep/2004.

SIEDLE, L. G.; GUSTAVSSOM, L.; JOHANSSON S.; MURILLO R.; CASTRO V.; BOHLIN L.; MERFORT I. The effect of sesquiterpene lactones on the release of human neutrophil elastase. *Biochem. Pharmacol.* 7559, 1-7, 2003.

SIMAS-TOSIN, F. F.; ABUD, A.P.R.; DE OLIVEIRA, C.C.; GORIN, P.A.J.; SASSAKI, G.L.; BUCCHI, D.F; IACOMINI, M. Polysaccharides from peach pulp: Structure and effects on mouse peritoneal macrophages. *Food Chemistry*, 2257–2260, 2012.

SIMÕES, C. M. O.; SHENKEL, E. P.; GOSMANN, G.; MELLO, J. C. P.; MENTZ, L. A.; PETROVICK, P. R. *Farmacognosia: da planta ao medicamento*. 5.ed. Porto Alegre, UFRGS, Florianópolis, UFSC, p. 403, 498, 2003.

SIMON, S. I.; CHERAPANOV, V.; NADRA, I.; WADDELL, T.K.; SEO, S. M.; WANG, Q.; DOERSCHUK, C. M.; DOWNEY, G.P. Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and localization with CD18. *Journal Immunology*. 163, 2891-2901, 1999.

SIMON, S. I.; CHERAPANOV, V.; NADRA, I.; WADDELL, T.K.; SEO, S. M.; WANG, Q.; DOERSCHUK, C. M.; DOWNEY, G.P. Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and localization with CD18. *J. Immunol.* 163, 2891-2901, 1999.

SRIVASTAVA, R.; KULSHVESHATHA, D. K. Bioactive polysaccharides from plants, *Phytochemistry*, v. 28, p. 2877-2883, 1989.

STEWART, T. S.; BALLOU, C. E. A comparison of yeast mannans and phosphomannans by actolysis. *Biochemistry*, 7: 1855-1863, 1968.

STIPP, M. C., BEZERRA, I.D. L., CORSO, C. R., DOS REIS LIVERO, F. A., LOMBA, L. A., CAILLOT, A. R. C., ZAMPRONIO, A. R., QUEIROZ-TELLES, J. E., KLASSEN, G., RAMOS, E. A. S., SASSAKI, G. L., ACCO, A. Necroptosis mediates the antineoplastic effects of the soluble fraction of polysaccharide from red wine in Walker-256 tumor-bearing rats. *Carbohydrate Polymers*, 160, 123–133. (2017).

STONE, C.W.; MILLS, D.V. Yeast products in the feed industry: a practical guide for feed professionals. 2007. Disponível em: <http://www.engormix.com/e_articles_view.asp?art=243&AREA=MAS>. Acesso em: 04 nov. 2017.

VARKI, A. Selectin ligands: will the real ones please stand up? *J. Clin Invest.*, 100, 31-35. 1997.

VIDAL, S. et al. Structural characterization of the pectic polysaccharide rhamnogalacturonan II: Evidence for the backbone location of the aceric acidcontaining oligoglycosyl side chain. *Carbohydrate Research*, v. 326, p. 277-294, 2000.

VIDAL, S.; WILLIAMS, P.; DOCO, T.; MOUTOUNET, M.; PELLERIN, P. The polysaccharides of red wine: total fractionation and characterization. *Carbohydrate Polymers*, 54 439–447, 2003.

VUKOVIC, R.; HUNDINA-DOMLADOVEC, M.; MRSA, V. Molecular organization of the *Saccharomyces cerevisiae* cell wall. *Prehrambeno-Tehnology Review*, 32: (2-3) 99-102. 1994.

WANG H, MA S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. *Am J Emerg Med*; 26:711–5. 2008.

WANG, L.; BROWN, J.R.; VARKI, A.; ESKO J.D. Heparin's anti-inflammatory effects require glucosamine 6-O-sulfation and are mediated by blockade of L- and P-selectins. *J Clin Invest.* 110: 127–136, 2002.

Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, et al. Extracellular histones are major mediators of death in sepsis. *Nat Med*;15:1318–21. 2009.

YADAV, R.; LARBI, K.Y.; YOUNG, R.E. & NOURSHARGH, S. Migration of leukocytes through the vessel wall and beyond. *Thrombosis Haemostasis*. 90: 598-606, 2003.

YAMADA, H. Pectic polysaccharides from Chinese herbs: structure and biological activity. *Carbohydrate Polymers*, v. 25, p. 269-276, 1994.

YANG, J.; MARTINSON, T. E.; LIU, R. H. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chemistry*. v. 116, n.1, p. 332-339. 2009.

ZOECKLEIN, B., FUGELSANG, K. C., GUMP, B. H., NURY, F. S. *Wine analysis and production*. New York: Chapman & Hall. (1995).

CAPITULO II

Este capítulo é composto pelo manuscrito “Structural Characterization of Polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: anti-inflammatory activity in LPS stimulated RAW 264.7 cells”. Publicado na Revista Carbohydrate Polymers em janeiro de 2018.

Structural Characterization of Polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: anti-inflammatory activity in LPS stimulated RAW 264.7 cells

Iglesias de Lacerda Bezerra^a, Adriana Rute Cordeiro Caillot^a, Lais Cristina Gusmão Ferreira Palhares^b, Arquimedes Paixão Santana Filho^a, Suely Ferreira Chavante^b, Guilherme Lanzi Sasaki^{a*}

^a *Department of Biochemistry and Molecular Biology, Federal University of Parana, Curitiba, Paraná, 81.531-980, Brazil*

^b *Department of Biochemistry and Molecular Biology, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, 59.078-970, Brazil*

Abstract

The structural characterization of the polysaccharides and *in vitro* anti-inflammatory properties of Cabernet Franc (WCF), Cabernet Sauvignon (WCS) and Sauvignon Blanc (WSB) wines were studied for the first time in this work. The polysaccharides of wines gave rise to three fractions of polysaccharides, namely (WCF) 0.16%, (WCS) 0.05% and (WSB) 0.02%; the highest one was chosen for isolation of polysaccharides (WCF). It was identified the presence of mannan, formed by a sequence of α -D-Manp (1 $\rightarrow$ 6)-linked and side chains O-2 substituted for α -D-mannan (1 $\rightarrow$ 2)-linked; type II arabinogalactan, formed by (1 $\rightarrow$ 3)-linked β -D-Galp main chain, substituted at O-6 by (1 $\rightarrow$ 6)-linked β -D-Galp side chains, and nonreducing end-units of arabinose 3-O-substituted; type I rhamnogalacturonan formed by repeating (1 $\rightarrow$ 4)- α -D-GalpA-(1 $\rightarrow$ 2)- α -L-Rhap groups; and traces of type II rhamnogalacturonan. The polysaccharide mixture and isolated fractions inhibited the production of inflammatory cytokines (TNF- α and IL-1 β) and mediator (NO) in RAW 264.7 cells stimulated with LPS.

Keywords: wines, polysaccharides, inflammation, NMR.

1. Introduction

Polysaccharides are present in wines and have important influence on several stages of the winemaking process, including fermentation, filtration, stabilization and are partially responsible for the organoleptic properties of wines (Gerbaud, Gabas, Blounin, Pellerin & Moutounet, 1997; Moine-Ledoux & Dubourdieu, 1999; Vernhet, Pellerin, Belleville, Planque & Moutounet, 1999). However, it has been shown that not all polysaccharides have the same behavior regarding wines. Their influence on wine processing and sensory properties depend on their quantity, class and structural features (Del Barrio-Galan, Pérez-Magarino, Ortega-Heras, Williams & Doco, 2011; Guadalupe & Ayestaran, 2007; 2008; Riou, Vernhet, Doco & Moutounet, 2002; Vidal et al., 2004).

The polysaccharides come from grape berries, yeast, bacterial and fungal grape microbiome. From the enological and quantitative perspective, polysaccharides from grapes and yeast are the most important. Their concentrations depend on many parameters, such as, cultivation, stage of maturity, wine-making techniques and the treatments leading to increased solubilization of the macromolecular components of the grape berry cell walls (Pellerin & Cabanis, 1998). Arabinose and galactose rich polysaccharides (AGs), such as type II

arabinogalactan-proteins (AGPs) and arabinans, rhamnogalacturonans type I (RG-I) and type II (RG-II), and homogalacturonans (HLs) come from grape berries, while glucans (GLs), mannans and mannoproteins (MPs) are released by yeast either during fermentation or by enzymatic activity during aging on yeast lees by autolysis (Ayestarán, Guadalupe, León, 2004; Belleville, Brillouet, Tarodo de la Fuente & Moutounet, 1991; Brillouet, Bosso & Moutounet, 1990; Doco & Brillouet, 1993; Doco, Vuchot, Cheynier & Moutounet, 2003; Pellerin, Vidal, Williams & Brillouet, 1995; Vidal, Williams, Doco, Moutounet & Pellerin, 2003).

Nowadays one of the main targets of the wine sector is to improve wine quality, elaborating wines that satisfy consumer demand, and expand the offer of quality wines. Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines, widely produced from grapes of *Vitis vinifera* originating in France, are very popular and quite consumed worldwide. Curiously, there is no information regarding their polysaccharides composition and structural characterization. Despite this, polysaccharides appear to be the most interesting molecules in enology due to their positive effects on the final quality of the wine (Doco et al., 2003; Fournairon, Camarasa, Moutounet, & Salmon, 2002).

Besides concerns related to the wine quality, there is also great interest in knowing the benefits that moderate consumption of wine can bring to health. So far, many other polysaccharides presented reported biological activities, such as antiviral, antitumor, immunostimulatory, anti-inflammatory, anticomplementary, anticoagulant, hypoglycemic, and anti-ulcer (Capek et al., 2003; Cipriani et al., 2008, 2009; Nergard et al., 2005; Srivastava & Kulshreshtha, 1989; Yamada, 1994). Nevertheless, there are very few reports regarding biological activities and modulation of inflammatory mediators by polysaccharides from wines.

The pathology of inflammation is a complicated process triggered by microbial pathogens, such as: viruses, bacteria, prion, and fungi (Vitaliti et al., 2014). Macrophages account for the first defense line of human body. LPS is usually employed as a model for inflammation due to its ability to stimulate macrophages. Different inflammatory mediators are secreted by macrophages induced by LPS: cytokines, such as tumor necrosis factor alpha (TNF- α) and interleukin-1 β (IL-1 β), and inflammatory mediator nitric oxide (NO) (Agarwal, Piesco, Johns & Riccelli, 1995; Lee et al., 2014). The LPS induced RAW 264.7 (mouse macrophages cell lines) is commonly employed as the anti-inflammation model for anti-inflammation candidate screening *in vitro* (Zhang et al., 2015).

Given the importance of polysaccharides in the wine making process and sensory properties of the beverage, understanding their content, identification and quantification is essential. Different analytical methodologies were developed to determine wine polysaccharides in this study. This preliminary work was aimed at characterizing the spectra of the purified polysaccharides. Such spectral identification of each polysaccharide family (AGs, MPs, and RGs) in Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines have not been reported previously as well as their *in vitro* involvement in the inflammatory process.

2. Materials and methods

2.1. Materials

Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines used in this study belonged to four vintages (2011, 2012, 2013 and 2014). All the wines were made from *Vitis vinifera* var. and that have evolved during the ageing time. They were stored under cellar conditions before the analyses were conducted. Oenological parameters such as pH or ethanol content were similar across the studied wines, and they were not included in the statistical data treatment.

2.2. Polysaccharides extraction and purification

The polysaccharides were precipitated by addition of cold EtOH (3 vol.), and separated by centrifugation (8.000 rpm at 4 °C, 20 min). The sediment was dissolved in H₂O, dialyzed against water for 72 h to remove the remaining low-molecular weight compounds, giving rise to a crude polysaccharide fraction: WCF (Cabernet Franc), WCS (Cabernet Sauvignon) and WSB (Sauvignon Blanc). For the purification of the polysaccharides, it was chosen the fraction with the highest yield. This fraction was frozen and then allowed to unfreeze at room temperature (Gorin & Iacomini, 1984), resulting in soluble and insoluble fractions which were separated by centrifugation as previously described. The insoluble fraction was not analyzed in this study due to its lower yield and difficult solubilization. The water-soluble fraction was treated with α -amylase and then with Fehling solution (Jones & Stoodley, 1965). The soluble fraction (FCF) was isolated from the insoluble fraction (PCF) by Cu²⁺ complex (Fehling solution) and centrifugation under the same conditions previously

described. The respective fractions were both neutralized with HOAc, dialyzed against water and deionized with mixed ion exchange resins and then freeze dried.

2.3. Monosaccharide analysis

WCF, WCS, WSB, FCF and PCF fractions (2 mg) were hydrolyzed with 1 M TFA at 100 °C for 14 h, the solution was then evaporated, and the residue dissolved in water (2 mL). The resulting monosaccharide mixture was examined by thin layer chromatography (TLC) silica-gel 60 (Merck), with ethyl acetate:acetic acid:n-propanol:water (4:2:2:1, v/v), then stained with orcinol-sulfuric acid (Sasaki, Souza, Cipriani, & Iacomini, 2008; Skipski, 1975). The monosaccharides were then reduced with 2 mg NaBH₄, yielding alditols, which were acetylated in Ac₂O-pyridine (1:1, v/v, 0.5 mL) at room temperature for 12 h (Wolfrom & Thompson, 1963a; Wolfrom & Thompson, 1963b). The resulting alditol acetates were extracted with CHCl₃ and analyzed by gas chromatography–mass spectrometry (GC–MS – Varian, Saturn 2000R, Ion-Trap detector), using a DB-225-MS column (30 m × 0.25 mm × 0.25 µm), programmed from 50 to 220 °C at 40 °C/min, with helium as carrier gas. Components were identified by their typical retention times and electron ionization (EI 70 eV) spectra. The uronic acids content of the fractions were determined using the colorimetric m-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991).

Carboxy-reduction of FCF (10 mg) was carried out by the carbodiimide method (Taylor & Conrad, 1972), using NaBH₄ as the reducing agent, having its uronic acid carboxyl groups reduced to primary alcohols and the results were given as mol % (Pettolino, Walsh, Fincher & Bacic, 2013).

2.4. Methylation analysis

Per-*O*-methylation of each isolated polysaccharide (10 mg) was carried out using NaOH-Me₂SO-MeI as described by Ciucanu and Kerek (1984). The process, after isolation of the products by neutralization (HOAc), dialysis and evaporation, was repeated, and the methylation was complete. The per-*O*-methylated derivatives were hydrolyzed with 72% (v/v) aq. H₂SO₄ (0.5 mL, v/v, 1 h, 0 °C), followed by dilution to 8% (v/v). The solution was kept at 100 °C for 17 h, then neutralized with BaCO₃, filtered and evaporated to dryness (Saeman, Moore, Mitchell, & Millet, 1954). The hydrolyzate was reduced with NaBD₄ (sodium borodeuteride) and then acetylated, giving rise to partially *O*-methylated alditol acetates and analyzed by GC–MS, as the previously described, but with a final temperature of 215 °C. The

compounds were identified by their typical retention times and electron impact spectra (Sasaki, Gorin, Souza, Czelusniak, & Iacomini, 2005).

2.5. Nuclear magnetic resonance (NMR) spectroscopy

1D and 2D NMR spectra were obtained with a 400 or 600 MHz Bruker spectrometer using 5 mm direct or inverse probeheads (Avance III and HD, Bruker, Billerica, Massachusetts, USA). 1D ^1H and ^{13}C -NMR at 600 MHz were collected after a 90° (p1) pulse calibration at 10.75 μs . The ^1H and ^{13}C chemical shifts were determined after ^1H assignments through 2D COSY and TOCSY analysis; bonding connections were performed by HMBC and $^1\text{H}/^{13}\text{C}$ chemical shift correlation mapping was finally determined by HSQCed (Heteronuclear Single Quantum Coherence edited spectroscopy CH, CH_3 positive phase CH_2 negative phase) performed at 30 $^\circ\text{C}$ in deuterium oxide (D_2O). 2D NMR experiments were recorded using quadrature detection at indirect dimension and acquired using 24 scans per series of 1024x320 data points, with zero filling in F1 (4096) prior to Fourier transformation, using the Software TOPSPIN version 3.2 pl6 (Bruker Biospin, Rheinstetten, Germany). The chemical shifts of the polysaccharide were expressed in δ (ppm) relative to trimethylsilyl propionic acid (TMSP). The ratios of the polysaccharides were calculated by integrating the area of specific anomeric signals corresponding to each cross-peak assignment on the 2D-HSQCed spectra, the volumes were normalized and calibrated to 1.00, using the most intense volume. The structural model was designed using the software ChemSketch v. 12.0 (ACD/Labs, Toronto, Ontario, Canada).

2.6. Determination of homogeneity and molar mass

The homogeneity and molar mass of the purified polysaccharide was determined by high performance steric exclusion chromatography (HPSEC), using a refractive index (RI) detector. Four columns were used in series, with exclusion sizes of 7×10^6 Da (Ultrasphere 2000, Waters), 4×10^5 Da (Ultrasphere 500, Waters), 8×10^4 Da (Ultrasphere 250, Waters) and 5×10^3 Da (Ultrasphere 120, Waters). The eluent was 0.1 M NaNO_3 , containing 0.5 g/L NaN_3 . The solutions were filtered through a membrane of 0.22 μm pore size (Millipore) and loaded (100 μL , loop) at a concentration of 1 mg/mL. The molar mass of the polymer was estimated using Astra software 4.70.

2.7. Cell Culture

RAW 264.7 macrophages were maintained at 37 °C in humidified atmosphere of 5% CO₂ in DMEM medium supplemented with 10% fetal bovine serum and penicillin/streptomycin/normocin (50 U/mL, 50 g/mL and 100 g/mL, respectively; InvivoGen, San Diego, CA, USA). Exponential phase cells were used throughout the experiments.

2.8. Analysis of cell viability

RAW 264.7 cells in exponential growth phase were seeded in 24-well plates at a density of 4.8×10^5 cells/well. The WCF, WCS, WSB, FCF and PCF were added at indicated concentrations (0.1, 1, 10 and 100 µg/mL). The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan was used to measure cell respiration as an indicator of cell viability (Mosmann, 1983). Briefly, after 24 h incubation with or without polysaccharides, 350 µL/well of MTT solution (1 mg/mL) was added and the cells were incubated for another 4 h at 37 °C. After removing the supernatant, 500 µL of DMSO (dimethyl sulfoxide) was added to the cells to dissolve the formazan. The absorbance of each group was measured by a microplate reader at wavelength of 570 nm. The control group consisted of untreated cells was considered as 100% of viable cells. Results were expressed as percentage of viable cells when compared with control group.

2.9. Measurement of cytokines production and NO production

The analysis was based on exposing cell line to fractions WCF, WCS, WSB, FCF and PCF (0.1, 1, 10 and 100 µg/mL) in the presence of lipopolysaccharide (LPS) in the wells. RAW 264.7 cells (4.8×10^5 cells/well) were plated into 96-well plates, then were stimulated with LPS (2 µg/mL) and after 1h, were treated with different concentrations of the mentioned fractions. After 24 h, the culture media were collected. The amount of cytokines in the supernatants for TNF-α and IL-1β, respectively, were determined by enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. Three replicates were carried out for each different treatment. The absorbance of each group was measured by a microplate reader at wavelength of 450 nm. To determine the total concentration of NO in the culture media, Griess reagent was added to 40 µL of supernatant and the absorbance at 545 nm was evaluated with an ELISA kit (BD Biosciences, San Jose, CA, USA) following the manufacture manual.

2.10. Statistical analysis

The results were expressed as mean $\pm$ SEM and were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's test as a post hoc test ($P < 0.001$). GraphPad Prism 5.0 (La Jolla, CA) program was used for both statistical analysis and figure design. Histological analysis was performed by qualitative observations.

3. Results and Discussion

3.1. Isolation and structural analysis of the polysaccharides

Approximately 750 mL of each wine were concentrated and the polysaccharides were recovered by ethanol precipitation followed by centrifugation and dialysis against water (Fig. 1). The solution was then freeze-dried, generating three fractions of polysaccharides namely (WCF) 0.16%, (WCS) 0.05% and 0.02% (WSB).

Cabernet Franc (WCF), Cabernet Sauvignon (WCS) e Sauvignon Blanc (WSB)

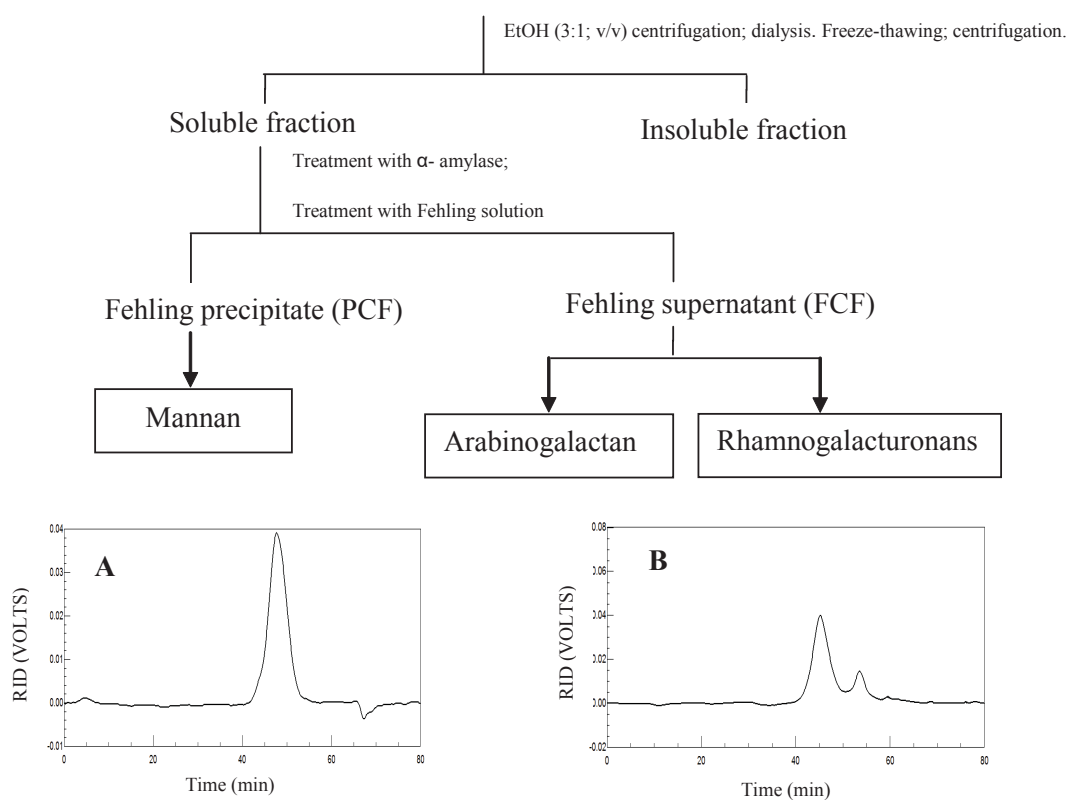
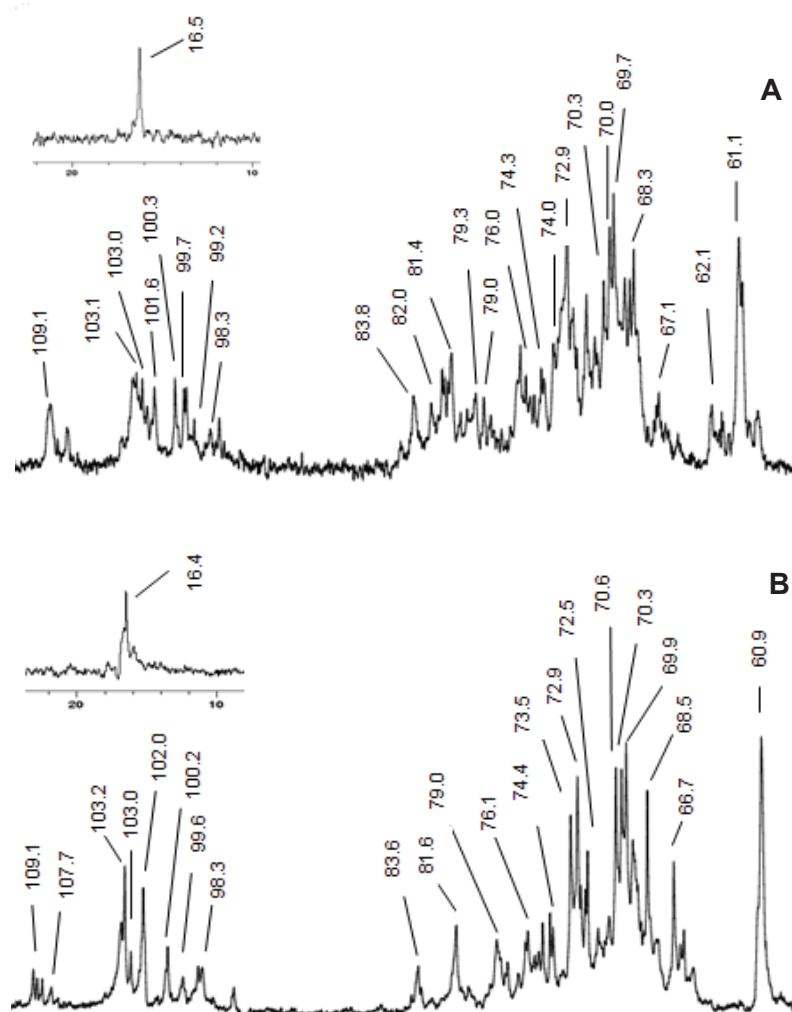


Fig. 1: Scheme of extraction and purification of polysaccharides; Elution profile of (A) PCF and (B) FCF fractions determined by HPSEC using light scattering and refractive index detectors.

The ^{13}C -NMR analysis of the WCF, WCS and WSB fractions showed characteristic signals of polysaccharides (Fig. 2). The anomeric region showed a complex profile, showing many peak overlaps signals due to mixture of polysaccharides.

Since all the polysaccharide fractions showed the same monosaccharide composition, but with different yields, the highest one was chosen for isolation of polysaccharides (WCF). The fractionation and purification of polysaccharides were carried out by a freeze-thawing procedure (Gorin & Iacomini, 1984), resulting in a cold water-soluble fraction. Therefore, this fraction was submitted to complexation with copper, resulting in insoluble (PCF) and soluble (FCF) fractions.



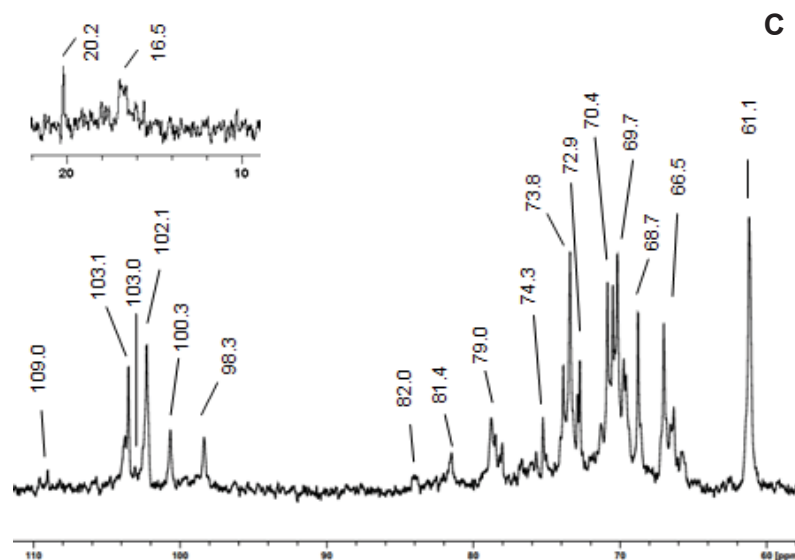


Fig. 2: ^{13}C -NMR spectra of (A) WCF, (B) WCS and (C) WSB in D_2O at 30°C (chemical shifts are expressed in δ ppm).

HPSEC analysis of PCF presented a homogeneous peak (Fig. 1a), with Mw 42,800 g/mol ($\text{dn/dc} = 0.156$). Monosaccharide composition exhibited exclusively mannose, as shown in the table S1 (supporting information), and absence of residual protein, indicating that the polysaccharide found in this sample is a mannan. In order to characterize the glycosidic linkages of the isolated polysaccharide, a methylation analysis was performed. The analysis showed a branched polysaccharide due the presence of non-reducing end units, as 2,3,4,6-Me₄-Manp and the derivatives 3,4-Me₂-Manp, 2,3,4-Me₂-Manp and 3,4,6-Me₃-Manp (table S2). Some mannose units were 6-*O* and 2,6-di-*O*- substituted (Komura et al., 2010). According to the methylation data, the branching points may be situated mainly at *O*-2 position, suggesting (1→2)-linked-Manp units, shown by the presence of the derivative 3,4,6-Me₃-Manp.

The presence of mannan was commonly found in wines deriving from yeast fermentation (Martínez, Guadalupe, Ayestarán & Perez, 2013), mainly presenting (1→2)-linked- α -D-Manp and main chain of *O*-6-substituted α -D-Manp units (Kobayashi et al., 1995; Vinogradov, Petersen & Bock, 1998).

The 2D NMR analysis of the mannan (Fig. 3) corroborated with monosaccharide composition (table S1 - supporting information) and with methylation data (table S2 – supporting information). The $^1\text{H}/^{13}\text{C}$ HSQC spectrum of PCF contain signals at δ 99.2/5.09

and 99.0/5.06 (C-1/H-1), δ 79.5/4.00 and 78.9/3.93 (C-2/H-2) typical of $\rightarrow 2,6$ - α -D-Manp-(1 $\rightarrow$). Also, the presence of signals at δ 101.4/5.27, 103.0/5.02 and 103.1/5.12 (C-1/H-1) and δ 79.3/4.09 (C-2/H-2) typical of $\rightarrow 2$ - α -D-Manp-(1 $\rightarrow$) (Kobayashi et al., 1995; Vinogradov, Petersen & Bock, 1998). In agreement, NMR results and methylation analysis suggest that PCF is a mannan formed by large sequences α -D-Manp (1 $\rightarrow$ 6) linked and side chains O-2 substituted for α -D-mannan (1 $\rightarrow$ 2) linked (Fig. S1 – supporting information).

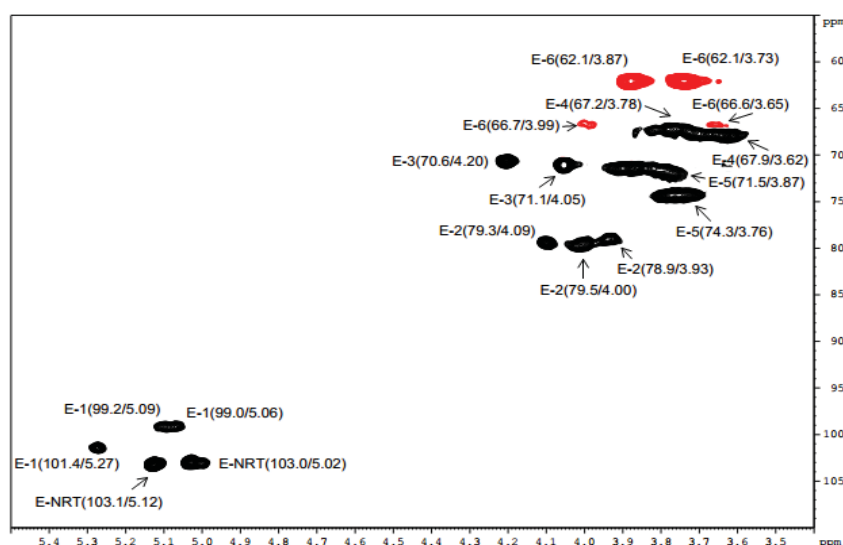


Fig. 3: $^1\text{H}/^{13}\text{C}$ HSQC NMR spectrum of PCF. Solvent D_2O at 30 $^\circ\text{C}$; numerical values are in δ ppm. E (Manp); NRT (non-reducing end units); The letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed, COSY, TOCSY and HMBC experiments.

FCF fraction showed a heterogeneous profile (Fig. 1b). It was treated with α -amylase for purification. After this procedure, the monosaccharide composition showed the presence of galactose (62.5%), arabinose (20.8%), rhamnose (8.2%) and galacturonic acid (7.5%) (table 1). Methylation analysis was performed with the carboxy-reduced FCF (Taylor & Conrad, 1972) and the uronic units of FCF were converted into the reduced form.

The methylation analysis of FCF presented a complex and branched polysaccharide by the presence of non-reducing end units of Galp, Araf and the derivatives 2,4-Me₂-Galp, 2,4,6-Me₃-Galp and 2,3,4-Me₃-Galp (table S3 – supporting information). The main chain is probably composed of β -D-Galp (1 $\rightarrow$ 3)-linked, due the presence of high amounts of 2,4,6-

Me₃Galp. According to the methylation data, the branching points may be situated mainly at the *O*-6 position of galactose residues. The side chains may be replaced at the *O*-3 position of non-reducing end units of α -L-Araf.

The polysaccharides are derived from grape berries, yeast, bacterial and fungal microbiome, thus the arabinogalactan is derived from the berries of grapes (Doco, Quéllec, Moutounet & Pellerin, 1999; Doco, Williams & Cheynier, 2007; Martínez, Guadalupe, Ayestarán & Perez, 2013). These heteropolysaccharides of FCF presented mainly β -D-Galp (1 $\rightarrow$ 3)-linked as main chain *O*-6-substituted by β -D-Galp (1 $\rightarrow$ 6)-linked units. The side chains may be replaced at the *O*-3 position of non-reducing end units of α -L-Araf (Carpita & Gibeaut, 1993).

In order to resolve the peak overlaps, edited HSQC experiment was performed. FCF showed cross peaks at δ 102.8/4.51 C-1/H-1 of β -D-Galp units (Fig. 4). The signals of C-3/H-3 at δ 81.9/3.93 and C-6/H-6 at δ 69.0/3.80 are from $\rightarrow$ 3,6)- β -D-Galp-(1 $\rightarrow$ units, whereas the resonances at δ 72.6/3.76 (C-3/H-3) and 61.1/3.76 (C-6/H-6) are from β -D-Galp units 3-*O*- and 6-*O*-substituted, respectively (Cipriani et al., 2006, 2009a; Delgobo, Gorin, Tischer & Iacomini, 1999; Fransen et al., 2000; Gorin & Mazurek, 1975; Renard, Lahaye, Mutter, Voragen & Thibault, 1998; Tischer, Gorin & Iacomini, 2002). Typical signals of nonreducing end of α -L-Araf of C-1/H-1 and C-5/H-5 were observed at δ 109.0/5.25 and δ 74.0/4.14, respectively (Delgobo, Gorin, Jones & Iacomini, 1998; Renard et al., 1998).

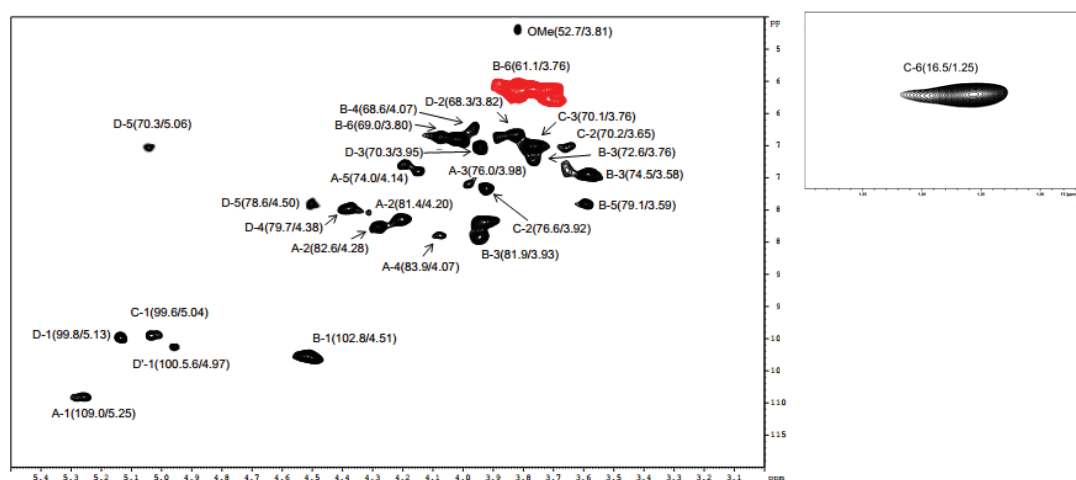


Fig. 4: $^1\text{H}/^{13}\text{C}$ HSQC NMR spectrum of FCF. Solvent D_2O at 30 °C; numerical values are in δ ppm. A (α -L-Araf); B (β -D-Galp); C (α -L-Rhap); D (α -D-GalpA); D' (6-OMe- α -D-GalpA). The letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed, COSY, TOCSY and HMBC experiments.

The arabinogalactans are classified according to their main chains. Therefore, the NMR result is in agreement with methylation analysis (table S3 – supporting information) and with monosaccharide composition shown in the table S1 (supporting information) that indicated the presence of galactose and arabinose in this fraction, suggesting that the polysaccharide FCF is a type II arabinogalactan, formed by a (1→3)-linked β -D-Galp main chain, substituted at HO-6 by (1→6)-linked β -D-Galp side chains and substituted by nonreducing end-units of arabinose 3-*O*-substituted α -L-Araf chains (Fig. S2).

Moreover, carboxy-reduced derivatives from FCF showed non-reducing end units of Rhap and the presence of GalpA (1→4)-linkages, due the derivative 2,3,6-Me₃-Galp (table S3 – supporting information) (Sasaki, Gorin, Souza, Czelusniak & Iacomini, 2005). The presence of 3,4-Me₂-Rhap, indicates *O*-2-substitution, which is often found in type I rhamnogalacturonan. According to the methylation data, this polysaccharide is formed by a chain of repeat units (1→4)-linked α -D-GalpA and (1→2)-linked α -L-Rhap (Fig. S3) (Carpita & Gibeaut, 1993).

The presence of pectins was confirmed by detection of the sequence of (1 → 4)-linked α - galacturonic acid residues, which gives a fingerprint cross peaks at δ 99.8/5.13 and 100.5/4.97 attributed to C-1/H-1(α -D-GalpA and 6-OMe- α -D-GalpA, respectively) and δ 79.7/4.38 (C-4/H-4), indicating this type of linkage (Fig. 4). The latter was confirmed by complete signaling of GalpA units at δ 68.3/3.82 (C-2/H-2), 70.3/3.95 (C-3/H-3), 70.3/5.06 (C-5/H-5 substituted) and 78.6/4.50 (C-5/H-5). Also, the methyl esterification of galacturonic acid is commonly found in type II ramnogalacturonans, being confirmed by the presence of a typical CO_2CH_3 cross peak at δ 52.7/3.81 (Renard et al., 1998; Ovodova et al., 2009; Popov et al., 2011). C-1/H-1 and C-6/H-6 of Rhap units (1→2)-linked were shown by resonances at δ 99.6/5.04 and 16.5/1.25, respectively (Renard et al., 1998). The combination of NMR data and methylation analysis suggests that the FCF also presents a type I rhamnogalacturonan, formed by large sequences of →4)-6-OMe- α -D-GalpA-(1→ units, interspersed with a few α -

L-Rhap units, that was also confirmed by the presence of rhamnose and galacturonic acid in the monosaccharide composition (table S1 – supporting information).

Further, the monosaccharide composition analysis of the FCF fraction by GC-MS revealed the presence of units of 2-*O*-methyl-xylose and 2-*O*-methyl-fucose (table S1 – supporting information), which are considered markers of type II rhamnogalacturonan. They also suggest a type II rhamnogalacturonan, although these signals were not observed in NMR spectrum, because of their small amounts in this polysaccharide, corresponding to less than 1% of the total. The 2-*O*-methyl substitution was characterized by electron ionization mass spectrometry, due the identification of fragments at m/z 117, 127, 243 and 289 from 2-*O*-methyl-xylitol and at m/z 117, 225, 229 and 275 from 2-*O*-methyl-fucitol (fig. S4 – supporting information).

3.2. Biological experiments

3.2.1. Analysis of cell viability

Macrophages are critical for the natural immune defense system of hosts, which has various immune regulatory functions (Del Carmen Juárez-Vázquez, Alonso-Castro & García-Carrancá, 2013; Schepetkin & Quinn, 2006). Therefore, it was important to evaluate the effects of WCF, WCS, WSB, FCF and PCF fractions on the viability of RAW 264.7 macrophages at the indicated concentrations of 0.1, 1, 10 and 100 $\mu\text{g/mL}$ for 24 h (Fig. 5). Results showed that RAW 264.7 macrophage cells viability was not significantly influenced by fractions at the indicated concentrations of 0.1, 1, 10 and 100 $\mu\text{g/mL}$ ($p > 0.001$). Results indicated that all the fractions were safe up to 100 $\mu\text{g/mL}$ to conduct the assay of anti-inflammatory activity.

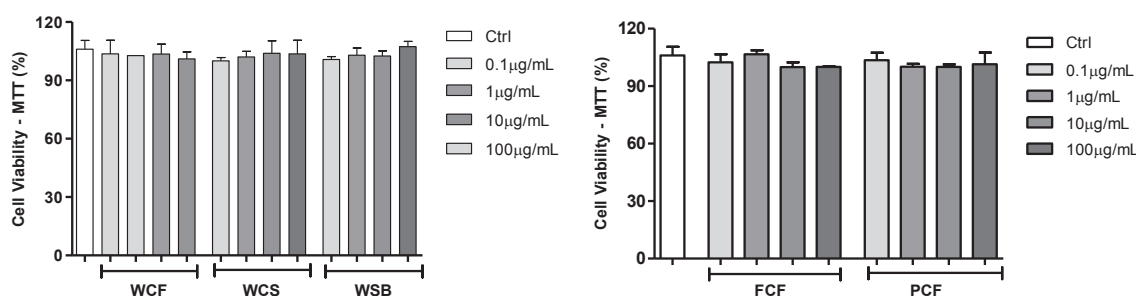


Fig. 5: Effect of WCF, WCS and WSB mixed fractions and the isolated fractions FCF and PCF on the viability of RAW 264.7 cells. The viability was measured by MTT assay. The values for each concentration tested (0.1, 1, 10 e 100 $\mu\text{g/mL}$) represent the average (mean $\pm$ S.D.); Ctrl (control).

3.2.2. Anti-inflammatory activity

Inflammation is a host response to foreign pathogens or tissue injury to eliminate harmful stimuli as well as to initiate the healing and repair process of the damaged tissue (Mariathasan & Monack, 2007). LPS is a major constituent of the cell wall of gram-negative bacteria, which can bind to the TLR 4 receptor of macrophages and induce inflammation (Rossol et al., 2011). In response to LPS, macrophages synthesize and release inflammatory mediators such as NO and produce pro-inflammatory cytokines, such as $\text{TNF-}\alpha$ and $\text{IL-1}\beta$, as already mentioned (Pan, Lin-Shiau & Lin, 2000). To test the inhibitory effects of WCF, WCS, WSB, FCF and PCF fractions on the production of the inflammatory cytokines ($\text{TNF-}\alpha$ and $\text{IL-1}\beta$) and mediator (NO) from LPS-stimulated RAW 264.7 cells, RAW 264.7 cells were treated with various concentrations of fractions (0.1, 1, 10 and 100 $\mu\text{g/mL}$) and then were incubated with or without 2 $\mu\text{g/mL}$ of LPS for another 24 h.

LPS is one of the leading activators of macrophages. The macrophages play important roles in inflammation through the production of several pro-inflammatory molecules, including NO. Production of excessive NO has been associated with a range of inflammatory diseases, including arteriosclerosis, ischemic reperfusion, hypertension and septic shock (Pacher, Beckman & Liaudet, 2007; Terao, 2009). Therefore, inhibition of NO production in LPS stimulated RAW 264.7 cells is one of the possible ways to screen for anti-inflammatory drugs or disease prevention thorough moderate wine intake. In this study WCF, WCS, WSB, FCF and PCF fractions showed the inhibition of NO production in cells, indicating the anti-inflammatory properties. As shown in figure 6 (K-O), while treatment both with the mixed fractions and fractions with isolated polysaccharides induced a significant inhibitory effect and it was observed that this effect was enhanced increasing the concentration of the fractions with a maximal inhibitory effect at the dose of 100 $\mu\text{g/mL}$.

Pro-inflammatory mediator NO plays a key role in the pathogenesis of many inflammatory diseases. The NO pathway is known to induce ROS production (Jung et al., 2010; Liu, Cheng, Chen & Yang, 2005). ROS is critical for LPS-induced inflammation

through the activation of NF- κ B related signaling (Asehnoune, Strassheim, Mitra, Kim & Abraham, 2004). Activated NF- κ B acts as a transcription factor, leading to increased production of pro-inflammatory cytokines such as TNF- α and IL-1 β (Janeway & Medzhitov, 2002). Thus, blocking the effects of pro-inflammatory mediators could be an effective therapeutic strategy. In figure 6 (A-J) it is shown that all fractions tested significantly decreased the TNF- α and IL-1 β production at all doses, indicating that the polysaccharides found in wine have anti-inflammatory properties. Thus, blocking the effects of pro-inflammatory mediators when drinking wine offers an attractive therapeutic strategy or preventive effect.

Statistical analysis was performed comparing the fractions isolated with the mixture fraction to evaluate which fraction would be exerting the anti-inflammatory effect, but no difference was observed between them in any of the doses tested, indicating that all of them had the same action. Our study showed that the mixture and the isolated polysaccharides (type II arabinogalactan, type I and II rhamnogalacturonans and mannan) significantly reduced production of these pro-inflammatory cytokines and mediator in the LPS-induced RAW 264.7 mouse macrophage cells *in vitro* and can contribute to the beneficial properties of wine, as well as resveratrol has been shown to exert its protective effect against cardiovascular disease, ischemia-reperfusion injury and diabetes mellitus through modulation of adipocyte/fibroblast biology, oxidative stress and inflammation (Farghali, Kutinova & Lekic, 2013; Gertz et al., 2012; Lakshminarasimhan et al., 2013). However, recently Chiu-Tsun Tang et al. (2014) reviewed the studies carried out with resveratrol and its biological effects and concluded that after more than 20 years of research there is still no evidence of biological activity of this compound in humans. Therefore, these benefits may be associated with other components of wines, such as polysaccharides.

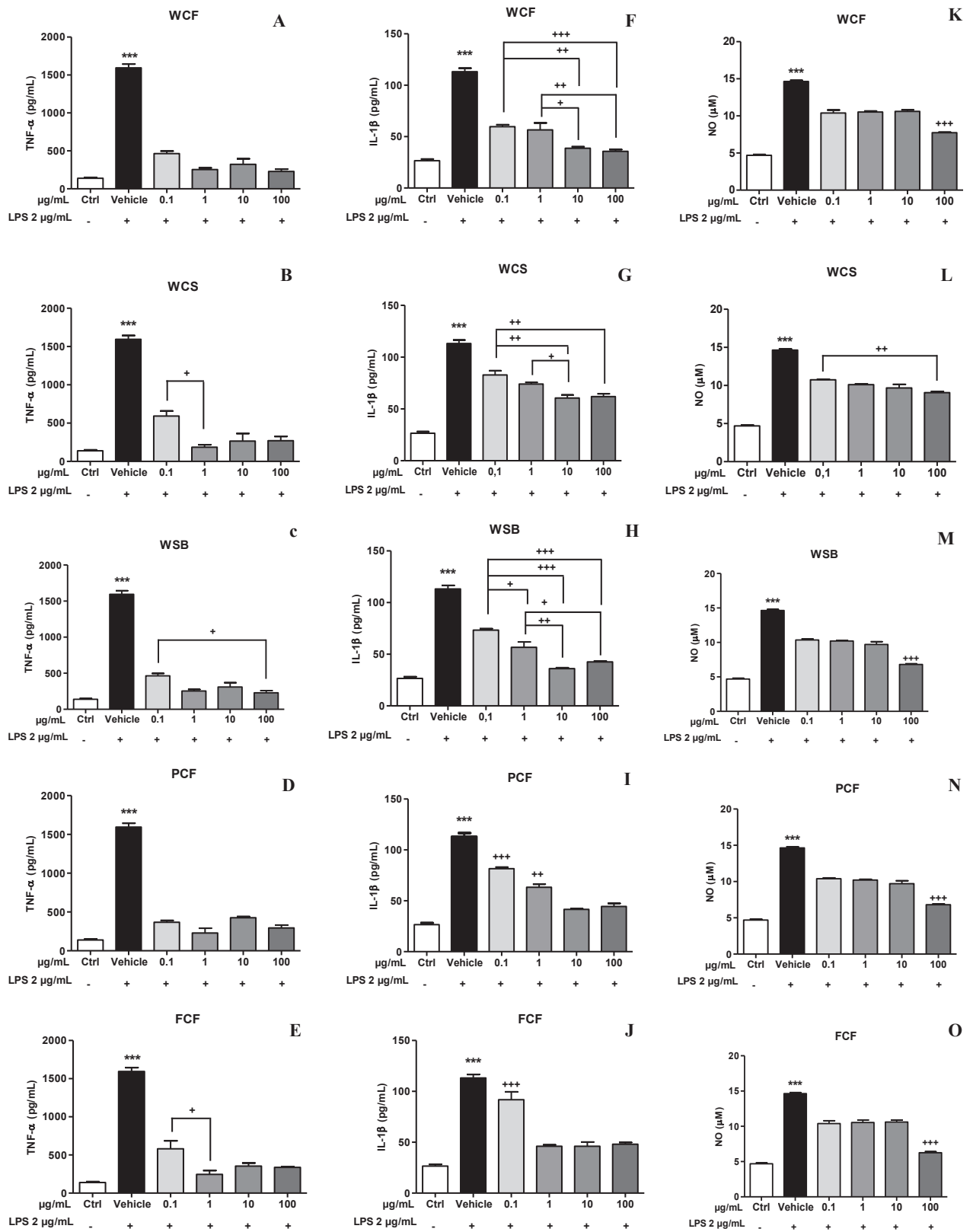


Fig. 6: Anti-inflammatory activities of WCF, WCS, WSB, PCF and FCF on RAW 264.7 cells. (A-E) Expression levels of TNF- α . (F-J) Expression levels of IL-1 β . (K-O) NO production levels. Ctrl (control).

4. Conclusions

In conclusion, on the basis of chemical data, the polysaccharides WCF, WCS and WCSB consist of: mannan, formed by large sequences α -D-Manp (1 $\rightarrow$ 6) linked and side chains O-2 substituted for α -D-mannan (1 $\rightarrow$ 2) linked; type II arabinogalactan, formed by a (1 $\rightarrow$ 3)-linked β -D-Galp main chain, substituted at HO-6 by (1 $\rightarrow$ 6)-linked β -D-Galp side chains, respectively and nonreducing end-units of arabinose 3-O-substituted α -L-Araf chains; type I rhamnogalacturonan, formed by a chain of repeat units (1 $\rightarrow$ 4)-linked α -D-GalpA and (1 $\rightarrow$ 2)-linked α -L-Rhap; and traces of type II rhamnogalacturonan.

The present study suggests that the mixed fractions (WCF, WCS and WSB) and fractions with isolated polysaccharides FCF (type II arabinogalactan/type I and II rhamnogalacturonans) and PCF (mannan) exerted anti-inflammatory effect on the LPS-induced RAW 264.7 mouse macrophage cells *in vitro*. These activities were mediated by decreasing inflammatory cytokines (TNF- α and IL-1 β) and mediator inflammatory (NO).

Acknowledgments

The authors would like to thank the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Grant number 449176/2014-2), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financiadora de Estudos e Projetos (FINEP) Fundação Araucária for financial support and UFPR NMR Center.

References

- Agarwal, S., Piesco, N. P., Johns, L. P., Riccelli, A. E. (1995). Differential expression of IL-1 β , TNF- α , IL-6, and IL-8 in human monocytes in response to lipopolysaccharides from different microbes. *Journal of Dental Research*, 74, 1057–65.
- Asehnoune, K., Strassheim, D., Mitra, S., Kim, J.Y., Abraham, E. (2004). Involvement of reactive oxygen species in toll-like receptor 4-dependent activation of NF- κ B. *The Journal of Immunology*, 172, 22–29.

Ayestarán, B., Guadalupe, Z., León, D. (2004). Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta*, 513, 29-39.

Belleville, M. P., Brillouet, J. M., Tarodo de la Fuente, B., Moutounet, M. (1991). Polysaccharide effects on cross-flow microfiltration of two red wines with a microporous alumina membrane. *Journal Food Science*, 55, 1598-1602.

Capek, P., Hribalová, V., Svandová, E., Ebringerová, A., Sasinková, V., Masarová, J. (2003). Characterization of immunomodulatory polysaccharides from *Salvia officinalis*. *International Journal Biology Macromolecules*, 33, 113-119.

Carpita, N. C., Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, 3, 1-30.

Chiu-Tsun, T. P., Ng Y-F., Ho S., Gyda M., Chan S-W. (2014). Resveratrol and cardiovascular health – promising therapeutic or hopeless illusion? *Pharmacological Research*, 90, 88–115.

Cipriani, T.R., Mellinger, C.G., Souza, L.M., Baggio, C.H., Freitas, C.S., Marques, M.C.A., Gorin, P.A.J., Sassaki, G.L., Iacomini, M., (2008). Acidic heteroxylans from medicinal plants and their anti-ulcer activity. *Carbohydrate Polymers*, 74, 274-278.

Cipriani, T. R., Mellinger, C. G., Bertolini, L. C., Baggio, C. H., Freitas, C. S., Marques, M. C. A., Gorin, P. A. J., Sassaki, G. L., Iacomini, M. (2009a). Gastroprotective effect of a type I arabinogalactan from soybean meal. *Food Chemistry*, 115, 687-690.

Ciucanu, I., Kerek, F., 1984. A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, 131, 209-217.

Brillouet, J. M., Bosso, C., Moutounet, M. (1990). Isolation, purification, and characterization of an arabinogalactan from a red wine. *American Journal of Enology and Viticulture*, 41, 29-36.

Del Barrio-Galan, R., Pérez-Magarino, S., Ortega-Heras, M., Williams, P., Doco, T. (2011). Effect of aging on lees and of three different dry yeast derivative products on Verdejo white wine composition and sensorial characteristics. *Journal of Agricultural and Food Chemistry*, 59, 12433–12442.

Del Carmen Juárez-Vázquez, M., Alonso-Castro, A. J., García-Carrancá, A. (2013). Kaempferitrin induces immunostimulatory effects in vitro. *Journal of Ethnopharmacology*, 148, 337-340.

Delgobo, C. L., Gorin, P. A. J., Jones, C., Iacomini, M. (1998). Gum heteropolysaccharide and free reducing mono- and oligosaccharides of *Anadenanthera colubrina*. *Phytochemistry*, 47, 1207-1214.

Delgobo, C. L., Gorin, P. A. J., Tischer, C. A., Iacomini, M. (1999). The free reducing oligosaccharides of angico branco (*Anadenanthera colubrina*) gum exudates: an aid for structural assignments in the heteropolysaccharide. *Carbohydrate Research*, 320, 167-175.

Doco, T., Brillouet, J. M. (1993). Isolation and characterization of a rhamnogalacturonan II from red wine. *Carbohydrate Research*, 243, 333-343.

Doco, T., Quellec, N., Moutounet, M., Pellerin, P. (1999). Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of Enology and Viticulture*, 50, 28-32.

Doco, T., Vuchot, P., Cheynier, V., Moutounet, M. (2003). Structural modification of arabinogalactan-proteins during aging of red wines on lees. *American Journal of Enology and Viticulture*, 54, 150-157.

Farghali, H., Kutinova C. N., Lekic N. (2013). Resveratrol and related compounds as antioxidants with an allosteric mechanism of action in epigenetic drug targets. *Physiological Research*, 62, 1-13.

Filisetti-Cozzi, T. M. C. C., Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry*, 197, 157-162.

Fournairon, C., Camarasa, C., Moutounet, M., & Salmon, J. M. (2002). New trends on yeast autolysis and wine ageing on lees. *Journal International de la Vigne et du Vin*, 36, 49-69.

Fransen, C. T. M., Haseley, S. R., Huisman, M. M. H., Schols, H. A., Voragen, A. G. J., Kamerling, J. P., Vliegthart, J. F. G. (2000). Studies on the structure of a lithium-treated soybean pectin: characteristics of the fragments and determination of the carbohydrate substituents of galacturonic acid. *Carbohydrate Research*, 328, 539-547.

Gerbaud, V., Gabas, N., Blounin, J., Pellerin, P., Moutounet, M. (1997). Influence of wine polysaccharides and polyphenols on the crystallization of potassium hydrogen tartrate. *Journal International Science Vigne et du Vin*, 31, 65-83.

Gertz M, Nguyen GT, Fischer F, Suenkel B, Schlicker C, Franzel B, Jana Tomaschewski, J., Aladini, F., Becker, C., Wolters, D., Steegborn, C. (2012). A molecular mechanism for direct sirtuin activation by resveratrol. *PLoS ONE*, 7(11): e49761.

Gorin, P. A. J., Mazurek, M. (1975). Further studies on the assignment of signals in ^{13}C magnetic resonance spectra of aldoses and derived methyl glycosides. *Canadian Journal of Chemistry*, 53, 1212-1222.

Gorin, P. A. J., Iacomini, M. (1984). Polysaccharides of the lichens *Cetraria islandica* and *Ramalina usnea*. *Carbohydrate Research*, 142, 119-132.

Guadalupe, Z., Ayestarán, B. (2008). Effect of commercial mannoprotein addition on polysaccharide, polyphenolic, and color composition in red wines. *Journal of Agricultural and Food Chemistry*, 56, 9022–9029.

Janeway, C. A. Jr., Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, 20, 197-216.

Jones, J. K. N., Stoodley, R. J. (1965). Fractionation using copper complexes. *Methods in Carbohydrate Chemistry*, 5, 36–38.

Jung, C.H., Kim, J.H., Park, S.J., Kweon, D.H., Kim, S.H., Ko, S.G. (2010). Inhibitory effect of *Agrimonia pilosa Ledeb.* on inflammation by suppression of iNOS and ROS production. *Immunological Investigations*, 39, 159-70.

Kobayashi, H., Watanabe, M., Komido, M., Matsuda, K., Ikeda-Hasebe, K., Suzuki, M., Shibata, N., Hisamichi, K., Suzuki, S. (1995). Assignment of ^1H and ^{13}C NMR chemical shifts of a D-mannan composed of α -(1→2) and α -(1→6) linkages obtained from *Candida kefyr* IFO 0586 strain. *Carbohydrate Research*, 267, 299-306.

Komura, D. L., Ruthes, A. C., Carbonero, E. R., Alquini, G., Rosa, M. C. C., Sasaki, G. L., Iacomini, M. (2010). The origin of mannans found in submerged culture of basidiomycetes. *Carbohydrate Polymers*, 79, 1052–1056.

Lakshminarasimhan M., Curth U., Moniot S., Mosalaganti S., Raunser S., Steegborn C. (2013). Molecular architecture of the human protein deacetylase Sirt1 and its regulation by AROS and resveratrol. *Bioscience Reports*, 33, e00037.

Lee, K. P., Sudjarwo, G.W., Kim, J.S., Dirgantara, S., Maeng, W.J., Hong, H. (2014). The anti-inflammatory effect of *Indonesian Areca* catechu leaf extract *in vitro* and *in vivo*. *Nutrition Research Practice*. 8, 267–271.

Liu, M.Y., Cheng, Y.J., Chen, C.K., Yang, B.C. (2005) Coexposure of lead- and lipopolysaccharide-induced liver injury in rats: involvement of nitric oxide-initiated oxidative stress and TNF- α . *Shock*, 23, 360–64.

Mariathasan S., Monack, D. M. (2007). Inflamma some adaptors and sensors: intracellular regulators of infection and inflammation. *Nature Reviews Immunology*, 7, 31–40.

Martínez-Lapuente, L., Guadalupe, Z., Ayestaran, B., Ortega-Heras, M., Perez-Magarino, S. (2013). Changes in polysaccharide composition during sparkling wine marking and aging. *Journal Agricultural and Food Chemistry*, 61, 12362-12373.

Moine-Ledoux, V., Dubourdieu, D. (1999). An invertase fragment responsible for improving the protein stability of dry white wines. *Journal of the Science of Food and Agriculture*, 79, 537–543.

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

Nergard, C.S., Diallo, D., Inngjerdigen, K., Michaelsen, T.E., Matsumoto, T., Kiyohara, H., Yamada, H., Paulsen, B.S., (2005). Medicinal use of *Cochlospermum tinctorium* in Mali: anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology*, 96, 255–269.

Ovodova, R. G., Golovchenko, V. V., Popov, S. V., Popova, G. Y., Paderin, N. M., Shashkov, A. S., Ovodov, Y. S. (2009). Chemical composition and anti-inflammatory activity of pectic polysaccharide isolated from celery stalks. *Food Chemistry*, 114, 610-615.

Pacher, P., Beckman, J.S., Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological Review*; 87, 315–424.

Pan, M. H., Lin-Shiau, S. Y., Lin, J. K. (2000). Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of I kappa B kinase and NF kappa B activation in macrophages. *Biochemical Pharmacology* 60, 1665–1676.

Pellerin, P., Vidal, S., Williams, P., Brillouet, J. M. (1995). Characterization of five type II arabinogalactan-protein complexes from red wine with increasing uronic acid content. *Carbohydrate Research*, 190, 183-197.

Pellerin, P., Doco, T., Vidal, S., Williams, P., Brillouet, J. M., O'Neill, M. A. (1996). Structural characterization of red wine rhamnogalacturonan II. *Carbohydrate Research*, 190, 183-197.

Pellerin, P., Cabanis, J-C. (1998). Les glucides. In *nologie: fondements scientifiques et technologiques*. Lavoisier, Tech. & Doc, France: Paris, 40-92.

- Pettolino, F.A., Walsh, C., Fincher, G. B., Bacic, A. (2012). Determining the polysaccharide composition of plant cell walls, *Nature Protocols*, 1590-1607.
- Popov, S. V., Ovodova, R. G., Golovchenko, V. V., Pupova, G. Y., Viatyasev, F. V., Shashkov, A. S., Ovodov, Y. S. (2011). Chemical composition and anti-inflammatory activity of a pectic polysaccharide isolated from sweet pepper using a simulated gastric medium. *Food Chemistry*, 124, 309-315.
- Renard, C. M. G. C., Lahaye, M., Mutter, M., Voragen, F. G. J., Thibault, J. F. (1998) Isolation and structural characterization of rhamnogalacturonan oligomers generated by controlled acid hydrolysis of sugar-beet pulp. *Carbohydrate Research*, 305, 271-280.
- Riou, V., Vernhet, A., Doco, T., Moutounet, M. (2002). Aggregation of grape seed tannins in model wine: effect of wine polysaccharides. *Food Hydrocolloids*, 16, 17–23.
- Rossol, M., Heine, H., Meusch, U., Quandt, D., Klein, C., Sweet, M.J., Hauschildt, S. (2011). LPS-induced cytokine production in human monocytes and macrophages. *Critical Reviews in Immunology*, 31, 379–446.
- Ruthes, A. C., Komura, D. L., Carbonero, E. R., Sasaki, G. L., Gorin, P. A. J., & Iacomini, M. (2010). Structural characterization of the uncommon polysaccharides obtained from *Peltigera canina* photobiont *Nostoc muscorum*. *Carbohydrate Polymers*, 81, 29–34.
- Saeman, J. F., Moore, W. E., Mitchell, R. L., Millet, M. A. (1954). Techniques for the determination of pulp constituents by quantitative paper chromatography. *Technical Association of Pulp and Paper Industry*, 37, 336-343.
- Sasaki, G. L., Gorin, P. A. J., Souza, L. M., Czelusniak, P. A., Iacomini, M. (2005). Rapid synthesis of partially *O*-methylated alditol acetate standards for GC-MS: some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation. *Carbohydrate Research*, 340, 731-739.
- Sasaki, G. L., Souza, L. M., Cipriani, T. R., Iacomini, M. (2008a). TLC of Carbohydrates in M. Waksmundzka-Hajnos, J. Sherma, T. Kowalska (Ed), *Thin Layer Chromatography in Phytochemistry*. CRC Press: Boca Raton, USA, 255-276.
- Schepetkin, I. A., & Quinn, M. T. (2006). Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *International Immunopharmacology*, 6(3), 317–333.
- Skipski, V. P. (1975). Thin layer chromatography of neutral glycolipids. *Methods in Enzymology*, 35, 396–425.
- Srivastava, R., Kulshreshtha, D.K. (1989). Bioactive polysaccharides from plants. *Phytochemistry* 28, 2877–2883.

Taylor, R. L., Conrad, H. E. (1972). Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry*, 11, 1383-1388.

Terao J. (2009). Dietary flavonoids as antioxidants. *Forum of Nutrition*; 61, 87–94.

Tischer, C. A., Gorin, P. A. J., Iacomini, M. (2002). The free reducing oligosaccharides of gum arabic: aids for structural assignments in the polysaccharide. *Carbohydrate Polymers*, 47, 151-158.

Vernhet, A., Pellerin, P., Belleville, M. P., Planque, J., Moutounet, M. (1999). Relative impact of major wine polysaccharides on the performances of an organic microfiltration membrane. *American Journal of Enology and Viticulture*, 50, 51–56.

Vidal, S., Williams, P., Doco, T., Moutounet, M., Pellerin, P. (2003). The polysaccharides of red wine: Total fractionation and characterization. *Carbohydrate Polymers*, 54, 439-447.

Vidal, S., Francis, L., Williams, P., Kwitkowski, M., Gawel, R., Cheynier, V., Waters, E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry*, 85, 519–525.

Vitaliti, G., Pavone, P., Mahmood, F., Nunnari, G., Falsaperla, R. (2014). Targeting inflammation as a therapeutic strategy for drug-resistant epilepsies: an update of new immune-modulating, Human Vaccines and Immunotherapeutics. 10, 868–875.

Wolfson, M. L., Thompson, A. (1963a). Acetylation. *Methods in Carbohydrate Chemistry*, 2, 211–215.

Wolfson, M. L., Thompson, A. (1963b). Reduction with sodium borohydride. *Methods in Carbohydrate Chemistry*, 2, 65–67.

Yamada, H. (1994). Pectic polysaccharides from Chinese herbs: structural and biological activity. *Carbohydrate Polymers*, 25, 269–276.

Zhang, X., Sun, J., Xin, W., Li, Y., Ni, L., Ma, X., Zhang, D., Zhang, D., Zhang, T., Du, G. (2015). Anti-inflammation effect of methyl salicylate 2-*O*-beta-D-lactoside on adjuvant induced-arthritis rats and lipopolysaccharide (LPS)-treated murine macrophages RAW264.7 cells, *International Immunopharmacology*, 25, 88–95.

Doco, T., Williams, P., Cheynier, V. (2007). Effect of flash release and pectinolytic enzyme treatments on wine polysaccharide composition. *Journal Agricultural and Food Chemistry*, 55 (16), 6643–6649.

Guadalupe, Z.; Ayestarán, B. (2007). Polysaccharide profile and content during the vinification and aging of tempranillo red wines. *Journal Agricultural nd Food Chemistry*, 55 (26), 10720–10728.

Vinogradov E.; Petersen B.; Bock K. (1998). Structural analysis of the intact polysaccharide mannan from *Saccharomyces cerevisiae* yeast using ^1H and ^{13}C NMR spectroscopy at 750 MHz. *Carbohydrate Research*, 307, 177-183.

Supporting Information

Table S1

Monosaccharide composition of fractions WCF, WCS, WSB, PCF and FCF.

Sample	Monosaccharides %							
	Gal	Ara	Rha	Man	Glc	Xyl ¹	Fuc ²	GalA [*]
WCF	42.7	11.1	6.7	20.2	13.6	0.4	0.3	5.0
WCS	35.3	10.0	7.0	28.4	12.7	0.3	0.2	6.1
WSB	24.5	9.2	6.2	46.4	7.5	0.2	0.2	5.8
PCF	--	--	--	100	--	--	--	--
FCF	62.5	20.8	8.2	--	--	0.6	0.4	7.5

(1) The xylose found is 2-*O*-methyl-xylose. (2) The fucose found is 2-*O*-methyl-fucose. *The galacturonic acid was determined by the spectrophotometric method of Filisetti-Cozzi and Carpita (1991).

Table S2

Profile of partially *O*-methylated alditol acetates obtained by methylation analysis of PCF.

<i>O</i> -Me-alditol acetate	Structure	Mol%
2,3,4,6-Me ₄ -Man	Man p -(1→	42.0
3,4-Me ₂ -Man	→2,6)-Man p -(1→	42.4
3,4,6-Me ₃ -Man	→2)-Man p -(1→	8.0
2,3,4-Me ₃ -Man	→6)-Man p -(1→	7.6

Table S3

Profile of partially *O*-methylated alditol acetates obtained by methylation analysis of FCF.

<i>O</i> -Me-alditol acetate	Structure	Native	Carboxyl-reduced
2,3,4-Me ₃ -Rha	Rhap-(1→	2.0	1.2
3,4-Me ₂ -Rha	→2)-Rhap-(1→	4.1	3.7
2,3,6-Me ₃ -Gal	→4)-Galp-(1→	--	4.1
2,4,6-Me ₃ -Gal	→3)-Galp-(1→	15.1	17.6
2,4-Me ₂ -Gal	→3,6)-Galp-(1→	38.3	36.2
2,3,4-Me ₃ -Gal	→6)-Galp-(1→	11.0	9.0
2,3,4,6-Me ₄ -Gal	Galp-(1→	9.8	10.2
2,3,5-Me ₃ -Ara	Araf-(1→	19.7	18.0

*The galacturonic acid content of FCF was 1% after carboxy-reduction, according to the spectrophotometric method of Filisetti-Cozzi and Carpita (1991).

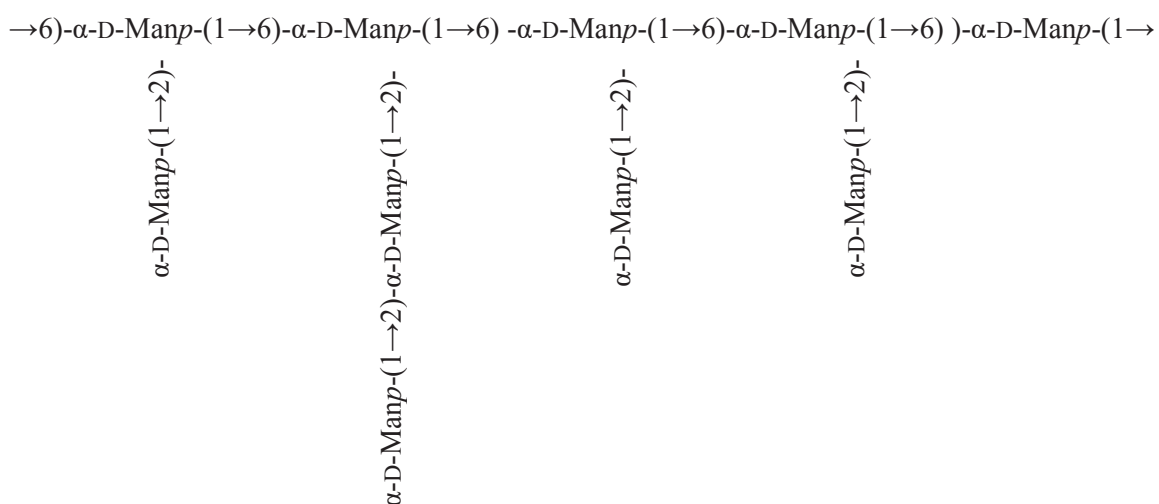


Figure S1: Suggested structure for mannan isolated.

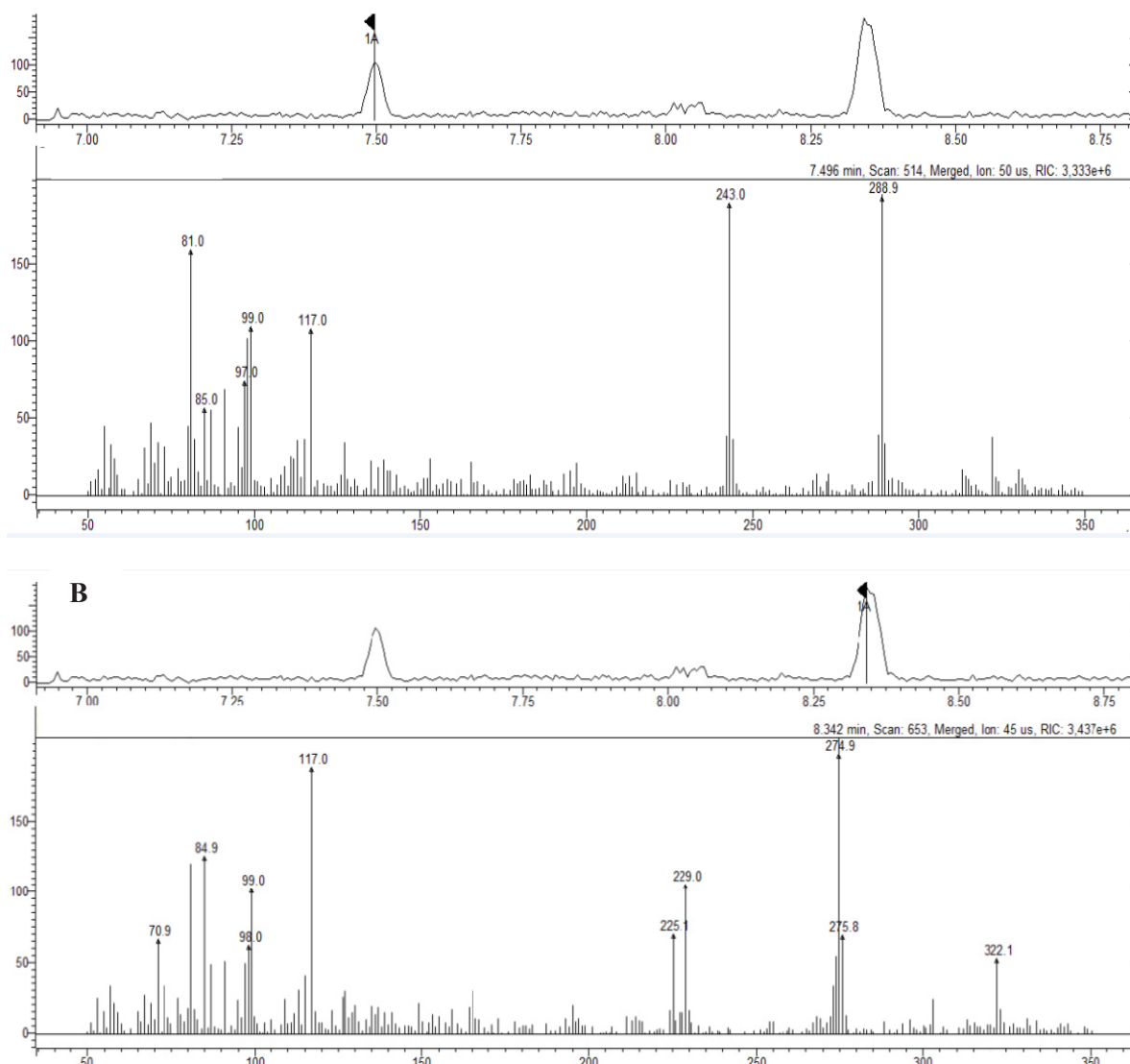


Figure S4: Chromatogram and mass spectra containing retention and fragmentation time from (A) 2-*O*-methyl-xylitol and those from (B) 2-*O*-methyl-fucitol, the identification of fragments at m/z .

CAPITULO III

Este capítulo é composto pelo manuscrito “Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice”. Publicado na Revista Carbohydrate Polymers em janeiro de 2019.

Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice

Iglesias de Lacerda Bezerra^a, Adriana Rute Cordeiro Caillot^a, Ana Flávia de Oliveira^a, Arquimedes Paixão Santana-Filho^a, Guilherme Lanzi Sassaki^{a*}

^a *Department of Biochemistry and Molecular Biology, Federal University of Parana, Curitiba, Paraná, 81.531-980, Brazil*

Abstract

Cabernet Sauvignon red wine is produced from *Vitis vinifera* grapes. The similarity between polysaccharides extracted from commercial Cabernet Sauvignon wines from three vintages and *in vivo* anti-inflammatory properties were studied in this work. It was demonstrated that the wines have similar polysaccharide patterns even though they were produced in different years. Also, it was investigated the effects of polysaccharide mixture and isolated fractions on the model of polymicrobial sepsis induced by cecal ligation and puncture in mice. Subcutaneous and oral administration of polysaccharides reduced the mice mortality rate. Treatment reduced leukocyte migration, inhibited pro-inflammatory cytokines and increased the IL-10 anti-inflammatory cytokine production when compared to the vehicle group. Complementarily, the polysaccharides decreased AST, ALT, bilirubin, urea and creatinine serum levels, with consequent protection against tissue damage. The polysaccharides demonstrated a potent anti-inflammatory effect in the sepsis model, which may indicate beneficial effects of moderate consumption of wine for human health.

Keywords: wine, Cabernet Sauvignon, polysaccharides, anti-inflammatory, NMR.

1. Introduction

Cabernet Sauvignon red wine, produced from *Vitis vinifera* grapes, originally from France, is very popular and quite consumed worldwide. Polysaccharides are one of the main macromolecules found in wine and coming mainly from grape berries, yeast, bacteria and fungal grape microbiome. Their concentrations depend on many parameters, such as, cultivation, stage of maturity, wine-making techniques and the treatments leading to increased solubilization of the macromolecular components of the grape berry cell walls (Carpita & Gibeaut, 1993; Martínez-Lapuente, Guadalupe, Ayestaran, Ortega-Heras & Perez-Magarino, 2013; Pellerin & Cabanis, 1998). Arabinogalactan-proteins, arabinogalactan, rhamnogalacturonans and homogalacturonans come from grape berries, while glucans, mannans and mannoproteins are released by yeast either during fermentation or by enzymatic activity during ageing on yeast lees by autolysis (Ayestarán, Guadalupe, & León, 2004; Brillouet, Bosso, & Moutounet, 1990; Doco & Brillouet, 1993; Doco, Vuchot, Cheynier, & Moutounet, 2003; Quijada-Morín, Williams, Rivas-Gonzalo, Doco & Escribano-Bailón, 2014; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003).

Bezerra et al. 2018 has already demonstrated that the Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines present of mannan, formed by large sequences 1,6-linked α -D-Man_p and side chains *O*-2 substituted for 1,2-linked α -D-mannan; type II arabinogalactan, formed by a 1,3-linked β -D-Galp main chain, substituted at HO-6 by 1,6-linked β -D-Galp side chains, which are 3-*O*-substituted α -L-Araf chains or nonreducing α -L-Araf; type I rhamnogalacturonan, formed by a chain of repeat units 1,4-linked α -D-GalpA and 1,2-linked α -L-Rhap; and traces of type II rhamnogalacturonan. In this work was proposed the evaluation of the reproducibility of polysaccharide similarity and its present yields among Cabernet Sauvignon wines from different vintages.

There are few studies regarding wine polysaccharides composition and structural characterization performed by NMR, and very few using the mixture of the polysaccharides. There is a great interest in knowing the benefits that wine moderate consumption can bring to health. Some authors had described important immunomodulatory, antioxidant, anti-septicemic, anti-inflammatory, antineoplastic and gastroprotective effects of polysaccharides (Bezerra et al, 2018; Cipriani et al., 2009; Cordeiro Caillot et al., 2018; Dartora et al., 2013; Inngjerdingen et al., 2014; Mellinger et al., 2008; Mueller & Anderer, 1990; Nascimento et al., 2013; Park et al., 2013; Stipp et al., 2016). Some beneficial effects of wine on health have been attributed to resveratrol, a polyphenol present in the skin of grapes (Diaz-Gerevini et al 2016; Gojkovic-Bukarica et al., 2018). Nevertheless, there are very few studies regarding biological activities and modulation of inflammatory mediators by wine polysaccharides.

Polysaccharides, such as rhamnogalactofuranan and arabinogalactan from various sources had an anti-inflammatory activity and decreased mice lethality submitted to polymicrobial sepsis induced by Cecal Ligation and Puncture (CLP) (Cordeiro et al., 2012; Scoparo et al., 2013). This biological model mimics sepsis in humans, caused by pathogens derived from the intestinal tract and is considered to closely simulate clinical situation (Rittirsch, Huber-Lang, Flierl, & Ward, 2009). Sepsis represents a state of pro-inflammatory mediators overproduction, a systemic inflammatory response that can cause organ dysfunction, especially in vital organs such as liver, kidney and lung, and consequently, multiple organ failure (Abraham et al, 1997; Angus, Linde-Zwirble, Lidicker, & Clermont, 2001; Cohen, 2002; Hotchkiss e Karl, 2003).

Therefore, this work also was analysis of Cabernet Sauvignon red wine polysaccharides and their anti-inflammatory activity against murine sepsis, evaluating effects

on lethality, leukocytes migration, anti- and pro-inflammatory cytokines levels, and biochemical, hematological and histopathological parameters.

2. Materials and methods

2.1. Materials

Cabernet Sauvignon polysaccharides were extracted from commercial wine belonged to three vintages (2016, 2015 and 2014). The wine was made from *Vitis vinifera* cv. Cabernet Sauvignon grapes grown in the Central Valley - Chile, semi-arid with strong influence of the Andes Mountain. Maturation of the wines of the three harvests was in French oak barrels. The wines have pH 3.62%, total acidity 3.41 g/L, alcoholic content 12.5% and residual sugar 3.19 g/L. These oenological parameters were similar across the studied wines and were not included in the statistical data treatment.

2.2. Polysaccharides extraction and purification

Polysaccharides were precipitated by addition of cold EtOH (3 vol.) and separated by centrifugation (8.000 rpm at 4 °C, 20 min). The sediment was dissolved in H₂O, dialyzed against distilled water with a 6-8 kDa cut-off membrane for 72 h to remove the remaining low-molecular weight compounds, giving rise to a crude polysaccharide fraction: WCS (Cabernet Sauvignon). This fraction was frozen and then allowed to unfreeze at room temperature (Gorin & Iacomini, 1984), resulting in soluble and insoluble fractions which were separated by centrifugation as previously described. The insoluble fraction in freeze-thawing process was not analyzed in this study due to its lower yield and difficult solubilization. The water-soluble fraction was treated with α -amylase and then with Fehling solution (Jones & Stoodley, 1965).

The fraction treated with Fehling solution was centrifuged under the same conditions previously described and the soluble fraction in Fehling solution (FCS) was isolated from the insoluble fraction in Fehling solution (PCS). The respective fractions were both neutralized with HOAc, dialyzed against distilled water with a 6-8 kDa cut-off membrane for 24 h and submitted to a cationic exchange resins (Amberlite-IR120-Sigma) and then freeze dried.

2.3. Monosaccharide analysis

WCS, FCS and PCS fractions (2 mg) were hydrolyzed with 1 M TFA at 100 °C for 14 h, the solution was then evaporated, and the residue dissolved in water (2 mL). The monosaccharides were then reduced with 2 mg NaBH₄, yielding alditols, which were acetylated in Ac₂O-pyridine (1:1, v/v, 0.5 mL) at room temperature for 12 h (Wolfrom & Thompson, 1963a; 1963b). The resulting alditol acetates were extracted with CHCl₃ and analyzed by gas chromatography–mass spectrometry (GC–MS – Varian, Saturn 2000R, Ion-Trap detector), using a DB-225-MS column (30 m × 0.25 mm × 0.25 µm), programmed from 50 to 220 °C at 40 °C/min, with He as carrier gas. Components were identified by their typical retention times and electron ionization (EI 70 eV) spectra. The uronic acid content of the fractions was determined using the colorimetric m-hydroxy biphenyl method (Filisetti-Cozzi & Carpita, 1991).

2.4. Structural identification by NMR

The NMR spectra were obtained with a 400 or 600 MHz Bruker spectrometer using 5 mm direct or inverse probe heads (Avance III and HD, Bruker, Billerica, Massachusetts, USA). 1D ¹H and ¹³C NMR at 600 MHz were acquired after a 90° (p1) pulse calibration at 10.75 µs. ¹H/¹³C chemical shift correlation mapping was finally determined by COSY, TOCSY HSQCed (Heteronuclear Single Quantum Coherence edited spectroscopy CH, CH₃ positive phase CH₂ negative phase) performed at 30 °C in deuterium oxide (D₂O) (Sasaki et al., 2013). 2D NMR experiments were conducted using quadrature detection at indirect dimension and acquired using 24 scans per series of 1024 × 320 data points, with zero filling in F1 (4096) prior to Fourier transformation, using the Software TOPSPIN version 3.2 pl6 (Bruker Biospin, Rheinstetten, Germany). The chemical shifts of the polysaccharide were expressed in δ (ppm) relative to trimethylsilyl propionic acid (TMSP).

2.5. *In vivo* experiments

Male Swiss mice (25–35 g) provided by Federal University of Paraná facilities were kept in a temperature-controlled room (20 ± 2 °C) on a 12 h light–dark cycle. Food and water were freely available. Experiments were performed after approval of the protocol by the university Institutional Ethics Committee (sepsis model, CEUA/BIO-UFPR, protocol number

1018). All experiments were conducted in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann, 1983). The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the treatment.

2.6. Sepsis induction by cecal ligation and puncture (CLP)

The WCS crude fraction was tested in two routes of administration: subcutaneous (abbreviation s.c.) and the fraction will be called CSS in this route of administration at doses of 0.1, 1 and 10 mg/kg and oral (abbreviation p.o.) and the fraction will be called CSO in this route of administration at doses of 3, 7 and 10 mg/kg. FCS and PCS isolated fractions were tested subcutaneously at doses of 0.1, 1 and 10 mg/kg. All doses (subcutaneous and oral) were prepared using sterile saline solution for solubilization of the polysaccharide fractions.

Mice were randomly divided into groups with 10 animals each: sham-operation, CLP plus saline (10 mL/kg s.c.), CLP plus CSS (0.1 mg/kg s.c.), CLP plus CSS (1 mg/kg s.c.), CLP plus CSS (10 mg/kg s.c.), CLP plus FCS (0.1 mg/kg s.c.), CLP plus FCS (1 mg/kg s.c.), CLP plus FCS (10 mg/kg s.c.), CLP plus PCS (0.1 mg/kg s.c.), CLP plus PCS (1 mg/kg s.c.), CLP plus PCS (10 mg/kg s.c.), CLP plus CSO (3 mg/kg, p.o.), CLP plus CSO (7 mg/kg, p.o.), CLP plus CSO (10 mg/kg, p.o.) and CLP plus dexamethasone - Dex (0.5 mg/kg s.c.). Saline was used as vehicle for dissolving the polysaccharide and dexamethasone was commercially purchased.

Ketamine (80 mg/kg) and xylazine (20 mg/kg) were injected intraperitoneally to anesthetize the mice before surgical procedures. Polymicrobial sepsis was induced by CLP as previously described (Rittirsch, Huber-Lang, Flierl, & Ward, 2009). A midline incision of ~1.5 cm was carried out on the abdomen. The cecum was carefully exposed and 50% of the distal moiety was ligated. The cecum was then punctured thrice with a sterile 16-gauge needle and squeezed to extrude the fecal material from the wounds. The cecum was replaced and the abdomen was stitched surgically. Sham-control animals were treated identically, but no cecal ligation or puncture was carried out. Each mouse received a subcutaneous sterile saline injection (1 mL) for fluid resuscitation after surgery. The mice were then kept on a heating pad (35 °C) until they recovered from the anesthesia. Food and water, *ad libitum*, were provided throughout the experiment. The survival rate was monitored for 7 days, every 12 h. During this period, water (vehicle) and drugs were orally or subcutaneously administered

daily. Dexamethasone was commercially purchased and subcutaneously administered at doses of 0.5 mg/kg (Lapa, Silva, Almeida Cabrini, & Santos, 2012; Longhi-Balbinot et al., 2012; Silva et al., 2012).

In this experimental set, mice were orally or subcutaneously treated with vehicle (1 h before surgery), sham-operation, CLP plus saline (10 mL/kg s.c.) and dexamethasone (0.5 mg/kg s.c.). The best dose of each fraction in the survival test was chosen for the anti-inflammatory evaluation tests, as follows: CSS (1 mg/kg, s.c.), CSO (10 mg/kg, p.o.), FCS (1 mg/kg, s.c.) and PCS (1 mg/kg, s.c.). For these doses, after a post-operation time of 6 h, mice were sacrificed. Liver, lung and kidney tissues were collected for histological analysis. Blood was collected for hematological and biochemical parameters evaluation.

2.7. Hematological assays

Blood was collected from the abdominal vena cava. Total leukocytes and mononuclear (MN) and polymorphonuclear (PMN) leukocytes were counted by an automated system, according to the manufacturer's instructions (Mindray BC-2800 vet, São Paulo, SP, Brazil).

2.8. Biochemical assays

The amount of TNF- α , IL-1 β and IL-10 cytokines in the blood plasma were determined by enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. Three replicates were carried out for each different treatment. The absorbance of each group was measured by a microplate reader at the wavelength of 450 nm. To determine the total concentration of nitric oxide (NO) in the blood plasma, Griess reagent was added to 40 μ L of plasma and the absorbance at 545 nm was evaluated with an ELISA kit (BD Biosciences, San Jose, CA, USA) following the manufacturer's manual.

Bilirubin, creatinine (CREA) and urea, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and activities were analyzed from plasma by an automated system, according to the manufacturer's instructions (Mindray BS-200, São Paulo, SP, Brazil).

2.9. Histopathological analysis

Lungs, liver and kidney tissue sections were fixed in ethanol at room temperature. After fixation, samples were dehydrated in a graded series of increasing ethanol before paraffin embedding. Thin sections (4 mm) from paraffin blocks were processed for histology, and the tissue was stained with hematoxylin eosin. The slides were then analyzed by light microscopy.

2.10. Statistical analysis

The results were expressed as mean $\pm$ SEM and were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's test as a post hoc test ($P < 0.001$). GraphPad Prism 5.0 (La Jolla, CA) program was used for both statistical analysis and figure design. Histological analysis was performed by qualitative observations.

3. Results and Discussion

3.1. Analysis of the polysaccharides of Cabernet Sauvignon wine

Approximately 750 mL from commercial wine was concentrated and precipitated with excess ethanol. The polysaccharides were recovered by ethanol precipitation and then centrifugated and dialysed against water. The solution was then freeze-dried, generating the WCS polysaccharide fraction with yield of 0.05% in the harvests of the three years evaluated and the table 2 show total polysaccharide yield in mass to 750 mL of wine.

Previous studies carried out by our group on polysaccharide structure demonstrated that fraction WCS from Cabernet Sauvignon is composed by a mixture of mannan, type II arabinogalactan, type I and II rhamnogalacturonans (Bezerra et al., 2018).

^1H NMR spectra analyses of the crude polysaccharide fractions from Cabernet Sauvignon wine from 2014 (WCS-2014), 2015 (WCS-2105) and 2016 (WCS-2016) were performed (Fig. 1). ^1H -NMR data of WCS of the three vintages showed characteristic polysaccharide NMR signals, showing a complex profile at the anomeric region (4.0~6.0 ppm). The ^1H NMR signals shown in figure 1 the anomeric region and the binding region

were similar for all three fractions. This experiment demonstrated that the wines have similar polysaccharide patterns even though they were produced in different years.

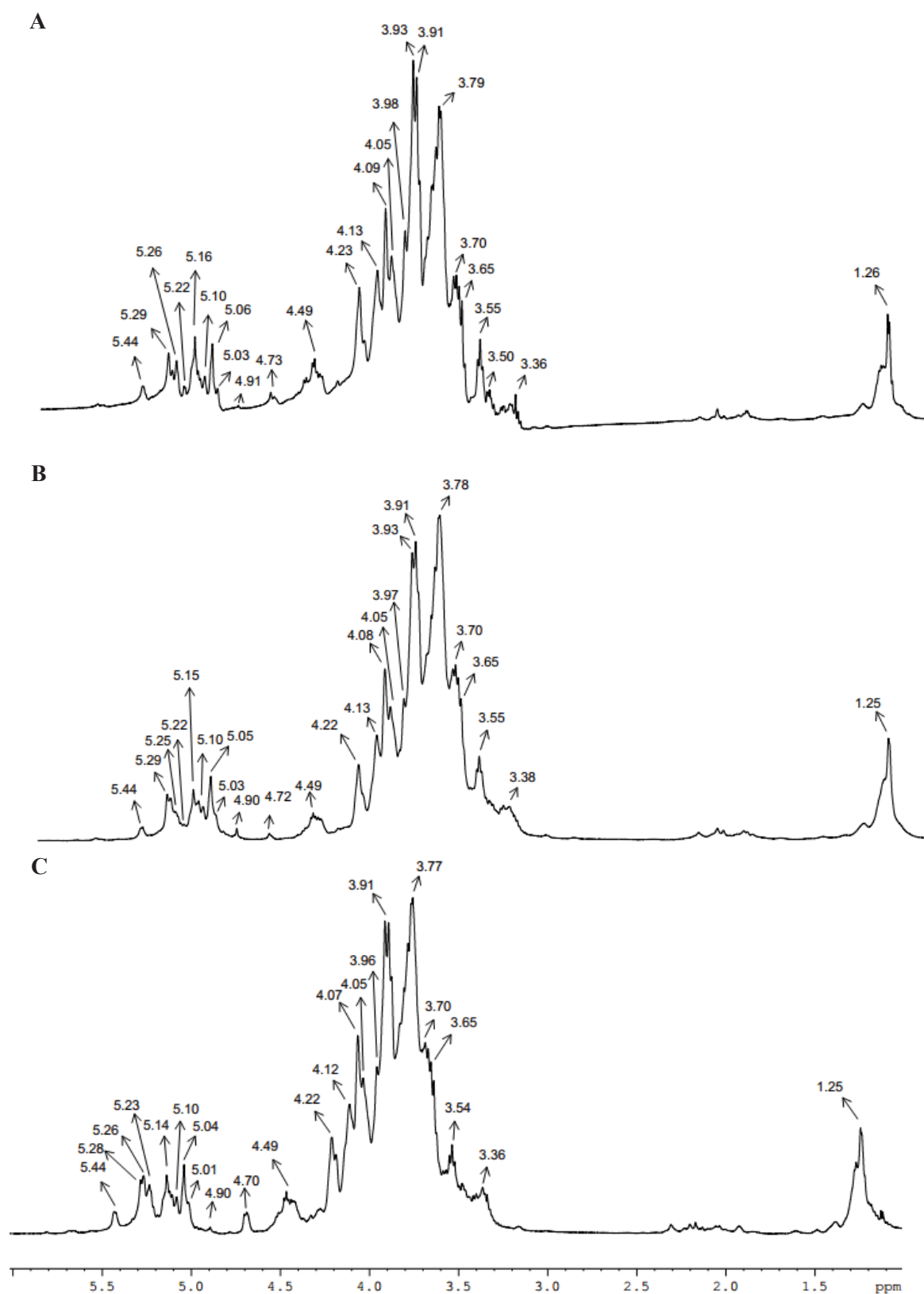


Figure 1: ^1H -NMR spectra of (A) WCS-2014, (B) WCS-2015 and (C) WCS-2016 in D_2O at $30\text{ }^\circ\text{C}$ (chemical shifts are expressed in δ ppm).

The monosaccharide composition was performed for the crude fractions extracted from wine bottles belonged to three vintages (table 1). All the fractions are composed by galactose, mannose, glucose, rhamnose, galacturonic acid, arabinose, 2-*O*-methyl-fucose and 2-*O*-methyl-xylose and it was observed that there was a minimum variation among the fractions analyzed, showing that all the fractions presented the same polysaccharides in different vintages.

Table 1

Composition Monosaccharide composition of the red wine polysaccharides of different vintages

Fraction	Monosaccharide %							
	Gal	Ara	Rha	Man	Glc	Xyl ¹	Fuc ²	GalA [*]
WCS-2014	36.5	11.2	8.1	23.9	12.9	0.3	0.2	6,9
WCS-2015	38.1	13.2	6.5	25.8	10.2	0.2	0.2	5,8
WCS-2016	35.3	10.0	7.0	28.4	12.7	0.3	0.2	6.1
PCS	nd	nd	nd	100	nd	nd	nd	nd
FCS	61.8	21.5	9.7	nd	nd	0.6	0.4	6.0

1) Xylose found is 2-*O*-methyl-xylose. (2) Fucose found is 2-*O*-methyl-fucose. *Galacturonic acid was determined by the spectrophotometric method of Filisetti-Cozzi and Carpita (1991). Not detected (nd).

Through of the ¹H NMR and NMR-HSQC analysis (Fig. S1 – supporting information), using the values obtained by the integrals of the monosaccharide signals found in the anomeric region of the spectra of the crude fraction of each wine and the retention time of the monosaccharides 2-*O*-methyl xylose and 2-*O*-methyl-fucose in GC-MS (Fig. S2 – supporting information) it was possible to quantify each polysaccharide in relation to the total amount obtained in each wine (table 2). In this analysis the reproductively of wine produced in different years was evaluated, the table 2 shows that the percentage of each polysaccharide and also show that the mass of each polysaccharide recovery remained practically the same in Cabernet Sauvignon wines of different vintages. The similar percentage in the monosaccharide composition, as well as the amount of polysaccharides may suggest

maintenance of quality of wine, indicating that the winery hold the same standard in the wine production process along the years.

Table 2

Quantification of each polysaccharide in relation to the total yield

Fraction	Polysaccharides								
	Total Yield	AG-II		RG-I/RG-II		Mannan		Dextrin	
	mg	%	mg	%	mg	%	mg	%	mg
WCS-2014	375.0	54.9	205.9	7.7	28.9	34.8	130.5	2.6	9.7
WCS-2015	390.0	55.1	214.9	6.8	26.5	35.7	139.2	2.4	9.4
WCS-2016	381.0	52.6	200.4	6.4	24.4	39.1	148.9	1.9	7.3

AG-II: type II arabinogalactan; RG-I/RG-II: type I and II rhamnogalacturonans.

The PCS and FCS fractions were studied by Bezerra et al (2018) through NMR, monosaccharide composition and methylation. The PCS fraction is a mannan, it is composed only by mannose (Table 1). The FCS polysaccharide is composed by galactose, rhamnose, galacturonic acid, arabinose, 2-*O*-Me-Fucose and 2-*O*-Me-Xylose (Table 1). This fraction presents a type II arabinogalactan, a type I rhamnogalacturonan and type II rhamnogalacturonan. These fractions (PCS and FCS) were used for the biological tests shown below in this work.

3.2. Biological experiments

Sepsis is one of the most prevalent diseases and one of the main causes of death among hospitalized patients. Uncontrolled severe infection often leads to sepsis, a systemic inflammatory response associated with fever, leukocytosis and multiple organ failure (Stearns-Kurosawa, 2011). This organ dysfunction can be represented by in the sequential organ failure assessment an increase including lung, kidney, and liver (Abraham et al., 1997; Bone et al., 1995; Singer et al., 2016).

In this work, we investigated the effect of Cabernet Sauvignon wine polysaccharides on survival of septic mice *in vivo*, as well as important hematological, biochemical and histopathological parameters related to organ dysfunction in sepsis. Here, it is suggested that

polysaccharides may exert anti-inflammatory action that may contribute to the benefits of wine consumption.

Polymicrobial sepsis was induced by CLP in mice to investigate WCS effects. This model mimics the human sepsis caused by pathogens derived from the intestinal tract and simulates a clinical situation (Otero-Anton et al., 2001). Many polysaccharides isolated from a variety of sources have shown immune responses *in vivo* and *in vitro*. Polysaccharides such as pectins and hemicelluloses had their anti-septicemic effect determined by being tested subcutaneously (Lim et al., 2002; Ruthes, Rattmann, Carbonero, Gorin & Iacomini, 2012; Tzianabos, Kasper, Cisneiros, Smith & Onderdonk, 1995) and orally (Dartora et al., 2013; Scoparo et al., 2013).

In this work, we treated orally the animals with the Cabernet Sauvignon wine polysaccharide mixture (CSO) to evaluate the protective effect of wine consumption and subcutaneously with the polysaccharide mixture (CSS) and the isolated fractions (FCS and PCS) to achieve very low doses and still be able to show the efficiency of the polysaccharides as anti-inflammatory and protective in an aggressive model of sepsis by CLP.

Lethality was found to be markedly reduced in mice receiving CSO, CSS, FCS and PCS treatments (Fig. 2). It was demonstrated that the CSO fraction prevents lethality caused by polymicrobial sepsis in mice. Lethality was markedly delayed in mice treated orally with CSO. The corresponding areas under the lethality curve increased to 9.120, 11.400 and 13.500 (arbitrary units) after 3, 7 and 10 mg/kg administration, respectively (Fig. 2A). The overall survival in the CSO group was 30%, 50% and 70%, increasing with the dose. The 10 mg/kg CSO dose was established for the analysis of this fraction effects in CLP.

The CSS fraction prevents lethality, obtaining a survival rate of 40% (0.1 mg/kg), 80% (1 mg/kg) and 78% (10 mg/kg) (Fig. 2B). Lethality was markedly delayed in subcutaneously treated mice. The corresponding areas under the lethality curve were 10.680, 14.160 and 14.040 (arbitrary units) after administering 0.1, 1 and 10 mg/kg, respectively. The 1 and 10 mg/kg doses presented similar effects and obtained better results when compared to dexamethasone (0.5 mg/kg), which promoted a 50% survival rate. Therefore, the 1 mg/kg CSS dose was established for the analysis of the CLP fraction effects.

Mice treated with FCS had a survival rate of 45% at the 0.1 mg/kg dose and 80% at 1 and 10 mg/kg doses (Fig. 2C). The 1 and 10 mg/kg doses presented similar effects. The corresponding areas under the lethality curve were 12.300, 14.860 and 15.120 (0.1, 1, 10

mg/kg, respectively). Dexamethasone, used as control, had an area under curve of 12.000, with an overall survival rate of 50% at the end of the observation period. As the doses of 1 and 10 mg/kg presented similar results, the 1 mg/kg FCS dose was established for the analysis of the effects of this fraction in CLP.

Lethality was markedly delayed in mice subcutaneously treated with PCS and their areas under the curve were 10.560, 13.020 and 12.900 after administrating 0.1, 1 and 10 mg/kg doses, respectively (Fig. 2D). At the end, the corresponding overall survival rates were 40%, 65% and 63%. The 1 and 10 mg/kg doses presented similar effects. Therefore, the 1 mg/kg PCS dose was established for the analysis of the effects of this fraction in CLP. In summary, considering survival rates, the following doses were chosen for following analyses in sepsis: 10 mg/kg for CSO and 1 mg/kg for CSS, FCS and PCS.

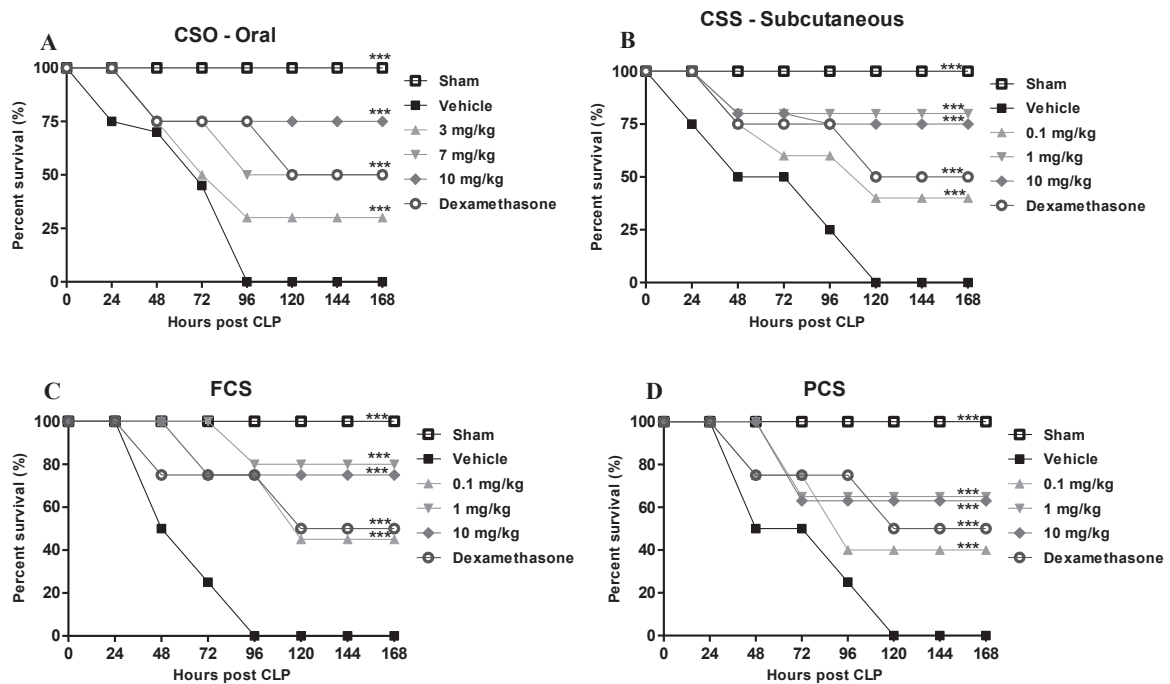


Fig. 2: Effects of CSO (A), CSS (B), FCS (C) and PCS (D) fractions on survival rate. Mice (10 animals/group) were orally administered various doses of CSO (3, 7 or 10 mg/kg) or subcutaneous administered various doses of CSS, FCS and PCS (0.1, 1, 10 mg/kg), vehicle (saline s.c.) and dexamethasone (0.5 mg/kg s.c.). Values represent means $\pm$ SEM. *** $p < 0.001$ when compared with CLP plus vehicle group; ANOVA followed by the Tukey's test.

In sepsis model, the acute immune response is organized and executed predominantly by innate immunity. Sepsis by CLP promotes migration of leukocytes through the bloodstream to the site of infection, peritoneal cavity and affected organs such as lung, liver and kidneys (Bone et al., 1992). Results showed that CSS, CSO, FCS, PCS and dexamethasone were able to reduce by 60%, 59%, 59%, 51% and 55% respectively, white blood cell (leukocyte count), when compared to the vehicle group (Table 3). Differential leukocyte counts demonstrated a decrease in the mononuclear leukocyte (MN) subset and the PMN subset in the bloodstream (Table 3). Mononuclear leukocyte subset reduction consisted in 62%, 59%, 59%, 58% and 60% for CSS, CSO, FCS, PCS and dexamethasone, respectively. For the PMN subset, these reductions were 51%, 53%, 50%, 46% and 47% (CSS, CSO, FCS, PCS, dexamethasone, respectively), when compared to the vehicle group. This exacerbated inflammatory response was observed on the vehicle group, in which there was an increased number of leukocytes, mononuclear leukocytes and PMN, whereas all fractions tested were able to reduce these effects significantly, reducing the amplification of the inflammatory response.

Neutrophils are the first cells to migrate to the site of infection, where they produce both reactive oxygen and nitrogen species that act to kill the infectious agents. These neutrophils and tissue resident cells also produce inflammatory cytokines, that can recruit and activate other inflammatory cells that, in turn, amplify and extend the initial systemic inflammatory response, as happened in the vehicle group. However, treatment with wine polysaccharides seems to reduce this effect (Yamashiro et al., 2001).

Table 3

Hematological parameters evaluated during sepsis in mice

Treatment	WBC (U/L)	PMN (U/L)	MN (U/L)
Sham	3316.7 ± 365.6	1450.0 ± 216.8	1850.0 ± 104.9
Vehicle	9317.9 ± 248.3***	3850.0 ± 225.8***	5283.3 ± 297.1***
CSS	3766.0 ± 266.5 [#]	1900.0 ± 322.5	2016.7 ± 271.4
CSO	3850.0 ± 176.1 [#]	1890.0 ± 206.6	2183.3 ± 183.3
FCS	3816.7 ± 343.0 ^{##}	1916.7 ± 292.7	2186.7 ± 116.0
PCS	4600.0 ± 346.4	2133.3 ± 311.6	2200.0 ± 282.8
Dex	4216.7 ± 242.7	2050.0 ± 242.9	2116.7 ± 216.1

*** $p < 0.001$ when vehicle was compared with all groups; # $p < 0.05$ and ## $p < 0.01$ when compared with PCS; ANOVA followed by the Tukey's test. WBC: White blood cell count; PMN: polymorphonuclear leukocytes; MN: mononuclear leukocytes; Dex: dexamethasone.

During sepsis, the immune response starts sensing of danger by pattern-recognition receptors on the immune competent cells, such as macrophages. The sensed danger signals, through specific signaling pathways, activate transcription factors and gene regulatory systems, which up-regulate the expression of proinflammatory mediators (Akira & Takeda, 2004). Inflammatory mediators, including proinflammatory cytokines TNF- α and IL-1 β , and anti-inflammatory cytokines, such as IL-10, generated by macrophages, induce the immune cells recruitment, mainly neutrophils (Overhaus et al., 2004; Pan, Lin-Shiau & Lin, 2000). Neutrophils damage tissue directly by releasing proinflammatory mediators, such as NO, which amplify the systemic inflammatory response and contribute to multiple organ failure (Landry & Oliver, 2001; Nieuwenhuijzen et al., 1996). This study shows that CSS, CSO, FCS, PCS and dexamethasone significantly decreased the production of the pro-inflammatory cytokines TNF- α (57%, 42%, 57%, 57% and 53%, respectively) and IL-1 β (54%, 52%, 53%, 53%, 53% and 52%, respectively) and increased the level of the anti-inflammatory cytokine IL-10 (3x for all fractions) when compared to the vehicle group (Fig. 3 A-C). In addition, production of excessive NO has been associated with a range of inflammatory diseases, including septic shock (Pacher, Beckman & Liaudet, 2007). As shown in figure 3D, treatments with CSS, CSO, FCS, PCS fractions and dexamethasone induced a significant inhibitory effect in NO production (60%, 57%, 59%, 57% and 54%, respectively), indicating anti-inflammatory properties. Reduction of the effects of pro-inflammatory mediators and increased levels of the anti-inflammatory mediator suggests that the all fractions exerted anti-inflammatory effect in sepsis. We performed assays regarding the phenol content of the polysaccharides sample using the Folin Ciocalteu method, and found that on the 1, 10, 100 and 1000 $\mu\text{g/mL}$ polysaccharides solutions, the polyphenols content, measured as galic acid equivalents, were of ~ 0 , 0.8, 3 and 7 $\mu\text{g/mL}$ respectively. Thus the data in the literature regarding macrophage activation by plant/fruit extract containing polyphenols (Hooshmand et al, 2015) suggest that only at concentrations between 100 and 1000 $\mu\text{g/mL}$ these compounds could significantly affect anti-inflammatory assays. Thus we can conclude that is unlikely that the observed biological activities demonstrated by the polysaccharide treatments could be somewhat influenced by their contents of polyphenols.

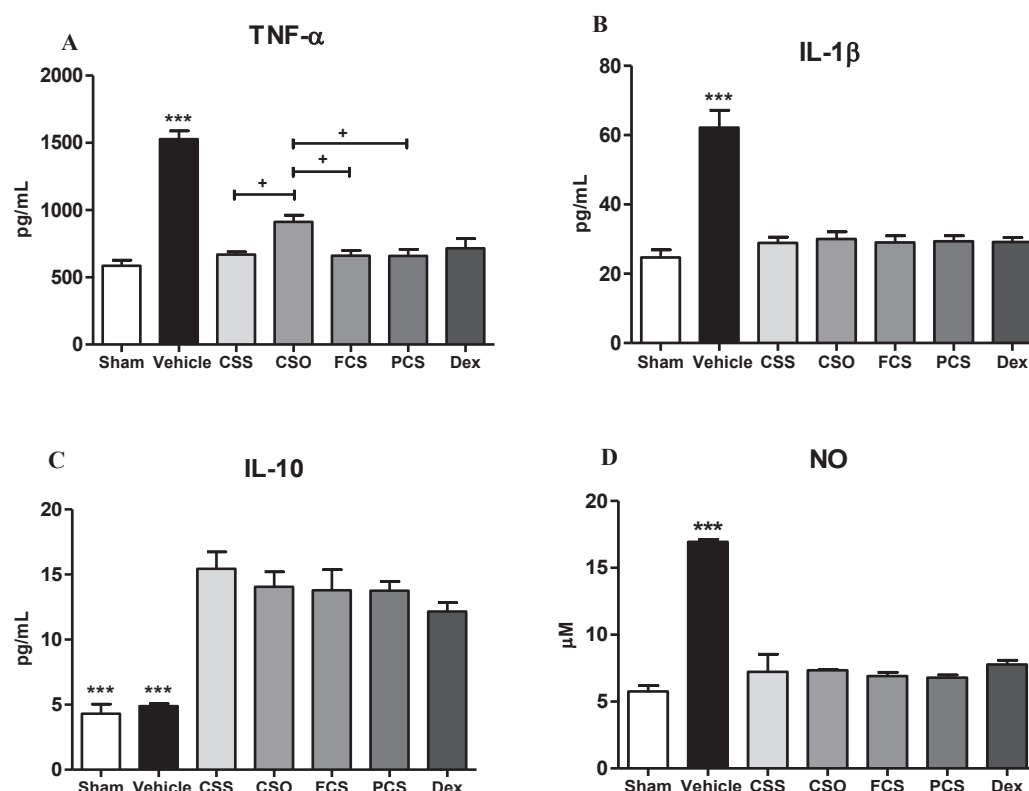


Fig. 3: Expression levels of TNF- α . (A), IL-1 β (B), IL-10 (C), and NO (D) production levels. Dex (dexamethasone). *** $p < 0.001$ when vehicle was compared with all groups; + $p < 0.05$ comparison between fractions. ANOVA followed by the Tukey's test.

Sepsis is characterized by a systemic inflammatory response to infection resulting in acute organ dysfunction and associated with a high mortality rate (Hotchkiss & Karl, 2003). There is progressive deterioration of various organs function, starting mostly with lung and followed by failure of liver and kidneys (Bau, Durhan & Faist, 1998). During sepsis, acute lung injury can be caused directly by bacterial damage or secondarily by the systemic inflammatory response of sepsis (Chen et al., 2016). The activated polymorphonuclear leukocytes play an important role in mediating microvascular damage and lung tissue injury. PMN leukocytes infiltrate the lung tissue and their persistence in the lungs is an important determinant of poor survival (Bhatia & Mochhala, 2004). Histopathological analysis showed the lungs of the treated and untreated mice after CLP (Fig. 4 H-N). Vehicle group showed characteristics of acute alveolar damage and inflammation, such as congestion, accumulation of neutrophils (Fig. 4I) and markedly thickened alveolar walls. CSS, CSO, FCS, PCS and dexamethasone groups showed reduced sepsis-induced damage and even normal lung histologic feature (Fig. 4 J-N). This study showed that the wine polysaccharides significantly

reduced the PMN number in blood and this may have reduced leukocyte infiltration in tissues and protected against lung injury.

Liver plays a major function in host defense in sepsis. Kupffer cells scavenge bacteria as well as clear cytokines from the liver. Hepatocytes modify metabolic pathways, increase gluconeogenesis, activate cellular repair and enzymes such as transaminases (AST and ALT) and alter bilirubin levels (Dhainaut, Marin, Mignon & Vinsonneau, 2001). ALT and AST transaminases are markers of hepatocyte injuries, so they were evaluated in this study. It was observed an increase in plasma AST and ALT activity in the septic group (Table 4). Treatment with CSS, CSO, FCS, PCS and dexamethasone showed a significant reduction of ALT in the blood in 59%, 64%, 57%, 58% and 58%, respectively, compared to the vehicle group (Table 4). Although ALT is an enzyme present in the hepatocyte cytoplasm, AST is present in mitochondria and at the cytoplasm. Moreover, AST has activity in the liver, heart, kidneys, pancreas, muscles and erythrocytes. Therefore, increased levels of plasma AST indicate the occurrence of major damage to the hepatocyte mitochondria or significant damage in a different organ. Thus, AST levels are higher than ALT in the vehicle group, but were not affected in all fractions treated groups and dexamethasone, which were able to maintain the low levels of the two enzymes. When compared to the vehicle group, it was observed the reduction in AST levels by 65% (CSS), 69% (CSO), 64% (FCS), 63% (PCS) and 63% (dexamethasone) (Table 4).

This study also evaluated liver function by measuring bilirubin levels. Bilirubin is recognized as an indicator of liver dysfunction, so severe elevations can cause damage to brain and erythrocytes (Brito, Silva & Brites, 2006; Fevery, 2008). Increased serum level of bilirubin was observed in the septic group, which was in accordance with other results already shown (Table 4). All treatments with fractions and dexamethasone showed a significant reduction of bilirubin serum in relation to septic group, consisting in 67% (CSS), 61% (CSO), 61% (FCS), 58% (PCS) and 58% (dexamethasone) (Table 4). Histopathological analyses demonstrated hepatic alterations in the vehicle group (Fig. 4B). Lesions were characterized by a reduction in sinusoids within the lumen, indicating tumefaction, and capsular infiltration of inflammatory cells. These lesions were found in septic group and could be prevented by treatments with each of all fractions, showing that the wine polysaccharides were able to preserve the liver, similar to dexamethasone (Fig. 4C-G) and sham group (Fig. 4A). These results showed that the wine polysaccharides were effective in protecting the liver and maintaining the organ functions during sepsis.

Table 4

Plasmatic parameters evaluate during sepsis in mice

Treatment	AST (U/L)	ALT (U/L)	Bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Sham	138.4 ± 28.1	73.9 ± 5.8	1.0 ± 0.1	60.7 ± 9.4	0.4 ± 0.1
Vehicle	459.8 ± 25.5***	248.7 ± 19.1***	3.6 ± 0.4***	209.1 ± 14.0***	1.3 ± 0.1***
CSS	161.2 ± 14.5	101.8 ± 5.7	1.2 ± 0.3	86.2 ± 9.6	0.4 ± 0.1
CSO	140.5 ± 14.9	90.7 ± 6.9	1.4 ± 0.2	89.9 ± 6.8	0.5 ± 0.1
FCS	166.0 ± 21.5	106.9 ± 9.1	1.2 ± 0.1	97.5 ± 10.6	0.4 ± 0.1
PCS	169.5 ± 24.6	104.6 ± 14.5	1.5 ± 0.3	101.8 ± 5.4	0.5 ± 0.1
Dex	168.7 ± 10.9	105.0 ± 2.8	1.5 ± 0.2	99.9 ± 4.1	0.5 ± 0.1

***p < 0.001 when vehicle was compared with all groups; There was no statistical difference between the treated groups. ANOVA followed by the Tukey's test. WBC:White blood cell count; Dex: dexamethasone.

Sepsis is a systemic inflammatory response syndrome caused by bacteria, viruses or fungi. It can cause organ damage far from the primary site of infection, leading to multiple organ failure and even death. Acute kidney injury (AKI) is one of the complications of sepsis that can cause increased mortality rate in patients (Rewa & Bagshaw, 2014; Yamada et al., 2013). Kidney infiltration by neutrophils and subsequent release of free radicals is one major mechanism leading to AKI (Huang et al., 2015). The ability of CSS, CSO, FCS and PCS to have a protective effect on the kidneys was evaluated through the analysis of creatinine and urea (Table 4), which are markers of renal function (Fig. 4 O-U and Fig. S3 - supporting information). There was a significant increase in serum urea and creatinine levels in mice in vehicle group, while all fractions showed a significant reduction of serum levels of these markers (Table 4). The observed reductions were 59% (CSS), 57% (CSO), 53% (FCS), 51% (PCS) and 52% (dexamethasone), in relation to the vehicle group for urea levels. For creatinine level, the reductions were of 69% (CSS), 62% (CSO), 69% (FCS), 62% (PCS) and 62% (dexamethasone). Histological analysis of kidney revealed a renal tubular dilatation, bleeding, leukocyte infiltrate and tubular epithelial cell vacuolar degeneration with presence of necrosis, Bowman's capsule and proximal convoluted tubule retraction with degeneration were observed in the vehicle group (Fig. 4P and Fig. S3B - supporting information). Pre-treatment with each of all fractions effectively improved these kidney histopathological

changes in mice with sepsis, as well as dexamethasone (Fig. 4Q-U and Fig. S3 C-G - supporting information) and sham group (Fig. 4O and Fig. S3A - supporting information). Wine polysaccharides were able to preserve the kidneys during sepsis and seem to have helped in maintaining kidney function.

Due to the results presented, we were able to show that pre-treatment with Cabernet Sauvignon wine polysaccharides prevented lethality caused by polymicrobial sepsis in mice, by both subcutaneous and oral administration. Polysaccharides demonstrated a potent anti-inflammatory effect in the sepsis model, what may indicate beneficial effects of wine moderate consumption for human health. The results were very satisfactory and encouraging, in future work we aim to include polysaccharides from commercial suppliers as controls. The anti-inflammatory effect of polysaccharides obtained from Cabernet Sauvignon wine and pure polysaccharide preparations (AGs, RGs and mannans) from commercial suppliers will be compared in the biological experiments, we will evaluate if the same polysaccharide obtained from different sources in which it presents small variations in its structure and different branching points may influence the biological response studied.

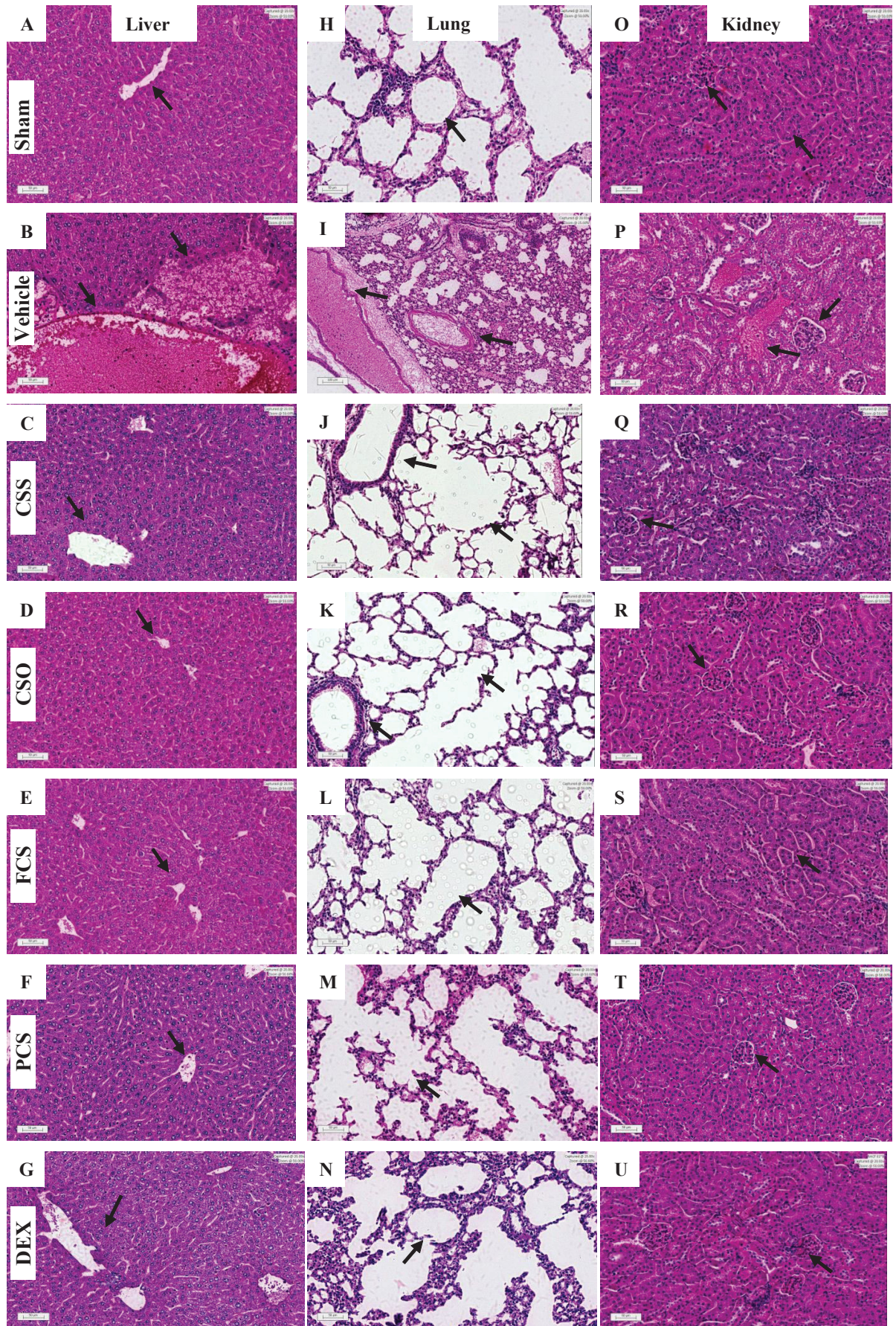


Fig. 4: Representative histological sections from mice in groups: sham, vehicle, CSS, CSO, FCS, PCS and DEX (dexamethasone). Liver (A-G), lung (H-N) and kidney (O-U). Original magnification $\times 50$. H&E. Scale bar = 50 μ m.

4. Conclusions

NMR and GC–MS analysis showed that the polysaccharides and their percentages were similar for Cabernet Sauvignon wine from different vintages. This has demonstrated the reproducibility of the different harvests wines from a polysaccharide perspective. The present study also suggests that wine polysaccharides exerted anti-inflammatory effect in sepsis induced by cecal ligation and puncture. It was demonstrated that polysaccharides prevent lethality caused by polymicrobial sepsis in mice. This beneficial effect seems to be due to a reduction in leukocyte infiltration, increased anti-inflammatory cytokine and inhibited production of pro-inflammatory cytokines, with consequent protection against tissue damage. The polysaccharide fractions were able to protect liver, lungs and kidney against injuries induced by CLP sepsis in mice.

Acknowledgements

The authors would like to thank the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Grant number 449176/2014-2), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financiadora de Estudos e Projetos (FINEP) and Fundação Araucária for financial support, and UFPR NMR Center. Special thanks to professor Dr Juliano Bordignon of the Laboratory of Molecular Virology (Carlos Chagas Institute – Curitiba, Brazil) for kindly donating the Cytokine ELISA Kits.

References

Abraham, E., Glauser, M. P., Butler, T., Garbino, J., Gelmont, D., Laterre, P. F., Kudsk, K., Bruining, H. A, Otto, C., Tobin, E., Zwingelstein, C., Lesslauer W. (1997). Leighton, p55

tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock. A randomized controlled multicenter trial. Ro 45-2081 study group, JAMA. 277, 1531–1538.

Angus, D. C., Linde-Zwirble, W. T., Lidicker, J., Clermont, G. (2001). Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Critical Care Medicine*, 29, 1303–1310.

Akira, S., Takeda, K. (2004). Toll-like receptor signalling. *Nature Reviews Immunology*. 4, 499– 511.

Ayestarán, B., Guadalupe, Z., León, D. (2004). Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta*, 513, 29-39.

Bau, A. E., Durhan, R., Faist, E. (1998). Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): Are we winning the battle? *Shock*, 10, 79-89.

Bezerra, I.L.; Caillot, A. R. C.; Palhares, L. C. G. F.; Santana-Filho, A. P.; Chavante, S. F. Guilherme Lanzi Sasaki, G. L. (2018). Structural characterization of polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: Anti-inflammatory activity in LPS stimulated RAW 264.7 cells. *Carbohydrate Polymers*, 186, 91-99.

Bhatia M, Moomchala S. (2004). Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *The Journal of Pathology*, 202(2), 145-56.

Bone, R. C., Balk, R. A, Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., Schein, R. M., Sibbald, W. J. (1992). American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis, *Chest*. 101, 1644-1655.

Bone, R. C., Balk, R. A., Fein, A. M., Perl, T. M., Wenzel, R. P. Reines, H. D., Quenzer, R.W., Iberty, T. J., Macintyre, N., Schein R. M. (1995). A second large controlled clinical study of E5, a monoclonal antibody to endotoxin: Results of a prospective, multicenter, randomized, controlled trial. The E5 Study Investigators. *Critical Care Medicine*, 23, 94–1006.

Brillouet, J. M., Bosso, C., Moutounet, M. (1990). Isolation, purification, and characterization of an arabinogalactan from a red wine. *American Journal of Enology and Viticulture*, 41, 29-36.

Brito, M. A., Silva, R. F., Brites, D. (2006). Bilirubin toxicity to human erythrocytes: a review, *Clinica Chimica Acta*. 374, 46-56.

Carpita, N. C., Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, 3, 1-30.

Chen, J. K., Wang, W. C, Zang, L., Zhao, J., Li, W., Jiang, T. (2016). Repression of a chromatin modifier aggravates lipopolysaccharide-induced acute lung injury in mouse. *Biochemical and Biophysical Research Communications*. 471, 515-521.

Cipriani, T. R., Mellinger, C. G., Souza, L. M., Baggio, C. H., Freitas, C. S., Marques, M. C. A., Gorin, P. A. J., Sassaki, G. L., Iacomini, M. (2008). Acidic heteroxylans from medicinal plants and their anti-ulcer activity. *Carbohydrate Polymers*, 74, 274-278.

Cipriani, T. R., Mellinger, C. G., Bertolini, L. C., Baggio, C. H., Freitas, C. S., Marques, M. C. A., Gorin, P. A. J., Sassaki, G. L., Iacomini, M. (2009). Gastroprotective effect of a type I arabinogalactan from soybean meal. *Food Chemistry*, 115, 687-690.

Cohen, J. The immunopathogenesis of sepsis. *Nature*. (2002). 420(6917), 885-91.

Cordeiro, L. M., Beilke, F., Bettim, F. L., Reinhardt, V de, F., Rattmann, Y. D., Iacomini, M. (2012). (1->2) and (1->6)-Linked beta-D-galactofuranan of microalga *Myrmecia biatorellae*, symbiotic partner of *Lobaria linita*. (2012). *Carbohydrate Polymers*. 90, 1779–1785.

Cordeiro Caillot, A. R.; Bezerra, I. L.; Palhares, L. C. G. F.; Santana-Filho, A. P.; Chavante, S.; Sassaki, G. L. (2018). Structural characterization of blackberry wine polysaccharides and immunomodulatory effects on LPS-activated RAW 264.7 macrophages. *Food Chemistry*, 257, 143-159.

Dartora, N.; Souza, L. M.; Paiva, S. M. M; Scoparo, C. T.; Iacomini, M.; Gorin Philip A. J., Rattmann, Y. D., Sassaki, G. L., (2013). Rhamnogalacturonan from *Ilex paraguariensis*: A potential adjuvant in sepsis treatment. *Carbohydrate Polymers*, 92, 1776-1782.

Delgobo, C. L., Gorin, P. A. J., Jones, C., & Iacomini, M. (1998). Gum heteropolysaccharide and free reducing mono- and oligosaccharides of *Anadenanthera colubrina*. *Phytochemistry*, 47, 1207–1214.

Delgobo, C. L., Gorin, P. A. J., Tischer, C. A., Iacomini, M. (1999). The free reducing oligosaccharides of angico branco (*Anadenanthera colubrina*) gum exudates: an aid for structural assignments in the heteropolysaccharide. *Carbohydrate Research*, 320, 167-175.

Dhainaut, J. F., Marin, N., Mignon, A., Vinsonneau, C. (2001). Hepatic response to sepsis: interaction between coagulation and inflammatory processes. *Critical Care Medicine*. 29, 42-47.

- Diaz-Gerevini, G. T., Repossi, G., Dain, A., Tarres, M. C., Das, U. N., Eynard, A. R. (2016). Beneficial action of resveratrol: How and why?. *Nutrition*, 2, 174-178.
- Doco, T., Brillouet, J. M. (1993). Isolation and characterization of a rhamnogalacturonan II from red wine. *Carbohydrate Research*, 243, 333-343.
- Doco, T., Quéllec, N., Moutounet, M., Pellerin, P. (1999). Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of Enology and Viticulture*, 50, 28-32.
- Doco, T., Vuchot, P., Cheynier, V., Moutounet, M. (2003). Structural modification of arabinogalactan-proteins during aging of red wines on lees. *American Journal of Enology and Viticulture*, 54, 150-157.
- Doco, T., Williams, P., & Cheynier, V. (2007). Effect of flash release and pectinolytic enzyme treatments on wine polysaccharide composition. *Journal of Agricultural and Food Chemistry*, 55(16), 6643–6649.
- Feverly, J. (2008). Bilirubin in clinical practice: a review. *Liver International*. 28, 592-605.
- Filisetti-Cozzi, T. M. C. C., Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry*, 197, 157-162.
- Fransen, C. T. M., Haseley, S. R., Huisman, M. M. H., Schols, H. A., Voragen, A. G. J., Kamerling, J. P., Vliegthart, J. F. G. (2000). Studies on the structure of a lithium-treated soybean pectin: characteristics of the fragments and determination of the carbohydrate substituents of galacturonic acid. *Carbohydrate Research*, 328, 539-547.
- Gojkovic-Bukarica, L., Markovic-Lipkovski, J., Heinle, H., Cirovic, S., Jovana Rajkovic, J., Djokic, V., Zivanovic, V., Ana Bukarica, A., Novakovic, R. (2018). The red wine polyphenol resveratrol induced relaxation of the isolated renal artery of diabetic rats: The role of potassium channels. *Journal of Functional Foods*, 52, 266–275.
- Gorin, P. A. J., Mazurek, M. (1975). Further studies on the assignment of signals in ^{13}C magnetic resonance spectra of aldoses and derived methyl glycosides. *Canadian Journal of Chemistry*, 53, 1212-1222.
- Gorin, P. A. J., Iacomini, M. (1984). Polysaccharides of the lichens *Cetraria islandica* and *Ramalina usnea*. *Carbohydrate Research*, 142, 119-132.
- Hooshmand, S., Kumar, A., Zhang, J. Y., Johnson, S. A., Chai, S. C., & Arjmandi, B. H. (2015). Evidence for anti-inflammatory and antioxidative properties of dried plum polyphenols in macrophage RAW 264.7 cells. *Food Funct*, 6(5), 1719-1725.
- Hotchkiss R.S., Karl, I. E. (2003). The pathophysiology and treatment of sepsis. *New England Journal of Medicine*, 348:138.

Huang, W., Liu, X., Liu, X. Y., Lu, Y., Li, Y., Zhang, Y. P., Kuang, Z. M., Cao, D., Chen, A.F., Yuan, H. (2015). Value of neutrophil counts in predicting surgery-related acute kidney injury and the interaction of these counts with diabetes in chronic kidney disease patients with hypertension. *Medicine*, 94, 1–7.

Inngjerdingen, K. T., Thöle, C., Diallo, D., Paulsen, B. S. Hensel, A. (2014). Inhibition of *Helicobacter pylori* adhesion to human gastric adenocarcinoma epithelial cells by aqueous extracts and pectic polysaccharides from the roots of *Cochlospermum tinctorium* A. Rich. And *Vernonia kotschyana* Sch. Bip. Ex Walp. *Fitoterapia*, 95, 127–132.

Jones, J. K. N., Stoodley, R. J. (1965). Fractionation using copper complexes. *Methods in Carbohydrate Chemistry*, 5, 36–38.

Kobayashi, H., Watanabe, M., Komido, M., Matsuda, K., Ikeda-Hasebe, K., Suzuki, M., Shibata, N., Hisamichi, K., Suzuki, S. (1995). Assignment of ¹H and ¹³C NMR chemical shifts of a D-mannan composed of α -(1→2) and α -(1→6) linkages obtained from *Candida kefyr* IFO 0586 strain. *Carbohydrate Research*, 267, 299–306.

Landry, D. W., Oliver, J. A.. (2001). The pathogenesis of vasodilatory shock. *The New England Journal of Medicine*, 345, 588–595.

Lapa, F. R., Silva, M. D., Almeida Cabrini, D., & Santos, A. R. (2012). Anti-inflammatory effects of purine nucleosides, adenosine and inosine, in a mouse model of pleurisy: Evidence for the role of adenosine A (2) receptors. *Purinergic Signal*, 8, 693–704.

Lim, D. S., Bae, K.G., Jung, I. S., Kim, C.H, Yun, Y.S., Song, J.Y. (2002). Anti-septicaemic effect of polysaccharide from *Panax ginseng* by macrophage activation, *Journal of Infection Prevention*, 45, 32–38.

Longhi-Balbinot, D. T., Lanznaster, D., Baggio, C. H., Silva, M. D., Cabrera, C. H., Facundo, V. A., et al. (2012). Anti-inflammatory effect of triterpene 3 β , 6 β , 16 β -trihydroxylup-20(29)-ene obtained from *Combretum leprosum* Mart & Eich in mice. *Journal of Ethnopharmacology*, 26, 59–64.

Martínez-Lapuente, L., Guadalupe, Z., Ayestaran, B., Ortega-Heras, M., Perez-Magarino, S. (2013). Changes in polysaccharide composition during sparkling wine marking and aging. *Journal Agricultural and Food Chemistry*, 61, 12362–12373.

Mellinger, C. G., Cipriani, T. R., Noleto, G. R., Carbonero, E. R., Oliveira, M. B. M., Gorin, P., Iacomini, M. (2008). Chemical and immunological modifications of an arabinogalactan present in tea preparations of *Phyllanthus niruri* after treatment with gastric fluid. *International Journal of Biological Macromolecules*, 43, 115–120.

Mueller, E. A., & Anderer, F. A. (1990). Synergistic action of a plant rhamnogalacturonan enhancing antitumor cytotoxicity of human natural killer and lymphokine-activated killer cells: Chemical specificity of target cell recognition. *Cancer Research*, 50(12), 3646–3651.

Nascimento, A. M., De Souza, L. M., Baggio, C. H., Werner, M. F. D. P., Maria-Ferreira, D., Da Silva, L. M., Cipriani, T. R. (2013). Gastroprotective effect and structure of a rhamnogalacturonan from *Acmella oleracea*. *Phytochemistry*, 85, 137–142.

Nieuwenhuijzen, G. A., Deitch, E. A., Goris, R. J. (1996). Infection, the gut and the development of the multiple organ dysfunction syndrome. *European Journal of Surgery*. 162, 259–273.

Otero-Anton, E., Gonzalez-Quintela, A., Lopez-Soto, A., Lopez-Bem, S., Llovo, J., & Pérez, L. F. (2001). Cecal ligation and puncture as a model of sepsis in the rat: Influence of the puncture size on mortality, bacteremia, endotoxemia and tumor necrosis factor alpha levels. *European Surgical Research*, 33, 77–79.

Overhaus, M., Togel, S., Pezzone, M. A., Bauer, A.J., (2004). Mechanisms of polymicrobial sepsis-induced ileus. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 287, G685–694.

Ovodova, R. G., Golovchenko, V. V., Popov, S. V., Popova, G. Y., Paderin, N. M., Shashkov, A. S., Ovodov, Y. S. (2009). Chemical composition and anti-inflammatory activity of pectic polysaccharide isolated from celery stalks. *Food Chemistry*, 114, 610-615.

Pacher, P., Beckman, J. S., Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological Review*; 87, 315–424.

Pan, M. H., Lin-Shiau, S. Y., Lin, J. K. (2000). Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of I kappa B kinase and NF kappa B activation in macrophages. *Biochemical Pharmacology* 60, 1665–1676.

Park, S. N., Noh, K. T., Jeong, Y.-I., Jung, I. D., Kang, H. K., Cha, G. S., Park, Y.-M. (2013). Rhamnogalacturonan II is a Toll-like receptor 4 agonist that inhibits tumor growth by activating dendritic cell-mediated CD8⁺ T cells. *Experimental & Molecular Medicine*, 45(2), e8.

Pellerin, P., Doco, T., Vidal, S., Williams, P., Brillouet, J. M., O'Neill, M. A. (1996). Structural characterization of red wine rhamnogalacturonan II. *Carbohydrate Research*, 190, 183-197.

Pellerin, P., Cabanis, J-C. (1998). Les glucides. In *nologie: fondements scientifiques et technologiques*. Lavoisier, Tech. & Doc, France: Paris, 40-92.

Popov, S. V., Ovodova, R. G., Golovchenko, V. V., Pupova, G. Y., Viatyasev, F. V., Shashkov, A. S., Ovodov, Y. S. (2011). Chemical composition and anti-inflammatory activity of a pectic polysaccharide isolated from sweet pepper using a simulated gastric medium. *Food Chemistry*, 124, 309-315.

Quijada-Morín, N., Williams, P., Rivas-Gonzalo, J. C., Doco, T., Escribano-Bailón. M. T. (2014). Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. *Food Chemistry*, 154, 44–51.

Renard, C. M. G. C., Lahaye, M., Mutter, M., Voragen, F. G. J., Thibault, J. F. (1998) Isolation and structural characterization of rhamnogalacturonan oligomers generated by controlled acid hydrolysis of sugar-beet pulp. *Carbohydrate Research*, 305, 271-280.

Rewa, O., Bagshaw S. M. (2014). Acute kidney injury-epidemiology, outcomes and economics, *Nature Reviews Nephrology*. 10, 193–207.

Rittirsch, D., Huber-Lang, M. S., Flierl, M. A., Ward, P. A., (2009). Immunodesign of experimental sepsis by cecal ligation and puncture. *Nature Protocols*. 4, 31–36.

Ruthes, A. C., Komura, D. L., Carbonero, E. R., Sassaki, G. L., Gorin, P. A. J., & Iacomini, M. (2010). Structural characterization of the uncommon polysaccharides obtained from *Peltigera canina* photobiont *Nostoc muscorum*. *Carbohydrate Polymers*, 81, 29–34.

Ruthes, A. C., Rattmann, Y. D., Carbonero, E. R., Gorin, A. J., & Iacomini, M. (2012). Structural characterization and protective effect against murine sepsis of fucogalactans from *Agaricus bisporus* and *Lactarius rufus*. *Carbohydrate Polymers*, 87, 1620–1627.

Sasaki, G. L., Elli, S., Rudd, T. R., Macchi, E., Yates, E. A., Naggi, A. (2013). Human (α -2,6) and avian (α -2,3) sialylated receptors of influenza A virus show distinct conformations and dynamics in solution. *Biochemistry*, 52(41), 7217–7230.

Scoparo, C. T., Souza, L. M., Rattmann Y. D., Dartora, N., Paiva, S. M. M., Sassaki, G. L., Gorin, P. A. J., Iacomini, M. (2013). Polysaccharides from green and black teas and their protective effect against murine sepsis. *Food Research International*, 53, 780–785.

Silva, M. D., Guginski, G., Werner, M. F. P., Baggio, C. H., Marcon, R., & Santos, A. R. (2012). (2011). Involvement of interleukin-10 in the anti-inflammatory effect of sanyinjiao (SP6) acupuncture in a mouse model of peritonitis. *Evidence-Based and Complementary and Alternative Medicine*, 217946.

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J. D., Coopersmith, C. M., Hotchkiss, R. S., Levy, M. M., Marshall, J. C., Martin, G. S., Opal, S. M., Rubenfeld, G. D., Poll, T., Vincent, J. L., Angus, D. C. (2016). The sepsis definitions task force the third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315, 801–810.

Stearns-Kurosawa, D. J., Osuchowski, M.F., Valentine, C., Kurosawa, S., Remick, D. G. (2011). The pathogenesis of sepsis. *Annual Review of Pathology*. 6, 19e48.

Stipp, M. C., Bezerra, I. L., Corso, C. R., Livero, F. A. R., Lomba, L. A., Caillot, A. R. C., Zampronio, A. R., Queiroz-Telles, J. E., Klassen, G., Ramos, E. A. S., Sasaki, G. L., Acco, A. (2016). Necroptosis mediates the antineoplastic effects of the soluble fraction of polysaccharide from red wine in Walker-256 tumor-bearing rats. *Carbohydrate Polymers*, 160, 123–133.

Tischer, C. A., Gorin, P. A. J., Iacomini, M. (2002). The free reducing oligosaccharides of gum arabic: aids for structural assignments in the polysaccharide. *Carbohydrate Polymers*, 47, 151-158.

Tzianabos, A. O., Kasper, D. L., Cisneiros, R. L., Smith, R. S., Onderdonk, A. B. (1995). Polysaccharide-mediated protection against abscess formation in experimental intra-abdominal sepsis. *Journal of Clinical Investigation*. 96, 2727–2731.

Vidal, S., Williams, P., Doco, T., Moutounet, M., Pellerin, P. (2003). The polysaccharides of red wine: Total fractionation and characterization. *Carbohydrate Polymers*, 54, 439-447.

Vinogradov E., Petersen B., Bock K. (1998). Structural analysis of the intact polysaccharide mannan from *Saccharomyces cerevisiae* yeast using ^1H and ^{13}C NMR spectroscopy at 750 MHz. *Carbohydrate Research*, 307, 177-183.

Wolf from, M. L., Thompson, A. (1963a). Acetylation. *Methods in Carbohydrate Chemistry*, 2, 211–215.

Wolf from, M. L., Thompson, A. (1963b). Reduction with sodium borohydride. *Methods in Carbohydrate Chemistry*, 2, 65–67.

Yamada, T., Ishikawa, S., Kataoka S., Uda, T., Iinuma, Y., Hattori, R., Yamada, T., Morikawa, M., Kaneuchi, M., Minakami, H. (2013). Coagulation fibrinolysis and laboratory characteristics of pregnant women with severely depressed antithrombin activity. *Hypertension in Pregnancy* 32, 235.

Yamashiro, S., Kamohara, H., Wang, J. M., Yang, D., Gong, W. H., Yoshimura, T. (2001). Phenotypic and functional change of cytokine activated neutrophils: inflammatory neutrophils

are heterogeneous and enhance adaptive immune response. *Journal of Leukocyte Biology*, 69, 698-704.

Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, 16, 109–110.

Supporting Information

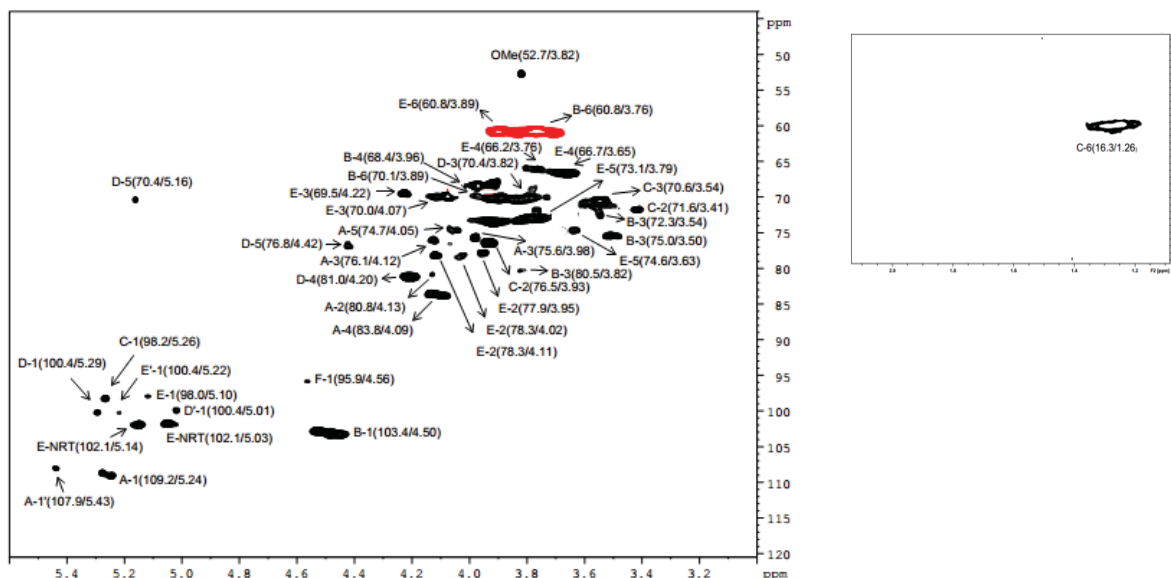


Figure S1: $^1\text{H}/^{13}\text{C}$ HSQC NMR spectrum of WCS. Solvent D_2O at 30 $^\circ\text{C}$; numerical values are in δ ppm. A (α -L-Araf); B (β -D-Galp); C (α -L-Rhap); D (α -D-GalpA); D' (6-OMe- α -D-GalpA), E (Manp); F (α -D-Glc). Letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed experiment.

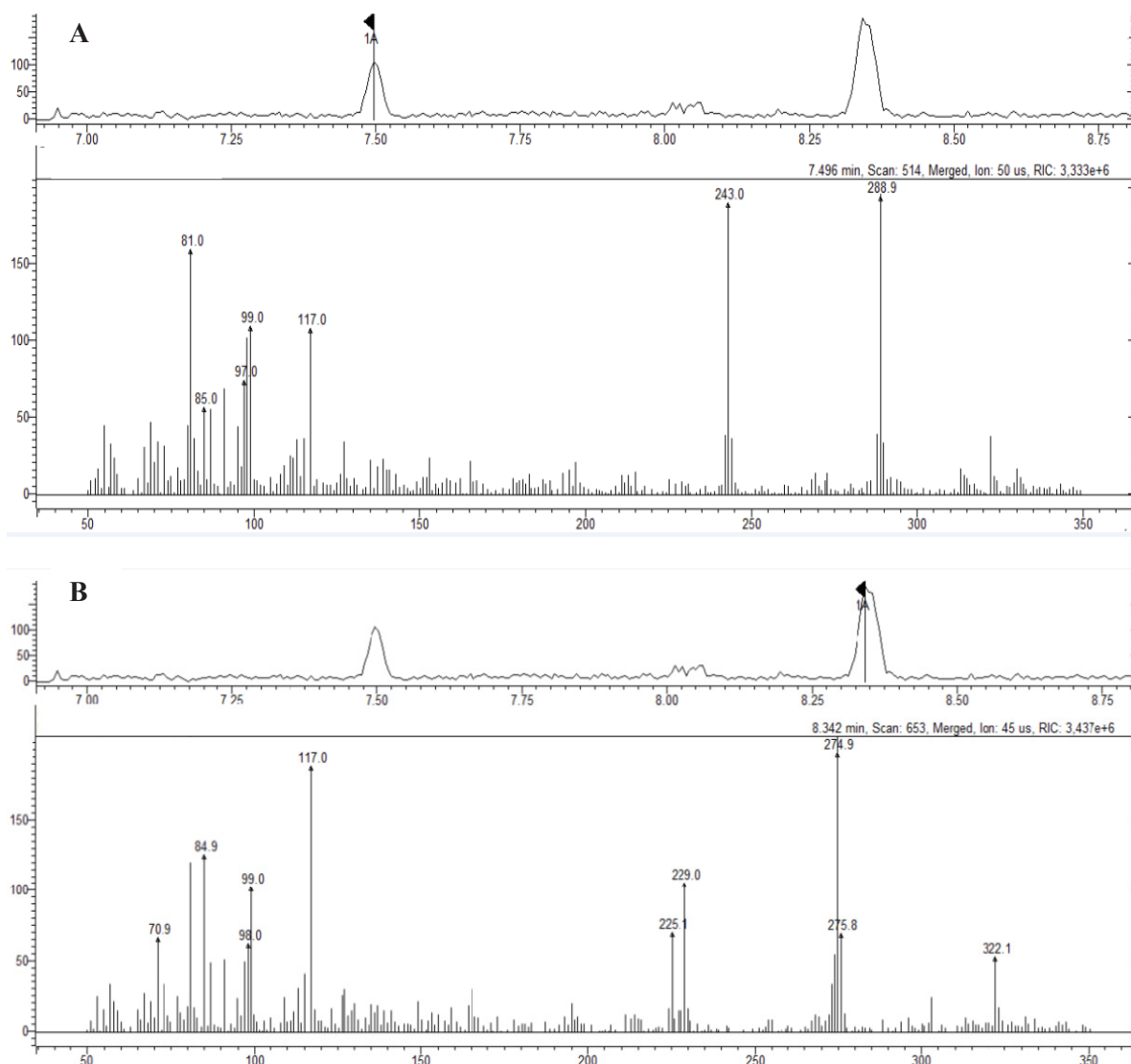


Figure S2: Chromatogram and mass spectra containing retention and fragmentation times from (A) 2-*O*-methyl-xylitol and those from (B) 2-*O*-methyl-fucitol. Identification of fragments at m/z .

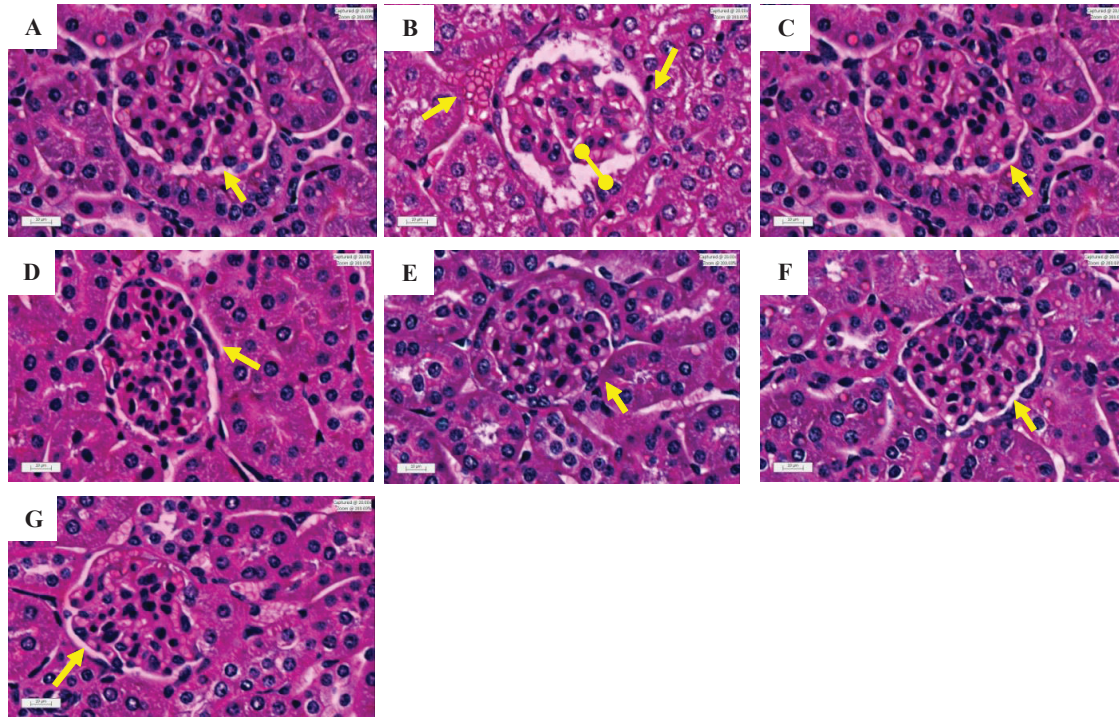


Fig. S3: Representative histological sections of kidney from mice in groups: sham (A), vehicle (B), CSS (C), CSO (D), FCS (E), PCS (F) and dexamethasone (G). Original magnification $\times 200$. H&E. Scale bar = 20 μ m.

CAPITULO IV

Este capítulo é composto pelo manuscrito “Biodistribution and anti-inflammatory activity of red wine polysaccharides: a sweet fluorescent trip in the mice body” que será submetido em uma revista.

Biodistribution and anti-inflammatory activity of red wine polysaccharides: a sweet fluorescent trip in the mice body

Iglesias de Lacerda Bezerra^a, Adriana Rute Cordeiro Caillot^a, Leociley Rocha Alencar Menezes^a, Rômulo dos Santos Cavalcante^b, Guilherme Lanzi Sassaki^{a*}

^a *Department of Biochemistry and Molecular Biology, Federal University of Parana, Curitiba, Paraná, 81.531-980, Brazil*

^b *Department of Morphology, Federal University of Rio Grande do Norte, Natal, RN, 59.064-741, Brazil*

Abstract

Polysaccharides are important components of wine, which show various biological activities, including anti-inflammatory, anti-tumor and immunomodulatory effects. However, no research has explored its absorption and biodistribution. This work developed a procedure for fluorescent labeling of wine polysaccharides, to evaluate their distribution *in vitro* and *in vivo*. It was demonstrated that the polysaccharide labeled with fluoresceinamine dye (WCF-F) exhibited high fluorescence without significant structural change. The migration and the anti-inflammatory effect of the labeled polymer on tissues and organs were observed following subcutaneous administration in mice after induction of sepsis by CLP. Moreover, the labeled polysaccharide showed no cytotoxicity in Raw 264.7 cells and it was observed the internalization of the polymer by macrophage as a function of time. This study showed that the wine polysaccharides demonstrated anti-inflammatory effect, the biodistribution by the body through the immune system of the mice.

1. Introduction

Cabernet Franc (*Vitis vinifera* L.) is considered as an ancient and traditional red wine grape variety. The chemical composition of grape allows them to ferment during vinification without the addition of sugars, acids, enzymes or other nutrients. The main macromolecules found in wine are polyphenols and polysaccharides. Polysaccharides come mainly from grapes and yeast during winemaking (Carpita & Gibeau, 1993; Martínez-Lapuente, Guadalupe, Ayestaran, Ortega-Heras & Perez Magarino, 2013; Pellerin & Cabanis, 1998). Bezerra et al. 2018 has already demonstrated the presence of mannan, type II arabinogalactan, type I and II rhamnogalacturonans from Carbernet Franc wine.

There is an increasing interest in knowing the benefits that moderate consumption of wine can bring to health. Studies show that polysaccharides, such as rhamnogalacturonan and arabinogalactan from various sources had an anti-inflammatory activity and were able to decrease mice lethality submitted to polymicrobial sepsis induced by Cecal Ligation and Puncture (CLP) (Bezerra et al., 2019; Cordeiro et al., 2012; Scoparo et al., 2013). The latter mimics sepsis in humans, caused by pathogens derived from the intestinal tract and is considered to closely simulate clinical situation (Rittirsch, Huber-Lang, Flierl, & Ward, 2009). Furthermore, polysaccharides from wine present various biological activities,

including anti-inflammatory, anti-tumor and immunomodulatory effects (Stipp 2017, Bezerra 2018; 2019; Codeiro-Caillot, et al., 2018).

However, these studies show that polysaccharides have beneficial health effects as an anti-inflammatory action, the mechanism of distribution of the molecule in the body are not clear. To date, fluorescence based techniques have been greatly encouraged in bioimaging due to their inherent advantages, such as high sensitivity, high selectivity, convenience and non-invasive character (Chen et al, 2014; Azhdarinia et al., 2012; Kikuchi, 2010). For examining the *in vitro* and *in vivo* biodistribution response, it is necessary to follow their cellular uptake and endocytosis. The internalization and penetration of the cells and tissues by polysaccharides can be efficiently monitored by fluorescent microscopy, if the polysaccharides are tagged with a stable fluorescent marker (Bae et al., 2012). The latter can offer a promising tool to obtain highly stable fluorescently labeled particles for an appreciable period of time (Abdel-Mottaleb et al., 205; Weiss et al., 2006; Dong et al., 2007). Thus, chemical derivatization of wine polysaccharides with fluorescent tags can make intracellular microscopic inspection possible.

Therefore, this work through this chemical derivatization it was possible to evaluate the biodistribution and the effect of polysaccharides extracted from Cabernet Franc wine during the inflammatory process *in vitro* through the cell uptake studies were carried out using Macrophage cell lines (RAW 264.7) by fluorescence microscopy to examine the dye uptake time and also *in vivo* through analysis of Cabernet Franc wine polysaccharides and their anti-inflammatory activity against sepsis, evaluating effects on lethality, leukocytes migration, anti- and pro-inflammatory cytokines levels, and biochemical, hematological and histopathological parameters and migration of fluorescent dye-labeled polysaccharides by the live animal organ by fluorescence camera.

2. Materials and methods

2.1. Materials

Cabernet Franc polysaccharides were extracted from commercial wine belonged to three vintages (2018, 2017 and 2016). The wine was made from *Vitis vinifera* cv. Cabernet Franc grapes grown in the Central Valley - Chile, semi-arid Mediterranean climate with strong influence of the Andes Mountain. Maturation of the wines of the three harvests was in

French oak barrels. The wines have pH 3.70%, total acidity 3.52 g/L, alcoholic content 13.0% and residual sugar 3.20 g/L. These oenological parameters were similar across the studied wines and were not included in the statistical data treatment.

2.2. Polysaccharides extraction

Polysaccharides were precipitated by addition of cold EtOH (3 vol.) and separated by centrifugation (8.000 rpm at 4 °C, 20 min). The sediment was dissolved in H₂O, dialyzed against distilled water with a 6–8 kDa cut-off membrane for 72 h to remove the remaining low-molecular weight compounds, giving rise to a mixture polysaccharide fraction: WCF (Cabernet Franc wine).

2.3. Labeling of WCF with fluoresceinamine

Sodium cyanoborohydride (300 mg) was added to a mixture of dimethyl sulphoxide (8.4 mL) and glacial acetic acid (3.6 mL). The reactant mixture was stirred for 30 min at room temperature. The Cabernet Franc wine polysaccharide mixture (140 mg) and Fluoresceinamine isomer 1 (70 mg) was added to the sodium borohydride cyanide solution. The mixture was stirred for 4 h at 400 rpm at room temperature. Then the reaction was continued at 75 °C for 8 h. The yellow colored product was collected by centrifugation at 4000 rpm for 10 min and washed with deionized distilled water to remove any free dye. The final yellow product was lyophilized using a freeze dryer at –50 °C under reduced pressure to produce cabernet Franc wine polysaccharide mixture labeled with fluoresceinamine (WCF-F) as powdered form.

2.4. Structural characterization

The structural features of WCF-F sample was examined by NMR, the spectra were obtained with a 600 MHz Bruker spectrometer using 5 mm inverse probe heads (QXI and N₂ cooled TCI) (Avance III and HD, Bruker, Billerica, Massachusetts, USA). 1D ¹H and ¹³C NMR at 600 MHz were acquired after a 90° (p1) pulse calibration at 10.75 μs. ¹H/¹³C chemical shift correlation mapping was finally determined by COSY, TOCSY HSQCed (Heteronuclear Single Quantum Coherence edited spectroscopy CH, CH₃ positive phase CH₂ negative phase) performed at 30 °C in deuterium oxide (D₂O) (Sasaki et al., 2014).

2D NMR experiments were conducted using quadrature detection at indirect dimension and acquired using 24 scans per series of 1024×320 data points, with zero filling in F1 (4096) prior to Fourier transformation, using the Software TOPSPIN version 3.2 pl6 (Bruker Biospin, Rheinstetten, Germany). The chemical shifts of the polysaccharide were expressed in δ (ppm) relative to trimethylsilyl propionic acid (TMSP). Diffusion measurement was carried out using stimulated echo and LED by bipolar gradient for diffusion and 2 spoil gradients. The spectrum was acquired using 8 scans per series of $8K \times 32$ data points and performed in a linear gradient of 32 steps 2-98%, diffusion time of 0.1 s and the length of the square diffusion encoding gradient pulses of 1 ms. 2D-DOSY spectrum was processed in exponential mode on Topspin 3.2 (Bruker BioSpin Corporation, Billerica, MA, USA). STD NMR experiments were performed at 303 K and 343 K. NMR samples were prepared in 0.5ml of 99.9% D₂O. The binding studies were prepared dissolving 70 mg fluoresceinamine in the polysaccharide solution to reach a 1:10 WCF/FLN molar ratio. 1D STD measurements were performed using the excitation sculpting with gradients for water suppression and 25 ms of $T_{1\rho}$ filter to suppress protein. The on-resonance irradiation was applied in up field carbohydrate resonances at H2-H6 of wine polysaccharides, and the off-resonance control irradiated at 40 ppm. The spectra were collected using a relaxation delay of 4 s to allow the ligands to relax between on- and off-resonance irradiation cycles and 2 s of saturation time. The total number of scans was 1024 and 16 dummy scans, using 16 ppm spectral widths.

The presence of fluoresceinamine of the WCF-F fraction was determined by high performance steric exclusion chromatography (HPSEC), using a refractive index (RI) detector and UV absorption at 495 nm of the fluoresceinamine. Four columns were used in series, with exclusion sizes of 7×10^6 Da (Ultrasphere 2000, Waters), 4×10^5 Da (Ultrasphere 500, Waters), 8×10^4 Da (Ultrasphere 250, Waters) and 5×10^3 Da (Ultrasphere 120, Waters). The eluent was 0.1 M NaNO₃, containing 0.5 g/L NaN₃. The solutions were filtered through a membrane of 0.22 μ m pore size (Millipore) and loaded (100 μ L, loop) at a concentration of 1 mg/mL. Furthermore, the interaction between wine polysaccharide and fluorescence was confirmed by ESI-MS.

2.5. Sepsis induction by cecal ligation and puncture (CLP)

The WCF-F fraction was tested in route of administration subcutaneous at doses of 0.1, 1 and 10 mg/kg. Mice were randomly divided into groups with 10 animals each: sham-operation, CLP plus saline (10 mL/kg s.c.), CLP plus WCF-F (0.1 mg/kg s.c.), CLP plus

WCF-F (1 mg/kg s.c.), CLP plus WCF-F (10 mg/kg s.c.), CLP plus FLN (1 mg/kg s.c.), and CLP plus dexamethasone - Dex (0.5 mg/kg s.c.). Saline was used as vehicle for dissolving the polysaccharide and dexamethasone was commercially purchased.

Ketamine (80 mg/kg) and xylazine (20 mg/kg) were injected intraperitoneally to anesthetize the mice before surgical procedures. Polymicrobial sepsis was induced by CLP as previously described (Rittirsch, Huber-Lang, Flierl, & Ward, 2009). This model mimics the human sepsis caused by pathogens derived from the intestinal tract and simulates a clinical situation (Otero-Anton et al., 2001). A midline incision of ~1.5 cm was carried out on the abdomen. The cecum was carefully exposed and 50% of the distal moiety was ligated. The cecum was then punctured thrice with a sterile 16-gauge needle and squeezed to extrude the fecal material from the wounds. The cecum was replaced and the abdomen was stitched surgically. Sham-operation animals were treated identically, but no cecal ligation or puncture was carried out. Each mice received a subcutaneous sterile saline injection (1 mL) for fluid resuscitation after surgery. The mice were then kept on a heating pad (35 °C) until they recovered from the anesthesia. Food and water, *ad libitum*, were provided throughout the experiment. The survival rate was monitored for 7 days, every 12 h. During this period, water (vehicle) and drugs were subcutaneously administered daily. Dexamethasone was commercially purchased and subcutaneously administered at doses of 0.5 mg/kg (Lapa, Silva, Almeida Cabrini, & Santos, 2012; Longhi-Balbinot et al., 2012; Silva et al., 2012).

In this experimental set, mice were subcutaneously treated with vehicle (1 h before surgery), sham-operation, CLP plus saline (10 mL/kg s.c.), FLN (1 mg/kg, s.c.) and dexamethasone (0.5 mg/kg s.c.). The best dose of WCF-F fraction in the survival test was chosen for the anti-inflammatory evaluation tests, that was 1 mg/kg, s.c. For these doses, after a post-operation time of 6 h, mice were sacrificed. Liver, lung and kidney tissues were collected for histological analysis. Blood was collected for hematological and biochemical parameters evaluation.

2.6. Hematological assays

Blood was collected from the abdominal vena cava. Total leukocytes and mononuclear (MN) and polymorphonuclear (PMN) leukocytes were counted by an automated system, according to the manufacturer's instructions (Mindray BC-2800 vet, São Paulo, SP, Brazil).

2.7. Biochemical assays

The amount of TNF- α , IL-1 β and IL-10 cytokines in the blood plasma were determined by enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. Three replicates were carried out for each different treatment. The absorbance of each group was measured by a microplate reader at the wavelength of 450 nm. To determine the total concentration of nitric oxide (NO) in the blood plasma, Griess reagent was added to 40 μ L of plasma and the absorbance at 545 nm was evaluated with an ELISA kit (BD Biosciences, San Jose, CA, USA) following the manufacturer's manual.

Bilirubin, creatinine (CREA) and urea, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and activity were analyzed from plasma by an automated system, according to the manufacturer's instructions (Mindray BS-200, São Paulo, SP, Brazil).

2.8. Histopathological analysis

Lungs, liver and kidney tissue sections were fixed in ethanol at room temperature. After fixation, samples were dehydrated in a graded series of increasing ethanol before paraffin embedding. Thin sections (4 μ m) from paraffin blocks were processed for histology, and the tissue was stained with hematoxylin eosin. The slides were then analyzed by light microscopy.

2.9. Cell culture

RAW 264.7 macrophages were maintained at 37 °C in humidified atmosphere of 5% CO₂ in DMEM medium supplemented with 10% fetal bovine serum and penicillin/streptomycin/normocin (50 U/mL, 50 g/mL and 100 g/mL, respectively; InvivoGen, San Diego, CA, USA). Exponential phase cells were used throughout the experiments.

2.10. Analysis of cell viability

RAW 264.7 cells in exponential growth phase were seeded in 24-well plates at a density of 4.8×10^5 cells/well. The WCF-F and FLN (only fluoresceinamine) were added at

indicated concentrations (0.1, 1, 10 and 100 µg/mL). The mitochondrial-dependent reduction of 3- (4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan was used to measure cell respiration as an indicator of cell viability (Mosmann, 1983). Briefly, after 24 h incubation with or without polysaccharides, 350 µL/well of MTT solution (1 mg/mL) was added and the cells were incubated for another 4 h at 37 °C. After removing the supernatant, 500 µL of DMSO (dimethyl sulfoxide) was added to the cells to dissolve the formazan. The absorbance of each group was measured by a microplate reader at wavelength of 570 nm. The control group consisted of untreated cells was considered as 100% of viable cells. Results were expressed as percentage of viable cells when compared with control group.

2.11. Macrophage internalization of WCF-F

The internalization of WCF-F and FLN by macrophages (Raw 264.7 cells) was analyzed by fluorescence microscopy. To this end, 2×10^5 cells were plated under glass coverslips (1.3 mm) in a 24-well plate. After complete adhesion, Raw 264.7 cells were treated with LPS (2 µg/mL) and maintained for 24 h. Successive PBS washes were made, and only then were the labeled polysaccharides added (100 µg/mL) and analyzed at different time intervals (0, 6, 24 and 48 h). At each end point the cells were washed twice with PBS and then fixed with 4 % buffered paraformaldehyde. Subsequently the coverslips were removed and rested in mounting medium containing DAPI on glass slides to counterstain the cell nuclei. Images were captured under a fluorescence microscope (Axio Observer Z1, ZEISS, Oberkochen, Germany) and processed with ZEN blue software (ZEISS). The tests were performed in triplicate and 5 random fields were analyzed, from which the average fluorescence intensity was obtained for graphing.

2.12. Imaging of Multi-wavelength fluorescence

To investigate the distribution of WCF-F during the inflammation process, we induced sepsis with the same method given in section 2.5. Then all groups were treated subcutaneously: Mice were randomly divided into groups with 10 animals each: sham-operation, CLP plus WCF-F (1 mg/kg s.c.) and CLP plus FLN (1 mg/kg s.c.). After treatment, the mice were evaluated after 1h, 3h and 6h by monochrome interlined, fixed lens (10x), CCD camera (13.8 x 13.8 cm / 2048 x 2048 px, 67 µmpx, 16 bit). The Imaging

Modalities of multi-wavelength fluorescence. Computer command center running Windows 7 Pro with Carestream MI acquisition and analysis software.

In addition, we also analyzed the presence WCF-F in tissues after 6h of treatment. The tissues (liver, kidney and lung) were fixed with OCT and then cooled to -80 ° C. Thin sections (1 mm) from blocks were processed with saponin 0.01% + DAPI (1:1000) for 40 min and then mounting the histology slides. The slides were then analyzed by confocal microscopy.

2.13. Statistical analysis

The results were expressed as mean $\pm$ SEM and were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's test as a post hoc test ($P < 0.001$). GraphPad Prism 5.0 (La Jolla, CA) program was used for both statistical analysis and figure design. Histological analysis was performed by qualitative observations.

3. Results

3.1. Structural characteristics of fluorescent labeled WCF-F

In order to understand how high molecular weight molecules are acting in the body during the inflammatory process, we labeled the molecule with fluoresceinamine (fig. 1a). The aldehyde group of terminal reducing ends form a schiff base in acidic medium and reductive ammination stabilize the product. In order to confirm the obtainment of the labeled product and exclude free residual fluoresceinamine, we performed ESI-MS in positive and negative ion modes. The MS spectra did not show the presence of expected ions at of free fluoresceinamine at m/z 348 and 346. The 2D DOSY spectrum of WCF-F corroborates with ESI-MS data, since there is no signal of fast tumbling molecules in the spectrum (fig. 1f). Despite that, extracting the spectrum of 2% and 98% of gradient we saw that both only have broad peaks and high molecular weight polysaccharides together with the fluorescent component. Combining all the spectroscopic NMR and MS data, plus the results of the high performance steric exclusion chromatography of the original and modified polysaccharide with fluoresceinamine which absorbed UV at 495 nm, shown in green in the figure (fig.1b-c), we conclude the polysaccharide labeling and the absence of free fluoresceinamine.

However it is important to notice that fluoresceinamine interacts with the polysaccharides, since it has positive signals on STD at 303 K. However, increasing the temperature to 343 K the exchange ratio of free and bound states are changed and the free state is predominant and no one signal is observed on STD spectrum (fig. 1d-e).

In addition, The $^1\text{H}/^{13}\text{C}$ HSQC spectrum of WCF-F shows in addition to typical signs in the anomeric and binding region of the polysaccharide mixture present of the Cabernet Franc wine, which are type II arabinogalactan, rhamnogalacturonans, mannose and dextrin which have been previously characterized by our group (Bezerra et al., 2018), also the presence of three signals δ 102.8/6.65, 111.3/6.58 and 131.2/7.00 (Kagairan et al., 2010), confirming the presence of linked fluoresceinamine in this fraction (fig. 1g).

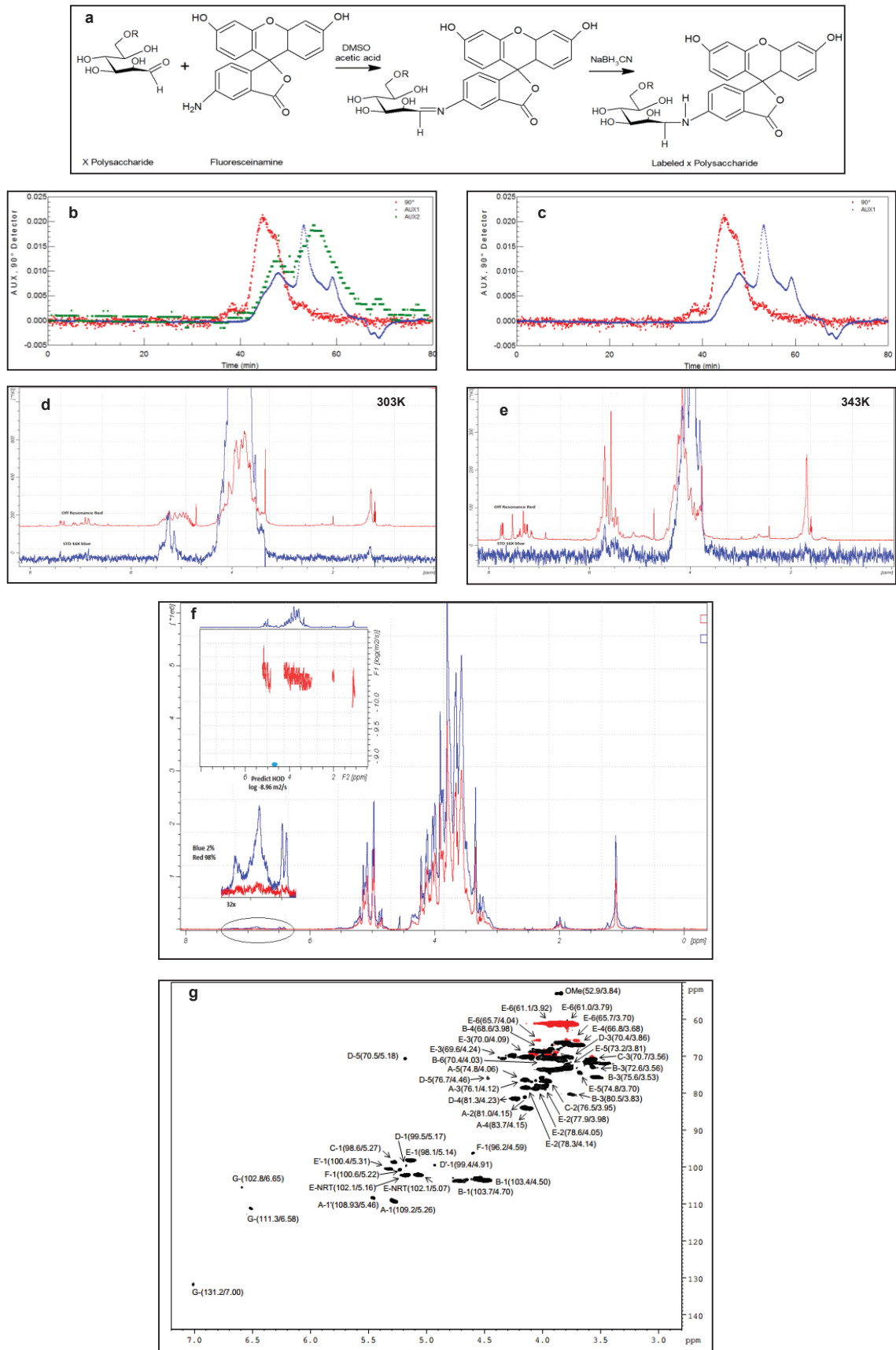


Figure 1: Structural characterization of fluorescence-labeled WCF-F. (a) Reaction of WCF with fluoresceinamine. (b) WCF-F and WCF (c) fractions determined by HPSEC using refractive index detector, λ at 495 nm (green). STD spectrum of WCF-F at 303 K (d) and 343 K (e). (f) 2D DOSY spectrum with 2% and 98% of gradient of WCF-F in D₂O at 30 °C (chemical shifts are expressed in δ ppm). (f) ¹H/¹³C HSQC NMR spectrum of WCF-F. Solvent D₂O at 30 °C; numerical values are in δ ppm. A (α -L-Araf); B (β -D-Galp); C (α -L-Rhap); D (α -D-GalpA); E (Manp); F (β -D-Glcp); G (fluoresceinamine). The letters are followed by the carbon number of the monosaccharide unit. Chemical shifts were determined by HSQCed, COSY, TOCSY and HMBC experiments.

3.2. Anti-inflammatory effect of wine polysaccharides

We treated subcutaneously the animals with WCF-F to evaluate the protective effect of wine consumption to show the efficiency of the polysaccharides as anti-inflammatory and protective in an aggressive model of sepsis by CLP. We investigated the effect of Cabernet Franc wine polysaccharides on survival of septic mice *in vivo*, as well as important hematological, biochemical and histopathological parameters related to organ dysfunction in sepsis. Here, it is suggested that polysaccharides may exert anti-inflammatory action that may contribute to the benefits of wine consumption.

Lethality was found to be markedly reduced in mice receiving subcutaneously treated with WCF-F, this fraction prevents lethality caused by pocymicrobial sepsis in mice (fig. S1). The corresponding areas under the lethality curve increased to 11.650, 12.890 and 12.900 (arbitrary units) after administrating 0.1, 1 and 10 mg/kg doses, respectively. At the end, the corresponding overall survival rates were 51%, 60% and 65%. The 1 and 10 mg/kg doses presented the same effect without statistical difference. The 1 mg/kg WCF-F dose was established for the analysis of this fraction effects in CLP.

Sepsis by CLP promotes migration of inflammatory cells and of leukocytes through the bloodstream to the site of infection, peritoneal cavity and affected organs such as lung, liver and kidneys (Bone et al., 1992). This study demonstrated that WCF-F was able to reduce by 63% white blood cell (leukocyte count), when compared to the vehicle group, being similar to dexamethasone, whose reduction was 56% (table 1) Differential leukocyte count demonstrated a decrease in the mononuclear leukocyte subset and PMN in the bloodstream (table 1). Mononuclear leukocyte subset reduction consisted in 64% and 62%

for WCF-F and dexamethasone, respectively. For de PMN subset, these reductions were 56% and 54% (WCF-F and dexamethasone, respectively), when compared to the vehicle group. This exacerbated inflammatory response was observed on the vehicle group, in which there was an increased number of leukocytes, mononuclear leukocytes and PMN, whereas all fractions tested were able to reduce these effects significantly, reducing the amplification of the inflammatory response.

Table 1

Hematological parameters evaluated during sepsis in mice

Treatment	WBC	PMN	MN
Sham	3384.2 ± 221.3	1560.2 ± 200.3	1700.0 ± 100.5
Vehicle	9408.1 ± 174.5***	3910.3 ± 211.4***	5450.4 ± 240.3***
FLN	9356.2 ± 126.3***	3857.4 ± 206.6***	5350.0 ± 159.5***
WCF-F	3452.8 ± 182.4	1740.6 ± 222.5	1980.9 ± 141.6
Dex	4149.1 ± 169.6	1800.3 ± 212.9	2106.8 ± 251.0

***p < 0.001 when vehicle was compared with all groups; ANOVA followed by the Tukey's test. WBC: White blood cell count; PMN: polymorphonuclear leukocytes; MN: mononuclear leukocytes; Dex: dexamethasone.

Neutrophils are the first cells to migrate to the site of infection, where they produce both reactive oxygen and nitrogen species that function to kill the infectious agents. These neutrophils and tissues resident cells also produce inflammatory cytokines that can recruit and activate other inflammatory cells that, in turn, amplify and extend the initial systemic inflammatory response (Yamashiro et al, 2001). This systemic inflammatory response was observed on the vehicle group, where there was an increase in the number of leukocytes mononuclear leukocyte and PMN, whereas the WCF-F fraction was able to reduce this effect significantly, avoiding the amplification of the inflammatory response.

In response to sepsis, macrophages synthesize pro-inflammatory cytokines, such as TNF- α and IL-1 β , and anti-inflammatory cytokines, such as IL-10 (Pan, Lin-Shiau & Lin, 2000). These inflammatory mediators generated by macrophages, induce the immune cells recruitment, mainly neutrophils (Overhaus et al., 2004; Pan, Lin-Shiau & Lin, 2000). Neutrophils damage tissue directly by releasing proinflammatory mediators, such as NO,

which amplify the systemic inflammatory response and contribute to multiple organ failure (Landry & Oliver, 2001; Nieuwenhuijzen et al., 1996). This study shows that WCF-F and dexamethasone significantly decreased the production of the pro-inflammatory cytokines TNF- α (53% and 52%, respectively) and IL-1 β (52% and 51%, respectively) and increased the level of the anti-inflammatory cytokine IL-10 (3x for all fractions) when compared to the vehicle group (Fig. 2 a-c). In addition, production of excessive NO has been associated with a range of inflammatory diseases, including septic shock (Pacher, Beckman & Liaudet, 2007). As shown in figure 2d, treatment with WCF-F fraction and dexamethasone induced a significant inhibitory effect in NO production (59% and 58%, respectively), indicating anti-inflammatory properties. Reduction of the effects of pro-inflammatory mediators and increased levels of the anti-inflammatory mediator suggests that the all fractions exerted anti-inflammatory effect in sepsis.

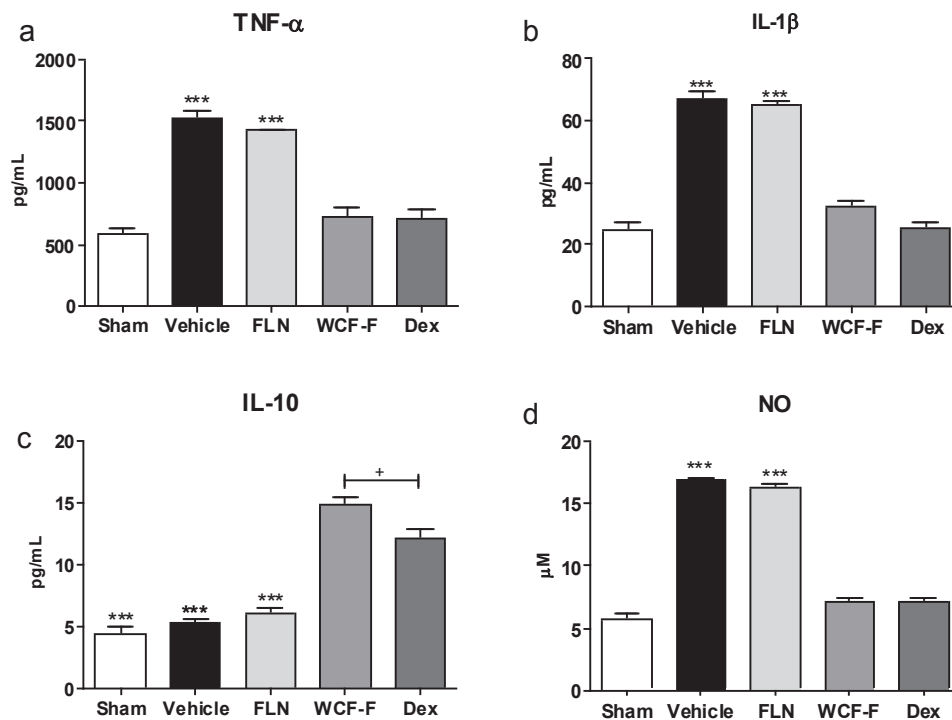


Figure 2: Expression levels of TNF- α . (a), IL-1 β (b), IL-10 (c), and NO (d) production levels. Dex (dexamethasone). ***p < 0.001 when vehicle was compared with all groups; + p < 0,05 comparison between fractions. ANOVA followed by the Tukey's test.

In sepsis, progressive deterioration of the function of various organs, starting mostly with lung which are followed by failure of the liver and kidneys, and/or circulation (Bau et al., 1998). During sepsis, hepatocytes modify metabolic pathways, increase gluconeogenesis, ensure cellular repair, increase the synthesis and release of coagulant factors, and enzymes such as transaminases (AST and ALT) and change in bilirubin levels (Dhainau et al., 2001). In the present study, we were directly addressed this possibility by analyzing several parameters related to liver function. Histologic analysis was stained with hematoxylin and eosin demonstrated hepatic alterations in the vehicle group (Fig. 3). The lesions were characterized by a reduction in sinusoids within the lumen, indicating tumefaction, and capsular infiltration of inflammatory cells (Fig. 3 a). These lesions were found in septic group and could be prevented by treatment with WCF-F fraction, which was observed to reduce these alterations, showing that this fraction was able to preserve the liver, similar to dexamethasone (Fig. 3 d-e).

Both ALT and AST transaminases are markers of hepatocyte injuries, so it had also evaluated them in this study. An increase in plasma AST and ALT activity in septic group, this increase can be reversed by treatment WCF-F and dexamethasone, which showed a significant reduction of ALT in the blood in 63% and 60%, respectively, compared to the vehicles group (table 2). Although ALT is an enzyme present in the hepatocyte cytoplasm, AST is present in the mitochondria and to the cytoplasm. Moreover, AST has activity in the liver, heart, kidneys, pancreas, muscles, and erythrocytes. Therefore, the increased level of plasma AST can indicate the occurrence of major damage to the hepatocyte mitochondria or significant damage to a different organ. Thus, AST levels are higher than ALT in the vehicle group, but were not affected in the groups treated with WCF-F and dexamethasone, which were able to maintain the low of the two enzymes. When compared to the vehicle group, it was observed the reduction in AST levels by 58% for WCF-F and 52% for dexamethasone.

Also, the bilirubin has long been recognized as an indicator of liver dysfunction, the severe elevations of unconjugated bilirubin cause damage to brain and erythrocytes (Feverly, 2008; Brito et al., 2006). This study also evaluated liver function taking into account the levels of bilirubin. It was observed an increased seric level of bilirubin in the septic group, which was in accordance with the other results already shown. The treatments WCF-F and dexamethasone, showed a significant reduction of bilirubin seric, in relation to septic group (table 2). All treatments with fractions and dexamethasone showed a significant reduction of bilirubin serum in relation to septic group, consisting in 69% (WCF-F) and 68%

(dexamethasone). These results showed that WCF-F was effective in protecting the liver and maintaining liver functions during sepsis.

Table 2

Plasmatic parameters evaluate during sepsis in mice

Treatment	AST	ALT	Bilirubin	Urea	Creatinine
Sham	132.7 ± 22.5	74.2 ± 4.9	1.0 ± 0.1	61.1 ± 8.3	0.3 ± 0.1
Vehicle	462.4 ± 19.8***	250.2 ± 15.3***	3.8 ± 0.2***	210.2 ± 8.8***	1.4 ± 0.1***
FLN	438.5 ± 12.8***	246.0 ± 7.6***	3.6 ± 0.2***	208.9 ± 6.4***	1.4 ± 0.1***
WCF-F	143.4 ± 12.7	93.6 ± 8.5	1.2 ± 0.3	79.4 ± 7.6	0.4 ± 0.1
Dex	158.2 ± 11.2	102.0 ± 3.2	1.2 ± 0.2	80.8 ± 5.2	0.5 ± 0.1

***p < 0.001 when vehicle was compared with all groups; There was no statistical difference between the treated groups. ANOVA followed by the Tukey's test. WBC: White blood cell count; Dex: dexamethasone.

During sepsis, acute lung injury can be caused directly by bacterial damage or secondarily by the systemic inflammatory response of sepsis (Chen et al., 2016). The activated polymorphonuclear leukocytes play an important role in mediating microvascular damage and lung tissue injury. PMN leukocytes infiltrate the lung tissue and their persistence in the lungs is an important determinant of poor survival (Bhatia & Moochhala, 2004). Therefore, we performed histological analysis of the lungs of the treated and untreated mice after CLP (Fig. 3 f-j). Vehicle group showed characteristics of acute alveolar damage and inflammation, such as congestion, accumulation of neutrophils (Fig. 3 f) and markedly thickened alveolar walls. WCF-F and dexamethasone groups showed reduced sepsis-induced damage and even normal lung histological feature (Fig. 3 i-j). This study showed that the wine polysaccharides significantly reduced the PMN number in blood and this may have reduced leukocyte infiltration in tissues and protected against lung injury.

In addition to the liver and lung, the kidney is also an important affected organ in sepsis. The acute kidney injury (AKI) is a common complication of sepsis (Rewa et al., 2014). Kidney infiltration by neutrophils and the subsequent release of free radicals is one major mechanism leading to AKI (Huang et al, 2015). Therefore, we evaluated the ability of WCF-F to have a protective effect on the kidneys through the analysis of creatinine and urea,

which are markers of renal function and histological analysis (Fig. 3 k-o). There was a significant increase in serum creatinine and urea levels in mice in vehicle group, this increase could be reversed by WCF-F and dexamethasone treatment, which showed a significant reduction of serum levels of these markers (table 2). For both there was a reduction of 71% (WCF-F) and 70% (dexamethasone) for creatinine level, while for urea levels, these observed reductions were 62% for WCF-F and dexamethasone, in relation to the vehicle group. Renal tubular dilatation, bleeding, leukocyte infiltrate and tubular epithelial cell vacuolar degeneration with presence of necrosis, Bowman's capsule and proximal convoluted tubule retraction retracted with degeneration were observed in the vehicle group (Fig. 3 k). The pretreatment with WCF-F fraction effectively improved these kidney histopathological changes in mice with sepsis, as well as dexamethasone (Fig. 3 n-o). WCF-F was able to preserve the kidneys during sepsis and seems to have helped in maintaining kidney function.

Taking into account all data presented, we were able to show that pretreatment with WCF-F fraction prevented the lethality caused by polymicrobial sepsis in mice. This beneficial effect seems to be, at least in part, due to a reduction in leukocyte migration, exerting an anti-inflammatory effect on the model tested, and consequent, protection against organ damage.

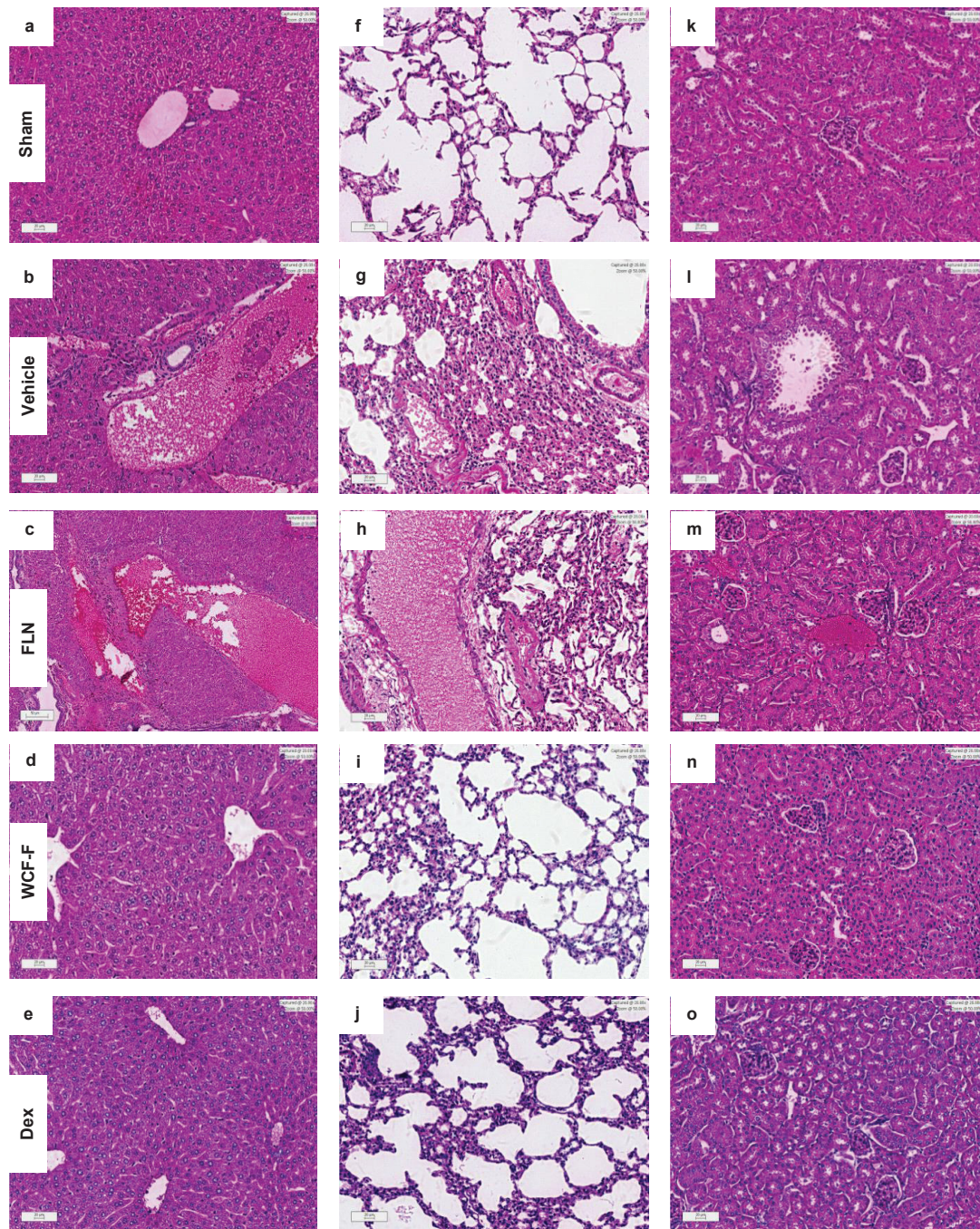


Figure 3: Representative histological sections from mice in groups: sham, vehicle, FLN, WCF-F and Dex (dexamethasone). Liver (a-e), lung (f-j) and kidney (k-o). Original magnification $\times 50$. H&E. Scale bar = $50\mu\text{m}$.

3.3. Analysis of cell viability

Macrophages are critical for the natural immune defense system of hosts, which various immune regulatory functions (Del Carmen Juárez-Vázquez, Alonso-Castro, & García-

Carrancá, 2013; Schepetkin & Quinn, 2006). Therefore, it was important to evaluate the effects of WCF-F and FLN fractions on the viability of RAW 264.7 macrophages for 24 h. Results showed that RAW 264.7 macrophage cells viability was not significantly influenced by fractions at the indicated concentrations of 0.1, 1, 10 and 100 $\mu\text{g/mL}$ ($p > 0.001$) (fig. S2).

3.4. Macrophage internalization of fluorescent labeled WCF-F

In order to investigate the cellular uptake behavior of dye labeled WCF-F as a function of time, we used fluorescence microscopy. Through the fluorescent labeling technique, the labeled product can be used to monitor the transport and action of target molecules within living cells (Zhang et al., 2011). The macrophage cell lines were treated with 100 $\mu\text{g/mL}$ of labeled WCF-F, then investigated after 6 h, then after 24 h and 48 h of treatment.

The internalization of labeled WCF-F followed the “passive delivery” method. Here, a non-specific cellular endocytosis results in WCF-F sequestration within the endocytic vesicles and leads to specific fluorescent points within the cytoplasm (fig. 4) (Ruedas-Rama et al., 2012). This is what happened to a lesser extent with the FLN negative control tested, which has only the dye and showed a statistically significant difference when compared to WCF-F ($p < 0.001$). This “passive delivery” method is one of the first mechanisms employed by cell lines. Major advantage of passive delivery is that further modification of the WCF surface is not required (Ruedas-Rama et al., 2012). Therefore, the fluorescent labeled WCF-F gave very intense images within murine macrophages cells with no significant background noise, proving that after subcutaneous administration, wine polysaccharides are internalized by macrophages and they probably lead to the inflammation site.

As part of the innate immune response, macrophages recognize and are activated by pathogen-associated molecular patterns microorganisms. Consequently, there is a significant change in transcriptional output in macrophage cell lines (Hayashizaki et al., 2006; Venter et al., 2014). The response to LPS is initiated via a signaling complex that includes CD14, toll-like receptor 4 (TLR4) and MD2 (Hayashizaki et al., 2006; Venter et al., 2014). Due to this transcriptional response to LPS in macrophages, there seems to be a change in the cellular uptake of labeled WCF-F after activation with LPS. That LPS activates macrophages and the activated macrophages have increased levels of lysosomal proteins and more passage in

cellular basal, layer that probably increases the more uptake of labeled WCF-F (Hayashizaki et al., 2006; Venter et al., 2014).

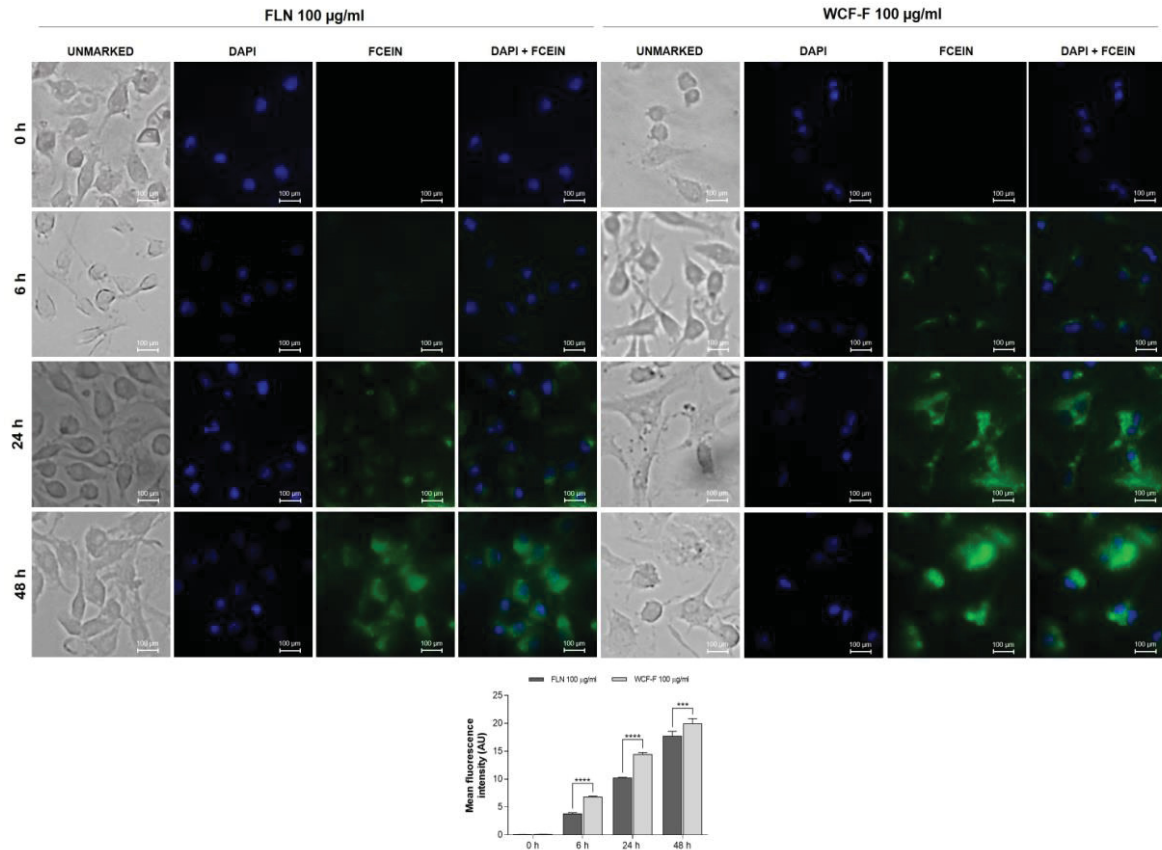


Figure 4: Fluorescence microscopy images of macrophage cell lines after incubation with 100 µg/mL of WCF-F or FLN fraction after 0 h 6h, 24h and 48 h. Average fluorescence intensity is plotted for WCF-F and FLN fractions according to time of analysis. ANOVA followed by the Tukey's test. ***p < 0.001.

3.5. Imaging of Multi-wavelength fluorescence *in vivo*

To investigate a distribution of dye-tagged polysaccharide by mouse organs during sepsis, we administered WCF-F, WCF or FLN subcutaneously, induced sepsis pr CLP and analyzed the distribution of treatments in live animals for 6 h by monochrome interlined with imaging Modalities of multi-wavelength fluorescence. Also, histopathological analysis to identify the presence of WCF-F fluorescence by confocal microscopy in the lungs, liver and kidneys, which are the most affected organs during sepsis we performed the same experiment of sepsis via subcutaneous and we can see the fluorescence in green (fig. 6). In the treated

mice we can see that the polymer is migrating in the sequence of affected tissues in sepsis, after 1 h the fluorescence is in the lungs first affected organ during sepsis, heart, liver and kidneys, and after 6 hours, it is apparently found all over the mice body, which corroborates the *in vitro* results that show growing fluorescence in the cells after 6 h of treatment (fig. 5). We also tested fluoresceinamine (FLN) and clearly a different behavior, the fluorescence is well reduced in the first hours of the treatment and after 6 h is located only in the kidney and bladder region probably being expelled by urine (fig. 5 e-h). Fluorescence of native polysaccharide, fluoresceinamine and sham are negative as expected. These results were confirmed in the histopathological analysis, the WCF-F fraction presented the average fluorescence intensity of 1979.76 (liver), 1303.44 (lung) and 1086.96 (kidney) per mm² of tissue (Fig. 6). The Fluorescence intensity of WCF-F treated organs was high compared to control groups.

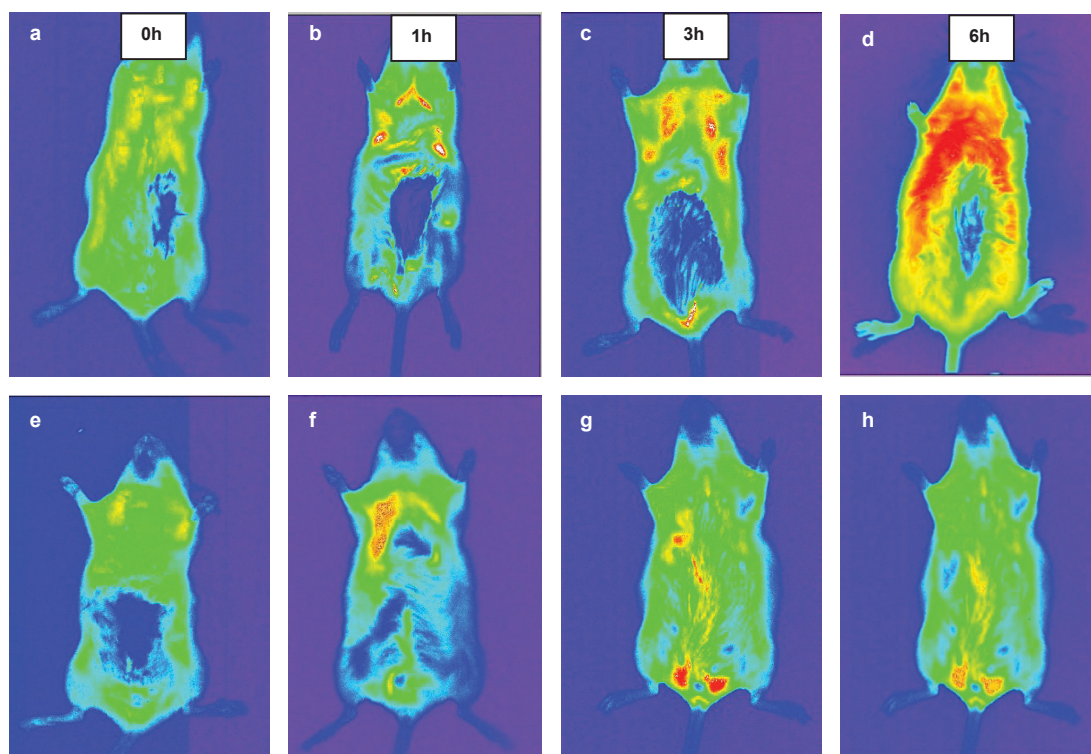


Figure 5: Imaging of Multi-wavelength fluorescence of WCF-F (a-d) and FLN (e-h) after 0 h 1h, 3h and 6h of treatment with live animals. Computer command center running Windows 7 Pro with Carestream MI acquisition and analysis software.

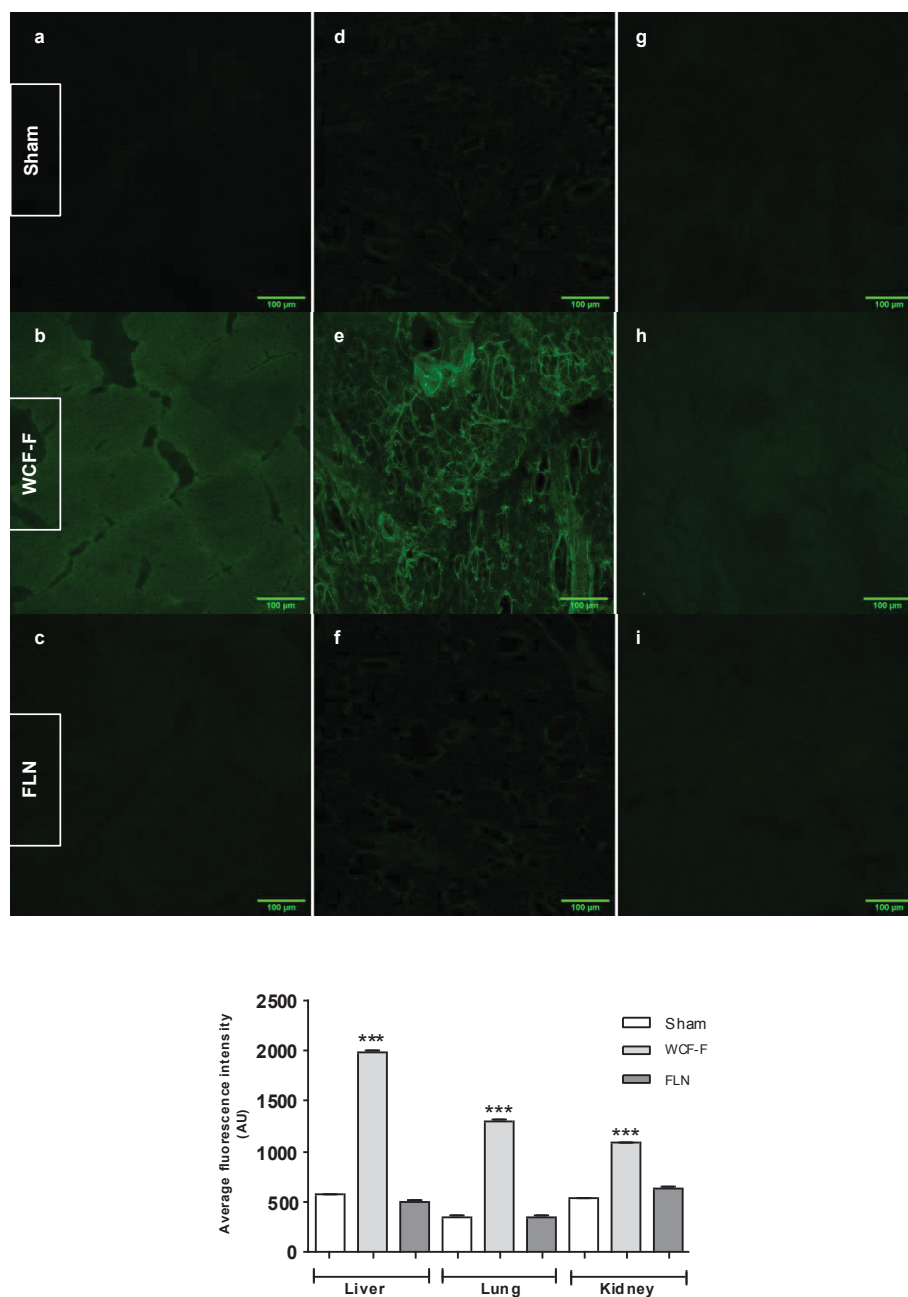


Figure 6: Representative histological sections from mice in groups: sham, WCF-F and FLN. Liver (a–c), lung (d–f) and kidney (g–i). Original magnification $\times 20$. Scale bar = 100µm. Average fluorescence intensity is plotted for WCF-F and FLN fractions of the liver, lung and kidney. ANOVA followed by the Tukey's test. *** $p < 0.001$.

4. Discussion

We have developed an efficient procedure for the fluorescent labeling of wine polysaccharides with fluoresceinamine based on the reductive amination method and

demonstrated the linkage through the ESI-MS, HPSEC, and NMR experiments, which showed that the method was effective for labeling and did not alter the original structure of wine polysaccharides. This labeling allowed the monitoring of high molecular weight polysaccharides during the inflammatory process through the screening of the flourishing molecule *in vitro* and *in vivo*.

Probably, the polysaccharide after being internalized by the macrophage blocks inflammatory process activation signaling pathways, since we observed that the animals treated with WCF-F reduced significantly the levels of inflammatory cytokines TNF- α and IL-1 β and also reduced the levels of the inflammatory mediator NO, which is responsible for causing tissue damage. We believe that macrophages with internalized polysaccharides go to the sites of inflammation, to the organs most affected by sepsis, as shown by the presence of dye-labeled polysaccharides in the liver, kidney and lung in histopathological analysis by confocal microscopy and migration analysis by camera. The anti-inflammatory effect of wine polysaccharides has also been observed by reduced levels of AST, ALT and bilirubin which are markers of liver function, also creatinine and urea which are markers of renal function. This was probably due to the reduction of leukocyte infiltration in the tissues and the reduction of the production of inflammatory mediators with consequent protection of the organs.

Due to the results presented, we were able to show that pre-treatment with Cabernet Franc) wine polysaccharides prevented lethality caused by polymicrobial sepsis in mice, by subcutaneous administration. This study showed the fluorescent polysaccharide migration through the animal is due immune system. Wine polysaccharides demonstrated a potent anti-inflammatory effect in the sepsis model, what may indicate and suggest the beneficial effects of wine moderate consumption for human health. This beneficial effect seems to be due to a reduction in leukocyte infiltration, increased anti-inflammatory cytokine and inhibited production of pro-inflammatory cytokines, with consequent protection against tissue damage. Moreover, the polysaccharide fractions were able to protect liver, lungs and kidney against injuries induced by CLP sepsis in mice.

Acknowledgements

The authors would like to thank the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Grant number 449176/2014-2), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financiadora de Estudos e Projetos (FINEP) and Fundação Araucária for financial support, and UFPR NMR Center.

References

Carpita, N. C. & Gibeaut, D. M. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, 3, 1-30 (1993).

Martínez-Lapuente, L., Guadalupe, Z., Ayestaran, B., Ortega-Heras, M., & Perez-Magarino, S. Changes in polysaccharide composition during sparkling wine marking and aging. *Journal Agricultural and Food Chemistry*, 61, 12362-12373 (2013).

Pellerin, P. & Cabanis, J-C. Les glucides. In *nologie: fondements scientifiques et technologiques*. Lavoisier, Tech. & Doc, France: Paris, 40-92 (1998).

Bezerra, I. L. et al. Structural characterization of polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: Anti-inflammatory activity in LPS stimulated RAW 264.7 cells. *Carbohydrate Polymers*, 186, 91-99 (2018).

Bezerra, I.L., Caillot, A. R. C., Oliveira, A. F., Santana-Filho, A. P. & Sassaki, G. L. Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice. *Carbohydrate Polymers*, 210, 254-263 (2019).

Cordeiro, L. M. et al. (1->2) and (1->6)-Linked beta-D-galactofuranan of microalga *Myrmecia biatorellae*, symbiotic partner of *Lobaria linita*. *Carbohydrate Polymers*, 90, 1779-1785 (2012).

Scoparo, C. T. et al. Polysaccharides from green and black teas and their protective effect against murine sepsis. *Food Research International*, 53, 780-785 (2013).

Rittirsch, D., Huber-Lang, M. S., Flierl, M. A. & Ward, P. A., Immunodesign of experimental sepsis by cecal ligation and puncture. *Nature Protocols*. 4, 31–36 (2009).

Chen, M. & Yin, M. Design and development of fluorescent nanostructures for bioimaging, *Prog. Polym. Sci*, 39 (2), 365–395 (2014).

Azhdarinia, A., Ghosh, P., Ghosh, S., Wilganowski, N. & Sevick-Muraca, E. M. Dual-labeling strategies for nuclear and fluorescence molecular imaging: a review and analysis, *Mol. Imaging Biol.*, 14 (3), 261–276 (2012).

Kikuchi, K. Design, synthesis and biological application of chemical probes for bio-imaging. *Chem. Soc. Rev.*, 39 (6), 2048-2053 (2010).

Bae, S. W., Tan, W. & Hong, J-I. Fluorescent dye-doped silica nanoparticles: new tools for bioapplications. *Chem. Commun.*, 48 (17), 2270-2282 (2012).

Abdel-Mottaleb, M. M., Beduneau, A., Pellequer, Y. & Lamprecht, A. Stability of fluorescent labels in PLGA polymeric nanoparticles: quantum dots versus organic dyes. *Int. J. Pharm.*, 494 (1), 471-478 (2015).

B. Weiss, B. et al. Nanoparticles made of fluorescence-labelled poly (L-lactide-co-glycolide): preparation, stability, and biocompatibility, *J. Nanosci. Nanotechnol.*, 6 (9–10), 3048-3056 (2006).

Dong, S. & Roman, M. Fluorescently labeled cellulose nanocrystals for bioimaging applications. *J. Am. Chem. Soc.*, 129 (45), 13810-13811 (2007).

Stipp, M. C. et al. Necroptosis mediates the antineoplastic effects of the soluble fraction of polysaccharide from red wine in Walker-256 tumor-bearing rats. *Carbohydrate Polymers*, 160, 123-133 (2016).

Cordeiro Caillot, A. R. et al. Structural characterization of blackberry wine polysaccharides and immunomodulatory effects on LPS-activated RAW 264.7 macrophages. *Food Chemistry*, 257, 143-159 (2018).

Sasaki, G. L., Guerrini, M., Serrato, R. V., Santana-Filho, A. P., Carlotto, J., Simas-Tosin, F., Cipriani, T. R., Iacomini, M., Torri, G., & Gorin, P. A. J. Monosaccharide composition of glycans based on Q-HSQC NMR. *Carbohydrate Polymers*, 104, 34-41. (2014).

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

Otero-Anton, E. et al. Cecal ligation and puncture as a model of sepsis in the rat: Influence of the puncture size on mortality, bacteremia, endotoxemia and tumor necrosis factor alpha levels. *European Surgical Research*, 33, 77-79 (2001).

Lapa, F. R., Silva, M. D., Almeida-Cabrini, D., & Santos, A. R. Anti-inflammatory effects of purine nucleosides, adenosine and inosine, in a mouse model of pleurisy: Evidence for the role of adenosine A (2) receptors. *Purinergic Signal*, 8, 693-704 (2012).

Longhi-Balbinot, D. T. et al. Anti-inflammatory effect of triterpene 3 β , 6 β , 16 β -trihydroxylup-20(29)-ene obtained from *Combretum leprosum* Mart & Eich in mice. *Journal of Ethnopharmacology*, 26, 59-64 (2012).

Silva, M. D. et al. Involvement of interleukin-10 in the anti-inflammatory effect of sanyinjiao (SP6) acupuncture in a mouse model of peritonitis. *Evidence-Based and Complementary and Alternative Medicine*, 2011, 217946 (2011).

Kazarian, A. A. et al. Development of a novel fluorescent tag O-2-[aminoethyl] fluorescein for the electrophoretic separation of oligosaccharides, *Anal. Chim. Acta*, 662 (2), 206–213 (2010).

Bone, R. C. et al. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest*, 101, 1644-1655. (1992).

Yamashiro, S. et al. Phenotypic and functional change of cytokine activated neutrophils: inflammatory neutrophils are heterogeneous and enhance adaptive immune responses. *J Leukoc Biol.*, 69, 698-704, (2001).

Pan, M. H., Lin-Shiau, S. Y. & Lin, J. K. Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of I kappa B kinase and NF kappa B activation in macrophages. *Biochemical Pharmacology*, 60, 1665–1676 (2000).

Overhaus, M., Togel, S., Pezzone, M. A. & Bauer, A.J., Mechanisms of polymicrobial sepsis-induced ileus. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 287, G685–694 (2004).

Landry, D. W. & Oliver, J. A. The pathogenesis of vasodilatory shock. *The New England Journal of Medicine*, 345, 588–595 (2001).

Nieuwenhuijzen, G. A., Deitch, E. A. & Goris, R. J. Infection, the gut and the development of the multiple organ dysfunction syndrome. *European Journal of Surgery*. 162, 259–273v (1996).

Pacher, P., Beckman, J. S., Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological Review*; 87, 315–424.

Bau, A. E., Durhan, R. & Faist, E. Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): Are we winning the battle? *Shock*, 10, 79-89 (1998).

Dhainaut, J. F., Marin, N., Mignon, A. & Vinsonneau, C. Hepatic response to sepsis: interaction between coagulation and inflammatory processes. *Crit Care Med.*, 29, 42-47 (2001).

Feverly, J. Bilirubin in clinical practice: a review, *Liver Int.*, 28, 592-605 (2008).

Brito, M. A., Silva, R. F. & Brites, D. Bilirubin toxicity to human erythrocytes: a review. *Clin. Chim. Acta.*, 374, 46-56 (2006).

Chen, J. K., Wang, W. C, Zang, L., Zhao, J., Li, W. & Jiang, T. Repression of a chromatin modifier aggravates lipopolysaccharide-induced acute lung injury in mouse. *Biochemical and Biophysical Research Communications*, 471, 515-521 (2016).

Bhatia M. & Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *The Journal of Pathology*, 202(2), 145-56 (2004).

Rewa, O. & Bagshaw, S. M. Acute kidney injury-epidemiology, outcomes and economics, *Nat. Rev. Nephrol.*, 10, 193-207 (2014).

Huang, W. Value of neutrophil counts in predicting surgery-related acute kidney injury and the interaction of these counts with diabetes in chronic kidney disease patients with hypertension., *Medicine*, 94, 1-7 (2015).

Del Carmen Juárez-Vázquez, M., Alonso-Castro, A. J. & García-Carrancá, A. Kaempferitrin induces immunostimulatory effects in vitro. *Journal of Ethnopharmacology*, 148, 337-340 (2013).

Schepetkin, I. A., & Quinn, M. T. Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *International Immunopharmacology*, 6 (3), 317–333 (2006).

Zhang, Y., Wang, Z., Zhang, X., Zhou, W. & Huang, L. One-pot fluorescent labeling of saccharides with fluorescein-5-thiosemicarbazide for imaging polysaccharides transported in living cells. *Carbohydr. Res.*, 346 (14), 2156–2164 (2011),

Ruedas-Rama, M. J., Walters, J. D., Orte, A. & Hall, E. A. Fluorescent nanoparticles for intracellular sensing: a review. *Anal. Chim. Acta*, 751, 1–23 (2012).

Hayashizaki, Y., Hume, D. A. & Sweet, M. J. LPS regulates proinflammatory gene expression in macrophages by altering histone deacetylase expression. *FASEB J.*, 20 (9), 1315-1327 (2006).

Venter, G. et al. Glucose controls morphodynamics of LPS-stimulated macrophages. *PLoS One*, 9 (5), e96786 (2014).

Supporting Information

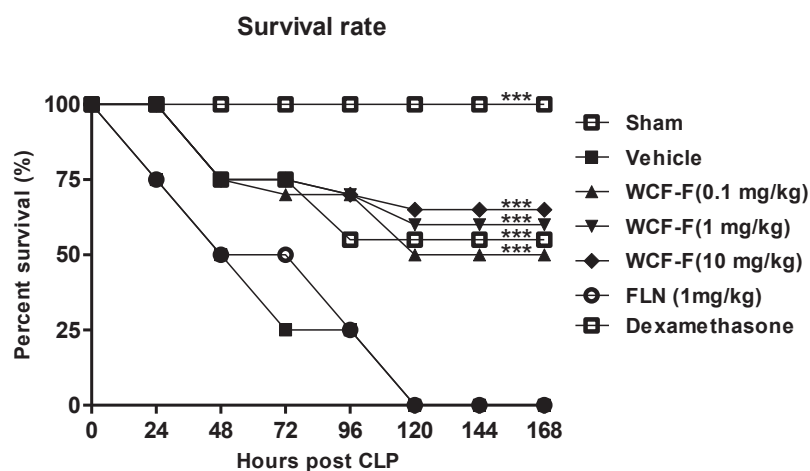


Figure S1: Effects of WCF-F and FLN fractions on survival rate. Mice (10 animals/group) were subcutaneous administered various doses (0.1, 1, 10 mg/kg), vehicle (saline s.c.) and dexamethasone (0.5 mg/kg s.c.). Values represent means $\pm$ SEM. *** $p < 0.001$ when compared with CLP plus vehicle group; ANOVA followed by the Tukey's test.

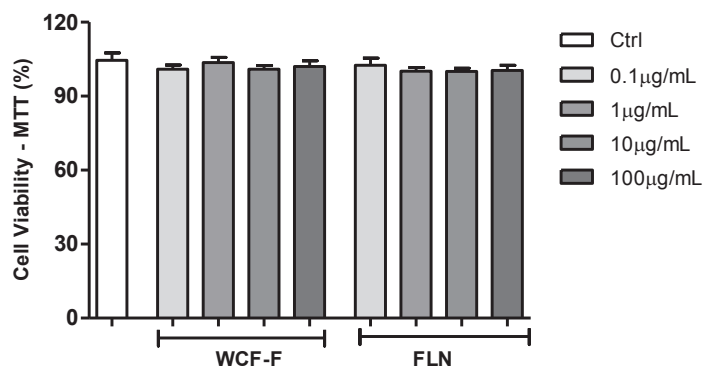


Figure S2: Effect of WCF-F and FLN fractions on the viability of RAW 264.7 cells. The viability was measured by MTT assay. The values for each concentration tested (0.1, 1, 10 e 100 μ g/mL) represent the average (mean $\pm$ S.D.); Ctrl (control).

CAPITULO V

Este capítulo é composto pelo manuscrito “Influence of red wine soluble polysaccharides profile on the flavanol composition and precipitationbody” que será submetido em uma revista.

Influence of red wine soluble polysaccharides profile on the flavanol composition and precipitation

Iglesias de Lacerda Bezerra^{ab}, Leociley Rocha Alencar Menezes^b, Guilherme Lanzi Sassaki^b, Ignacio García Estévez^a, María Teresa Escribano Bailón ^{a*}

^a *Grupo de Investigación en Polifenoles, Unidad de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, E-37007 Salamanca, Spain*

^b *Department of Biochemistry and Molecular Biology, Federal University of Parana, Curitiba, Paraná, 81.531-980, Brazil*

Abstract

Polysaccharides and phenolic compounds are very important quality parameters of wines because of their impact on color, taste and health properties, and together contribute to the chemical stability and sensory perception of the products. In this context, the flavanols and polysaccharide composition of Cabernet Sauvignon and Tempranillo wines and the relationship with precipitation were investigated. It was demonstrated that the wines have similar polysaccharide and flavanols patterns even, but they have different concentrations. Statistical analyses revealed the existence of relationships between polysaccharide composition and procyanidins precipitation. Some polysaccharide families showed clear opposition to solubilization of flavanols according to principal component analysis (PCA) results, being stronger for mannan, mannoproteins, xylan and detrin.

Keywords: wine, polysaccharides, flavanols, interaction, precipitation

1. Introduction

Polysaccharides and tannins are the main macromolecules that are found in wine, and influence the elaboration process and sensory properties. Their concentrations depend on many parameters, such as, grape culture conditions, degree of maturity, fermentation yeast, wine-making techniques and the treatments leading to increase solubilization of the macromolecular components of the grape berry cell walls (Carpita & Gibeaut, 1993; Martínez-Lapuente, Guadalupe, Ayestaran, Ortega-Heras, & Perez-Magarino, 2013; Pellerin & Cabanis, 1998).

Among wine polysaccharides, arabinose and galactose rich polysaccharides, such as type II arabinogalactans (AGII) and arabinans, rhamnogalacturonans type I and type II, and homogalacturonans come from grape berries; while glucans, mannans and mannoproteins are released by yeast during fermentation (Ayestarán, Guadalupe, & León, 2004; Belleville, Brillouet, Tarodo de la Fuente & Moutounet, 1991; Bezerra, Caillot, Palhares, Santana-Filho, Chavante & Sasaki, 2018; Brillouet, Bosso, & Moutounet, 1990; Doco & Brillouet, 1993; Doco, Vuchot, Cheynier, & Moutounet, 2003; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, Escribano-Bailón, 2014; Pellerin, Vidal, Williams, & Brillouet, 1995; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003). Arabinogalactans, mannans and rhamnogalacturonans

are polysaccharides which were detected as the main polysaccharides in red wine (Bezerra, Caillot, Oliveira, Santana-Filho & Sassaki, 2019).

Flavanols are a wide family of phenolic compounds, as monomers, oligomers and polymers, commonly found in plants, foods and beverages, especially in wine (Monagas, Gómez-Cordovés, Bartolomé, Laureano, & Ricardo-Da-Silva, 2003). The presence of different linkage positions, galloylation, and combinations between the subunits lead to a great number of structures with different capacities for interactions, i.e. with polysaccharides and with proteins, and this can influence the organoleptic properties of a wine, such as perception astringency (Kennedy, Ferrier, Harbertson, & Peyrot des Gachons, 2006; Brossaud, Cheynier, & Noble, 2001; Preys et al., 2006).

The polysaccharides interact with phenolic compounds through hydrophobic forces, which have been shown to drive the main associations that occur between tannins and cell wall polysaccharides. The strength of such interactions depends on the structure and conformation of both the tannins and the cell wall material (Bindon, Smith & Kennedy, 2010; Le Bourvellec, Bouchet & Renard, 2005; Lin, Fischer & Wicker, 2016; Renard, Baron, Guyot, Drilleau, 2001). The interactions between tannins and soluble and insoluble cell wall components are, in part, responsible for the low quantities of tannins found in wines compared with the quantities in grapes (Osete-Alcaraz, Bautista-Ortín & Gómez-Plaza, 2019). Osete-Alcaraz et al (2019) showed the capacity of interaction of tanins with mannans and pectins, and Ruiz-García et al (2014) showed that pectins and hemicelluloses also have binding capacity.

In this work, we have investigated the composition in polysaccharides and flavanols of Cabernet Sauvignon and Tempranillo red wines to determine if there is some relationship between their contents in red wines. To obtain further insights helping to discuss the results obtained, we have studied in model solution the interaction between polysaccharides and flavanols.

2. Materials and methods

2.1. Materials

Four Cabernet Sauvignon (CSCM, CSVA, CSFA, CSJA) and one Tempranillo (TPHO) commercial wines corresponding to 2017 and 2018 vintages were used in this study.

All the wines were stored under cellar conditions before analyzes were conducted. Oenological parameters such as pH, ethanol content, zone of production and vintage are described in the table S1 of the supporting information.

Vitis vinifera var. Cabernet Sauvignon grapes were collected at harvest from a vineyard located in Ribera de Duero AOC (Spain). In total, 200 berries were collected. Grape samples were immediately frozen and stored until analyses were performed.

2.2. Polysaccharides extraction

The polysaccharides from each wine were isolated by precipitation by the addition of cold EtOH (3 vol.), and separated by centrifugation (8,000 rpm at 4 ° C, 20 min). The sediment was dissolved in H₂O, dialyzed against water for 72 h to remove the remaining low-molecular weight compounds and then freeze dried, giving rise to polysaccharide extracts: CSCM, CSJA, CSFA and CSVA were obtained from the corresponding Cabernet Sauvignon wines and TPHO from the corresponding Tempranillo wine.

2.3. Polysaccharide identification by NMR

The NMR spectra were obtained with a 400 or 600 MHz Bruker spectrometer using 5 mm direct or inverse probe heads (Avance III and HD, Bruker, Billerica, Massachusetts, USA). 1D ¹H NMR at 600 MHz was acquired after a 90° (p1) pulse calibration at 10.75 μs. ¹H/¹³C chemical shift correlation mapping was finally determined by COSY, TOCSY HSQCed (Heteronuclear Single Quantum Coherence edited spectroscopy CH, CH₃ positive phase CH₂ negative phase) performed at 30 °C in deuterium oxide (D₂O) (Sasaki, et al, 2014). 2D NMR experiments were conducted using quadrature detection at indirect dimension and acquired using 24 scans per series of 1024 × 320 data points, with zero filling in F1 (4096) prior to Fourier transformation, using the Software TOPSPIN version 3.2 pl6 (Bruker Biospin, Rheinstetten, Germany). The chemical shifts of the polysaccharide were expressed in δ (ppm). The quantification of each polysaccharide in relation to the total obtained for each sample is performed from the values obtained by the integrals of the monosaccharide signals found in the anomeric region of the ¹H NMR and NMR-HSQC spectra of the sample of each wine following the procedure previously described (Bezerra et al., 2019).

2.4. Determination of molecular weight distribution of polysaccharides

The molecular weight distribution of polysaccharide extracts was estimated by using high-performance size-exclusion chromatography (HPLC-SEC) equipped with two serial ShodexTM OHpakTM SB-803 HQ and SB-804 HQ columns (8 x 300 mm) (Showa Denko Europe GmbH, Germany). Elution was performed with a flow of 1 mL min⁻¹ using 0.1 M LiNO₃ (aq.) as previously described (Guadalupe & Ayestarán 2007). HRSEC was performed using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a refractive index detector (RID). Calibration was carried out with several pullulan molecular weight standards (P1, Mw = 342 D; P2, Mw = 1320 D; P3, Mw = 6200 D; P4, Mw = 10000 D; P5, Mw = 21700 D; P6, Mw = 48800 D; P7, Mw = 113000 D; P8, Mw = 200000 D; P9, Mw = 348000 D; P10, Mw = 805000 D). All analyses were performed in triplicate.

2.5. Analysis of phenolic compounds in wines

To perform the identification and quantification of flavanols, 2 mL wine was added to 2 mL HCl 0,1N. Then, 2 mL of this solution were loaded into a mixed-mode cation exchange / reversed phase SPE cartridge Oasis MCX (60 mg, 3 mL) from Waters (Milford, MA, USA), previously conditioned with 2 mL methanol followed by 2 mL ultrapure water. After washing with 4 mL water, flavanols were eluted with 8 mL methanol. The eluate was brought to dryness under vacuum at 30 °C and then reconstituted in 500 µL ultrapure water to obtain the flavanol wine extract. Quantification was performed by HPLC – DAD – ESI / MS (García-Estévez, Alcalde-Eon & Escribano-Bailón, 2017). To do this, 390 µL of this extract was mixture with 10 µL of chlorogenic acid (1 mg/mL), which was used as internal standard, prior to the injection into the chromatographic system.

2.6. Flavanols extraction from grape seeds

Procyanidin extraction was carried out as described in Ferrer-Gallego, García-Marino, Hernández-Hierro, Rivas-Gonzalo and Escribano-Bailón (2010). Briefly, Cabernet Sauvignon grape seeds were separated manually and freeze-dried. Seeds were ground to obtain a homogeneous powder, which was extracted with 75% ethanol. The ethanolic extracts were concentrated at low pressure until an aqueous extract was obtained, giving rise to a flavanol

extract: FSE. The extracts were analyzed following the afore mentioned methodology (section 2.5). All analyses were performed in triplicate. The composition of the obtained flavanol extract is shown in Table S2.

2.7. Interaction assays

Interaction test were performed between the polysaccharides extracted from Cabernet Sauvignon and Tempranillo wines and the FSE extract. The following mixtures were prepared, maintaining 10:1 flavanols/polysaccharide molar ratio: control (FSE + water), ICSCM (CSCM + FSE), ICSJA (CSJA + FSE), ICSVA (CSVA + FSE), ICSFA (CSFA + FSE) and ITPHO (TPHO + FSE). Samples were incubated for 15 min at room temperature and then centrifuged for 7 min at 13,000 rpm. The flavanols remained in the supernatant were analyzed by HPLC – DAD – ESI / MS (García-Estévez, Alcalde-Eon & Escribano-Bailón, 2017).

2.8. Statistical analysis

Unsupervised methods were applied to observe patterns in the data indicating relationships between samples and/or between variables (Brereton, 2003). The unsupervised pattern recognition method used for data analysis was principal components analysis (PCA), which was applied to the correlation matrix of the original variables. The software packing for Windows IBM SPSS 23 (SPSS, Inc. Chicago, IL, USA) was used for data processing. Statistical error was determined by one-way ANOVA; the post hoc test was Tukey. Calculations were performed with Graphpad Prism 5.0. *p*-values < 0.001 were considered significant.

3. Results and Discussion

3.1. Analysis of the polysaccharide extracts from wines

The ¹H-NMR analysis of the CSCM, CSJA, CSVA, CSFA and TPHO samples showed characteristic signals of polysaccharides (Fig. S1 – supporting information). The anomeric region presented a complex profile (4.0~6.0 ppm), with many overlapped signals due to mixture of polysaccharides (Bezerra et al, 2018). All wines analyzed had similar

polysaccharide patterns, containing AGII, RGs, mannan, mannoprotein, xylan and dextrin; but in a different concentration. Total polysaccharide content ranged from 773.34 to 1025.10 mg/L (Table 1) which was noticeably greater than some of the contents reported in the literature (Barrio-Galán, Cáceres-Mella, Medel-Maraboli & Peña-Neira, 2015); but in the same range of other studies that use the same methodology of analysis of Bezerra et al., 2018..

Through of the NMR analysis, using the values obtained by the integrals of the monosaccharide signals found in the anomeric region of the spectra of the crude fraction of each wine, it was possible to quantify each type of polysaccharide in relation to the total amount obtained in each wine (Table 1). AGII was the most abundant polysaccharide family in all the studied samples, ranging from 49 to 63%. RGs presented values ranging from 6 to 14%. CSFA sample presented the highest value of RGs (13.95%). All the samples studied presented low xylan and dextrin percentages, xylan presented values ranging from 4 to 6% and dextrin from 2 to 5.5%. Although all samples showed the same polysaccharide pattern, the percentages of polysaccharides varied significantly between samples.

Polysaccharides are mainly derived from grape berries and yeasts, therefore, during winemaking, various factors such as grape variety and the winemaking technique used by each winery can affect the final amount of polysaccharides in wine. Since they were extracted from wines produced in different wineries, probably the winemaking technique used in each one had a great influence on the concentration of polysaccharides. In our study, the percentage of AGII in the Tempranillo variety of the wine form (62.33%) is significantly higher than the percentage of this polysaccharide in the analyzed Cabernet Sauvignon wines. This is in accordance with studies by Apolinar-Valiente et al. (2014) that reported that the grape variety and winemaking technique have a significant impact on the quantity and composition of polysaccharides extracted from grapes into wine. In addition, the results showed that the percentage of mannan/manoprotein varied from 24% to 36%. Mannans and mannoproteins are released mainly from cell walls during fermentation and aging (Apolinar-Valiente et al., 2013; Ayestaran et al., 2004; Doco et al., 2007), and one of the factors that may have influenced this variation between samples is the type of yeast strain used by the winery during winemaking. Barrio-Galán et al. (2014) reported that different *Saccharomyces cerevisiae* yeast presents different capabilities of polysaccharide liberation during alcoholic fermentation. Thus, our study is in accordance with that literature which states that the final concentration of each polysaccharide in wine appears to be strongly influenced by the technique applied during winemaking.

Table 1: Percentage of polysaccharides determined in Cabernet Sauvignon and Tempranillo wines

Sample	Polysaccharides					Total (mg/L)
	MP (%)	AGII (%)	RG (%)	Xylan (%)	Dextrin (%)	
CSCM	24.31 ^b	56.78 ^b	11.14 ^{ab}	5.21 ^a	2.55 ^{bc}	941.00
CSJA	34.62 ^a	48.99 ^c	6.04 ^c	4.86 ^{ab}	5.49 ^a	773.34
CSVA	25.23 ^b	55.86 ^b	9.12 ^b	5.88 ^a	3.90 ^b	1090.61
CSFA	22.69 ^c	57.68 ^b	13.95 ^a	4.17 ^b	1.51 ^c	1025.10
TPHO	25.35 ^b	62.33 ^a	6.30 ^c	3.90 ^b	2.11 ^c	1016.62

The mean percentage was calculated from the individual percentage of each compound analyzed in triplicate. MP: mannans + mannoproteins; AGII: type II arabinogalactans; RG: rhamnogalacturonans. Different letters indicate significant differences ($p < 0.001$; Tukey test) between samples.

The HPLC-SEC analysis of the samples showed a heterogeneous profile with the presence of 4 predominant chromatographic peaks (Table 2). In addition, the correlation between the polysaccharidic composition and the molecular weight was investigated by means of Pearson's coefficient and its significance. The polysaccharidic composition showed significant correlations with molecular weight. It is possible to observe a positive correlation between P1 (208.82 - 243.96 kDa) and RGs, P2 (59.82 - 96.88 kDa) and AGII, P3 (11.07 - 13.48 kDa) and mannan/mannoprotein and dextrans and P4 (6.38 - 7.06 kDa) and dextrans and also xylan, but, in this last case, correlation is not significant (see Table S3). Furthermore, relating the tables 1 and 2, the percentage of each molecular weight obtained and the percentage each polysaccharide for the same sample surprisingly corroborates. This evidence is greater when it is observed that P2, which presents a higher overall percentage among molecular weights as shown in the table 2, has significantly positive relationship with AGII according to table S3, which also has a higher overall percentage among polysaccharides and interestingly. P3 from CSJA sample has higher percentage of molecular weight (33.70%) and coincides with the percentage of mannan/mannoprotein (34.62%) from the same sample, which is also the highest percentage among samples for this polysaccharide y corroborate the positive relationship between the percentage P3 in relation to the mannan/mannoprotein indicated in the table S3. Additional experiment is necessary to confirm these data, but it is notable that there is a great correlation between the percentages obtained for each molecular

weight and for each of the corresponding polysaccharides in the samples, suggesting that each size found refers to the correlated polysaccharides.

Table 2: Molecular weights of the polysaccharides determined in Cabernet Sauvignon and Tempranillo wines

Sample	Molecular Weight							
	P1		P2		P3		P4	
	kDa	%	kDa	%	kDa	%	kDa	%
CSCM	208.82	18.27 ^{ab}	90.25	35.10 ^b	13.48	25.48 ^b	7.03	21.15 ^b
CSJA	243.96	12.89 ^c	60.92	29.53 ^c	11.07	33.70 ^a	6.38	23.88 ^a
CSVA	232.03	16.17 ^b	86.06	32.48 ^b	12.10	26.80 ^b	6.67	24.34 ^a
CSFA	295.79	20.20 ^a	96.88	36.71 ^{ab}	12.78	22.59 ^c	6.82	20.50 ^b
TPHO	211.36	15.66 ^b	59.82	38.54 ^a	13.19	25.05 ^b	7.06	20.76 ^b

The mean percentage was calculated from the individual percentage of each compound analyzed in triplicate. Different letters indicate significant differences ($p < 0.001$; Tukey test) between samples.

3.2. Analysis of flavanols extract of wines

HPLC-MS analysis of the phenolic compounds in wines showed a major concentration of procyanidins (PC), between 62 and 275 mg /L in Cabernet Sauvignon wines, and 58 mg/L for Tempranillo wine (table 3). Prodelphinidin (PD) content was lower, ranking between 4 and 7 mg/L for Cabernet Sauvignon wines and 8 mg/L for Tempranillo wine. The average degree of polymerization between the samples was between 1.87 and 2.13. Dimmers were the more abundant procyanidins in all the studied samples, their content ranged from 60 to 70%. (Epi)Catechins (monomers) presented values ranging from 13 to 20% for all samples, except the TPHO sample, which presented a low value of 2%. All the samples showed pentamers PC and epicatechin gallate with percentages values below 1%.

Noticeable differences were observed between percentages of each type of phenolic compound for the different wines. The CSJA and TPHO wines, which were elaborated in 2018, showed lower concentrations of total flavanols (68.4 and 66.5 mg/L, respectively). Interestingly, these samples showed a higher percentage of mannans and mannoproteins, as

shown in the Table 1. Our study is in accordance with Barrio-Galán et al. (2015) that reported that a higher liberation of polysaccharides reduce the concentration of the phenolic families. Probably, this variation is influenced by the interaction of flavanols with families of polysaccharides that presents in greater quantity during winemaking, affecting not only the amount of soluble flavanols but also that of polysaccharides in the final product, which, consequently, may influence the organoleptic properties of the wine (Le Bourvellec, Guyot, & Renard, 2009; Renard, Watrelot, Le Bourvellec, 2017; Ramos-Pineda, García-Estévez, Brás, Martín del Valle, Dueñas, & Escribano-Bailón, 2018; Lei et al., 2019).

Table 3: Flavanols determined in Cabernet Sauvignon and Tempranillo wines

Sample	Procyanidin							Prodelphinidin		mDP
	Mon	Dim	Trim	Tetra	Pent	ECG	Total	Total	Total	
	%	%	%	%	%	%	mg/L	%	mg/L	
CSCM	20.79 ^a	66.52 ^{ab}	9.18 ^{bc}	1.58 ^b	0.04 ^b	0.15 ^a	274.70	1.77 ^d	4.95	1.91
CSJA	22.00 ^a	67.48 ^{ab}	2.33 ^d	1.00 ^b	0.02 ^b	0.03 ^b	62.89	6.69 ^b	5.53	1.90
CSVA	23.95 ^a	61.85 ^b	9.93 ^b	1.31 ^b	0.02 ^b	0.01 ^c	212.20	3.05 ^c	6.68	1.87
CSFA	13.96 ^b	74.32 ^a	6.95 ^c	1.37 ^b	0.03 ^b	0.03 ^b	122.80	3.32 ^c	4.01	1.95
TPHO	2.18 ^c	70.41 ^a	10.07 ^a	4.70 ^a	0.19 ^a	0.04 ^b	58.28	12.38 ^a	8.29	2.13

The mean percentage was calculated from the individual percentage of each compound analyzed in triplicate. Mon: Monomers; Dim: Dimmers; Trim: Trimmers; Tetra: Tetramers; Pent: Pentamers; ECG: epicatechin gallate. mDP: mean degree of polymerization. Different letters indicate significant differences between samples ($p < 0.001$; Tukey test).

The figure 1 shows the score plot on the plane defined by the first and second principal components resulting from the principal component analysis performed with the flavanic and polysaccharidic composition of the studied wines. The first principal component (PC 1) describes 49.00% of the data variability and the second (PC 2) 33.80%, then explaining almost 83% of data variability. The flavanol composition did not shown an important correlation neither with AGII or RG, which may indicate that the presence of these type of polysaccharide has not significant influence on its composition. On the other hand, it can be seen a clear opposition of flavanol variables to polysaccharides variables such as mannan,

mannoprotein, dextrin and xylan, which may suggest that the presence of these polysaccharides can negatively influence the concentration of flavanols in the wine. In addition, the figure 1B shows the distribution of the wine samples in the plane defined by the two first components due to their phenolic and polysaccharidic composition and to the molecular weight of polysaccharides. It can be observed that Cabernet Sauvignon wines are separated from the Tempranillo wine along PC1, mainly due to the higher percentages of procyanidin oligomers, prodelphinidins and AGII and to the lower percentage of xylan of the latter.

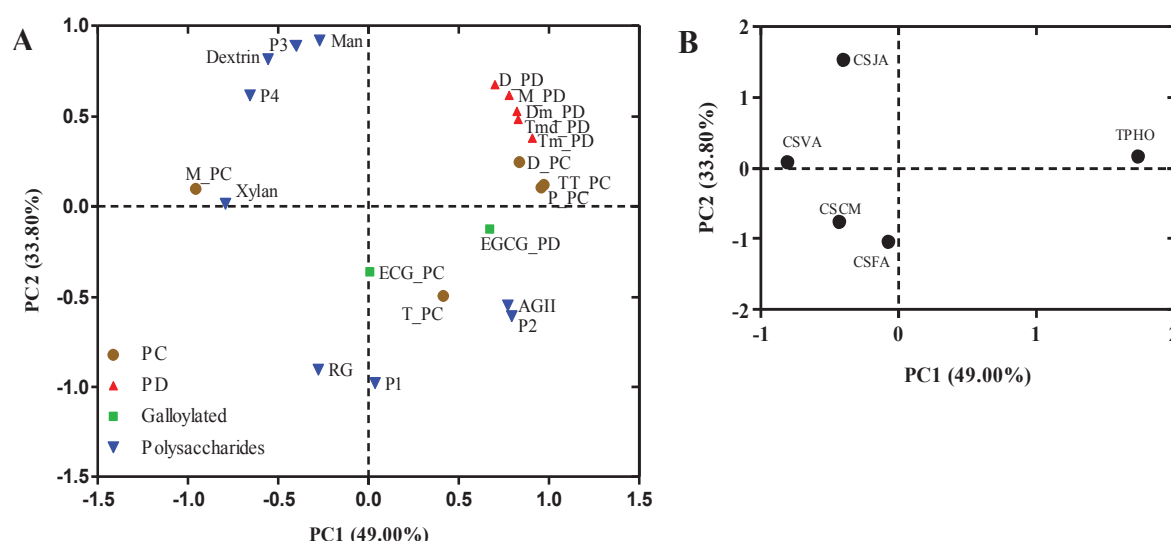


Figure 1: The score plot of the main component analysis relating (A) polysaccharide families percentage, flavanols percentage and molecular weight percentage (B) samples profile CSCM, CSJA, CSVA, CSFA and TPHO. PC: procyanidin; PD: Prodelphinidin.

3.3. Interaction experiments

Table 4 shows the percentage of precipitation of procyanidins after interaction with the different polysaccharides extracted from wines. Results indicated precipitation between 19% and 50% of non-galloylated procyanidins in the analyzed samples. ICSJA had the highest percentage of precipitation (50.1%) and ITPHO had the lowest one (19.09%) for this type of procyanidins. In addition, a precipitation between 17% and 38% was observed for the galloylated procyanidins. The ICSVA interaction had the highest precipitation (37.7%) and ITPHO had the lowest precipitation (17.1%) for this last type of procyanidins. Some

procyanidins have greater interaction with polysaccharides than others. ICSJA sample showed the highest percentage of precipitation of dimers (76%), and almost 50% of precipitation of pentamers.

As mentioned above, the TPHO polysaccharides used for the interaction test had high percentage of AGII (62.3%). This polysaccharide does not seem to have a significant influence on the precipitation of flavanols. This is also observed in the interaction analysis, since ITPHO was the sample with the lowest percentage of procyanidin precipitation. On the other hand, in agreement with works of Bindon et al. (2010) and Ozete-Alcaraz et al. (2019) that show the reduction of tannin solubilization in the presence of mannan, our results showed that the CSJA polysaccharides, which has a percentage of mannan and mannoproteins of 34.6%, showed a high affinity towards flavanol interaction, since they strongly led to the procyanidins precipitation (34.3% precipitation for no galloylated and 37.7% for galloylated) (Le Bourvellec, Bouchet & Renard, 2005; Le Bourvellec et al., 2009). Osete-Alcaraz et.al, (2019) and Mateus et al. (2004) stated that the average degree of polymerization of flavanols can be reduced after interaction with polysaccharides. However, we observed the same precipitation average among all classes of non-galloylated procyanidins in the different samples, so the precipitation seems to have occurred in a similar way regardless of the degree of polymerization, which would explain why the degree of polymerization did not significantly change when the procyanidins of the grape seeds interacted with the soluble polysaccharides in our study.

Table 4: Percentage of procyanidins precipitated after polysaccharide interaction assay

Flavanols	Sample (%)				
	ICSCM	ICSJA	ICSVA	ICSFA	ITPHO
Monomers	30.68 ^a	26.73 ^{ab}	26.05 ^{ab}	9.89 ^c	20.82 ^b
Monomers Galloylated	32.71 ^a	34.41 ^a	39.64 ^a	16.99 ^b	19.02 ^b
Dimers	41.43 ^b	76.02 ^a	43.04 ^b	28.77 ^c	23.50 ^c
Dimers Galloylated	29.57 ^{ab}	32.50 ^a	36.23 ^a	22.74 ^b	17.90 ^c
Dimers Digalloylated	29.61 ^b	40.95 ^a	43.98 ^a	15.71 ^c	20.66 ^{bc}
Trimmers	18.47 ^{ab}	27.18 ^a	23.18 ^a	11.30 ^{bc}	8.73 ^c
Trimmers Galloylated	26.93 ^b	39.53 ^a	38.93 ^a	13.95 ^c	17.46 ^c
Trimmers Digalloylated	20.61 ^b	21.86 ^b	37.63 ^a	6.64 ^c	0.54 ^d
Tetramers	27.06 ^b	27.13 ^b	32.64 ^a	20.66 ^c	6.99 ^d
Tetramers Galloylated	36.55 ^b	36.64 ^b	42.28 ^a	22.43 ^c	16.23 ^c
Tetramers Digalloylated	12.56 ^c	35.89 ^a	23.48 ^b	19.32 ^b	16.67 ^{bc}
Pentamers	43.22 ^a	48.62 ^a	48.24 ^a	34.31 ^b	20.52 ^c
Pentamers Galloylated	44.11 ^{ab}	35.69 ^b	52.79 ^a	14.44 ^c	6.43 ^d
Non-Galloylated	33.62 ^b	50.19 ^a	34.31 ^b	20.07 ^c	19.09 ^c
Galloylated	29.87 ^a	34.00 ^a	37.71 ^a	20.70 ^b	17.65 ^b
mDP	2.20	2.20	2.16	2.17	2.20

The mean percentage was calculated from the individual percentage of each compound analyzed in triplicate. mDP: mean degree of polymerization. Different letters indicate significant differences between samples ($p < 0.001$; Tukey test).

To better visualize the relationship between polysaccharide composition and flavanol precipitation, principal components analysis (PCA) was carried out by using three groups of compositional variables (polysaccharide families, molecular weight distribution of the polysaccharides and percentage of each type of procyanidins precipitated after the interaction test) (Fig. 2). The first principal component (PC 1) describes 71.30% of the variability of the data and the second (PC 2) 18.10%, thus explained almost 90% of the data variability. Regarding the variable loading-plot, in which procyanidin precipitation and polysaccharide composition can be related (Fig. 2A), it can be observed a strong relationship between flavanol precipitation and the polysaccharides mannan, mannoprotein and dextrin. This suggested that these polysaccharides are prone to interact and cause the precipitation of

procyanidins. Mannoproteins have already been described as compounds able to interact with polyphenols aggregates and to act as steric stabilizers (Poncet-Legrand et al., 2007). On the other hand, the AGII and RGs content showed an important opposition with precipitated procyanidin percentage along PC1, suggesting that these macromolecules have no significant influence on the precipitation of procyanidins, contrary to what is described in the literature about the strong interaction between pectic polysaccharides and flavanols (Lin, Fischer, & Wicker, 2016; Renard, Baron, Guyot & Drilleau, 2001). Figure 2B shows the sample score plot of the PCA performed (ICSCM, ICSJA, ICSVA, ICSFA and ITPHO), whose distribution is related to the percentage of procyanidin precipitated, the polysaccharide composition and the molecular weight. The interaction samples are clearly separated, which shows a different precipitation profile for each test according to the percentages of polysaccharides (described in the table 1) that were present in each sample used in the tests. Therefore, the results suggest that some polysaccharide families can significantly affect the solubilization of flavanols in wine.

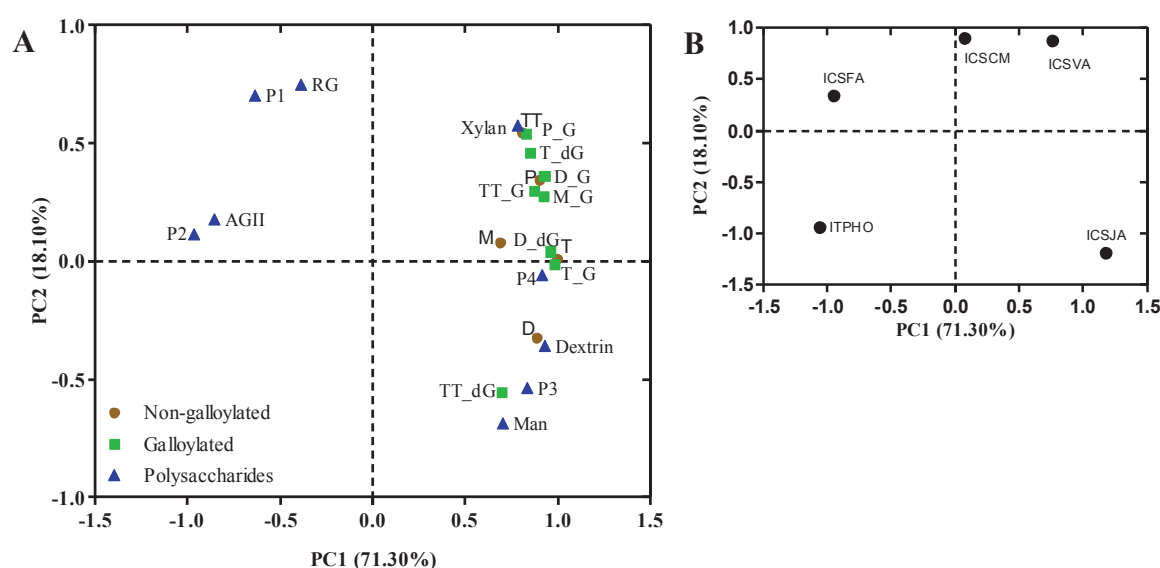


Figure 2: The score plot of the main component analysis relating (A) polysaccharide families percentage, molecular weight percentage, and percentage of procyanidins precipitated after interaction test (B) samples profile ICSCM, ICSJA, ICSVA, ICSFA and ITPHO. RG: rhamnogalacturonans; Man: mannans + mannoproteins; AGII: type II arabinogalactans; PC: procyanidins; PD: Prodelphinidin. M: monomers galloylated; M_G: Monomers galloylated; D: Dimers; D: Dimers Digalloylated; T: Trimmers; T_G: Trimmers Galloylated; T_dG:

Trimmers digalloylated; T_G: Tetramers Galloylated; TT_G: Tetramers galloylated TT_dG: Tetramers digalloylated; P: Pentamers; P_G: Pentamers galloylated. P1, P2, P3 and P4: molecular weight of polysaccharides.

4. Conclusions

A comprehensive study of chemical composition was conducted for Cabernet Sauvignon and Tempranillo wines. Results obtained showed the polysaccharides AGII, RGs, mannan, mannoproteins, xylan and dextrin in different concentrations for each sample of Cabernet Sauvignon and Tempranillo wines. As well as the presence of flavanols at different concentrations, with a majority of procyanidins.

The data obtained provide an interesting insight, on the impact of polysaccharide composition in the precipitation of procyanidins. Results showed the ability of polysaccharides to interact with flavanols. The association strength of interactions seems to have varied according to the polysaccharide family. Mannan, mannoproteins, xylan and dextrin showed highest affinity towards the interaction with flavanols that lead to their precipitation.

This work will contribute to further understand the characteristic of polysaccharides and flavanols in wine and the role of polysaccharides in modulating astringency.

Acknowledgments

Authors thank the Spanish MINECO (project ref AGL2017-84793- C2-1-R cofunded by FEDER) and Strategic Research Programs for Units of Excellence from Junta de Castilla y León (ref CLU-2018-04). IGE thanks the Spanish MICINN (Ministry of Science, Innovation and Universities) for the Juan de la Cierva-incorporación postdoctoral contract (IJCI-2017-31499). The authors also thank the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Grant number 449176/2014-2), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financiadora de Estudos e Projetos (FINEP) Fundação Araucária for financial support and UFPR NMR Center.

References

- Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., Rivas-Gonzalo, J. C. (2004). Separation of pyranoanthocyanins from red wine by column chromatography. *Analytica Chimica Acta*, 513(1), 305–318. <https://doi.org/10.1016/j.aca.2003.10.076>
- Apolinar-Valiente, R., Williams, P., Romero-Cascales, I., Gomez-Plaza, E., Lopez-Roca, J. M., Ros-Garcia, J. M., et al. (2013). Polysaccharide composition of Monastrell red wines from four different Spanish Terroirs: Effect of wine-making techniques. *Journal of Agricultural and Food Chemistry*, 61(10), 2538–2547. <https://doi.org/10.1021/jf304987m>
- Apolinar-Valiente, R., Romero-Cascales, I., Williams, P., Gómez-Plaza, E., López-Roca, J. M., Ros-García, J. M., Doco, T. (2014). Effect of winemaking techniques on polysaccharide composition of Cabernet Sauvignon, Syrah and Monastrell red wines. *Australian Journal of Grape and Wine Research*, 20, 62-71.
- Ayestarán, B., Guadalupe, Z., León, D. (2004). Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta*, 513, 29-39. <https://doi.org/10.1016/j.aca.2003.12.012>
- Belleville, M. P., Brillouet, J. M., Tarodo de la Fuente, B., Moutounet, M. (1991). Polysaccharide effects on cross-flow microfiltration of two red wines with a microporous alumina membrane. *Journal Food Science*, 55, 1598-1602. <https://doi.org/10.1111/j.1365-2621.1990.tb03579.x>
- Barrio-Galán, del R., Cáceres-Mella, A., Medel-Marabolí, M., Peña-Neira, A. Effect of selected *Saccharomyces cerevisiae* yeast strains and different aging techniques on the polysaccharide and polyphenolic composition and sensorial characteristics of Cabernet Sauvignon red wines. (2015). *Journal of the Science of Food and Agriculture*, 95, 2132-2144. <https://doi.org/10.1002/jsfa.6932>
- Bezerra, I. d. L., Caillot, A. R. C., Palhares, L. C. G. F., Filho, A. P. S., Chavante, S. F., Sasaki, G. L. (2018). Structural Characterization of Polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: anti-inflammatory activity in LPS stimulated RAW 264.7 cells. *Carbohydrate Polymers*, 186, 91-99, 2018. <https://doi.org/10.1016/j.carbpol.2017.12.082>

Bezerra, I. L., Caillot, A. R. C., Oliveira, A. F., Santana-Filho, A. P. & Sassaki, G. L. (2019). Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice. *Carbohydrate Polymers*, 210, 254-263. <https://doi.org/10.1016/j.carbpol.2019.01.025>

Bindon, K.A.; Smith, P.A.; Kennedy, J.A. (2010) Interaction between grape-derived proanthocyanidins and cell wall material. Effect on proanthocyanidin composition and molecular mass. *J. Agric. Food Chem*, 58, 2520–2528. <https://doi.org/10.1021/jf9037453>

Brereton, R. G. (2003). *Chemometrics: Data analysis for the laboratory and chemical plant*. Chichester, West Sussex, England: J. Wiley. <https://doi.org/10.1021/op030005r>

Brillouet, J. M., Bosso, C., Moutounet, M. (1990). Isolation, purification, and characterization of an arabinogalactan from a red wine. *American Journal of Enology and Viticulture*, 41, 29-36.

Brossaud, F., Cheynier, V., & Noble, A. C. (2001). Bitterness and astringency of grape and wine polyphenols. *Australian Journal of Grape and Wine Research*, 7(1), 33–39. <https://doi.org/10.1111/j.1755-0238.2001.tb00191.x>

Carpita, N. C., Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, 3, 1-30. <https://doi.org/10.1111/j.1365-313X.1993.tb00007.x>

Doco, T., Brillouet, J. M. (1993). Isolation and characterization of a rhamnogalacturonan II from red wine. *Carbohydrate Research*, 243, 333-343. [https://doi.org/10.1016/0008-6215\(93\)87037-S](https://doi.org/10.1016/0008-6215(93)87037-S)

Doco, T., Vuchot, P., Cheynier, V., Moutounet, M. (2003). Structural modification of arabinogalactan-proteins during aging of red wines on lees. *American Journal of Enology and Viticulture*, 54, 150-157.

Ferrer-Gallego, R., García-Marino, M., Hernández-Hierro, J. M., Rivas-Gonzalo, Escribano-Bailón, M. T. (2010). Statistical correlation between flavanolic composition, colour and sensorial parameters in grape seed during ripening. *Analytica Chimica Acta* 660, 22–28. <https://doi:10.1016/j.aca.2009.09.039>

García-Estévez, I., Alcalde-Eon, C., Escribano-Bailón, M. T. (2017). Flavanol Quantification of Grapes via Multiple Reaction Monitoring Mass Spectrometry. Application to Differentiation among Clones of *Vitis vinifera* L. cv. Rufete Grapes. *Journal of Agricultural and Food Chemistry* , 65 (31) , 6359-6368. DOI: 10.1021/acs.jafc.6b05278.

Kennedy, J. A., Ferrier, J., Harbertson, J. F., & Peyrot des Gachons, C. (2006). Analysis of tannins in red wine using multiple methods: Correlation with perceived astringency. *American Journal of Enology and Viticulture*, 57(4), 481-485.

Le Bourvellec, C., Bouchet B., Renard, C. M. G. C. (2005). Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochimica et Biophysica Acta*, 1725,10-18. <https://doi.org/10.1016/j.bbagen.2005.06.004>

Le Bourvellec, C., Guyot, S., Renard, C. M. G. C. (2009). Interactions between polyphenols and cell-walls: Modification of polysaccharide extractability. *Carbohydrate Polymers*, 75, 251-261.

Lei, X., Zhu, Y., Wang, X., Zhao, P., Liu, P., Zhang, Q., Chen, T., Yuan, H., Guo, Y. (2019). Wine polysaccharides modulating astringency through the interference on interaction of flavan-3-ols and BSA in model wine. *International Journal of Biological Macromolecules*, 139, 896-903. <https://doi.org/10.1016/j.ijbiomac.2019.08.050>

Lin, Z., Fischer, J., Wicker, L. (2016) Intermolecular binding of blueberry pectin-rich fractions and anthocyanin. *Food Chem.* 194, 986–993. <https://doi.org/10.1016/j.foodchem.2015.08.113>

Garcia-Marino, M., Rivas-Gonzalo, J. C., Ibañez, E., Garcia-Moreno, C. (2006). Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. *Analytica Chimica Acta*, 563, 44-50. <https://doi.org/10.1016/j.aca.2005.10.054>

Martínez-Lapuente, L., Guadalupe, Z., Ayestaran, B., Ortega-Heras, M., Perez-Magarino, S. (2013). Changes in polysaccharide composition during sparkling wine marking and aging. *Journal Agricultural and Food Chemistry*, 61, 12362-12373. <https://doi.org/10.1021/jf403059p>

Mateus, N., Carvalho, E., Luís, C., de Freitas, V. (2004). Influence of the tannin structure on the disruption effect of carbohydrates on protein–tannin aggregates. *Anal. Chim. Acta*. 513, 135–140. <https://doi.org/10.1016/j.aca.2003.08.072>

Monagas, M., Gomez-Cordove, C., Bartolomé, B., Laureano, O., Ricardo da Silva, J. M. (2003). Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem*, 51, 6475–6481. <https://doi.org/10.1021/jf030325+>

Osete-Alcaraz, A., Bautista-Ortín, A. A., Gómez-Plaza, E. (2019). The Role of Soluble Polysaccharides in Tannin-Cell Wall Interactions in Model Solutions and in Wines. *Biomolecules*, 10, 36. <https://doi.org/10.3390/biom10010036>

Pellerin, P., Vidal, S., Williams, P., Brillouet, J. M. (1995). Characterization of five type II arabinogalactan-protein complexes from red wine with increasing uronic acid content. *Carbohydrate Research*, 190, 183-197. [https://doi.org/10.1016/0008-6215\(95\)00206-9](https://doi.org/10.1016/0008-6215(95)00206-9)

Pellerin, P., Cabanis, J-C. (1998). Les glucides. In *nologie: fondements scientifiques et technologiques*. Lavoisier, Tech. & Doc, France: Paris, 40-92.

Ramos-Pineda, A. M., García-Estévez, I., Brás, N. F., Martín del Valle, E. M., Dueñas, M., & Escribano Bailón, M. T. (2017). Molecular approach to the synergistic effect on astringency elicited by mixtures of flavanols. *Journal of Agricultural and Food Chemistry*, 65, 6425–6433. <https://doi.org/10.1021/acs.jafc.7b01600>

Renard, C. M., Baron, A., Guyot, S., Drilleau, J. F. (2001). Interactions between apple cell walls and native apple polyphenols: Quantification and some consequences. *Int. J. Biol. Macromol.*, 29, 115–125. [https://doi.org/10.1016/S0141-8130\(01\)00155-6](https://doi.org/10.1016/S0141-8130(01)00155-6)

Renard, M. G. C. C, Watrelot, A. A., Le Bourvellec, A. (2017). Interactions between polyphenols and polysaccharides: Mechanisms and consequences in food processing and digestion. *Trends in Food Science & Technology*, 63, 43-51. <https://doi.org/10.1016/j.tifs.2016.10.022>

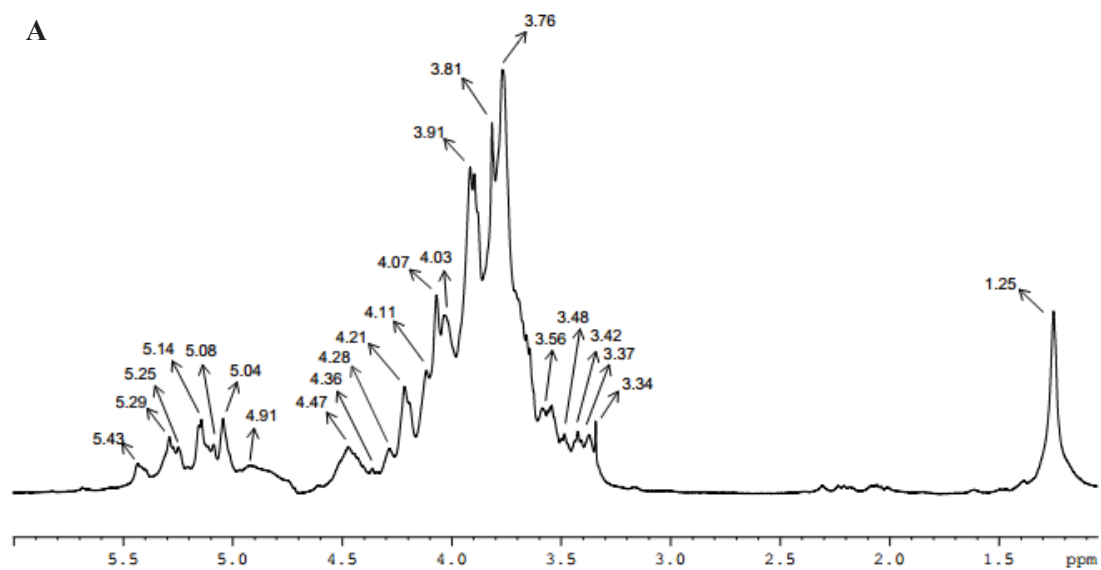
Ruiz-García, Y., Smith, P. A., Bindon, K. A. (2014) Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydr. Polym.* 114, 102–114. <https://doi.org/10.1016/j.carbpol.2014.07.024>

Sasaki, G. L., Guerrini, M., Serrato, R. V., Santana-Filho, A. P., Carlotto, J., Simas-Tosin, F., Cipriani, T. R., Iacomini, M., Torri, G., & Gorin, P. A. J. (2014). Monosaccharide composition of glycans based on Q-HSQC NMR. *Carbohydrate Polymers*, 104, 34-41. <https://doi.org/10.1016/j.carbpol.2013.12.046>

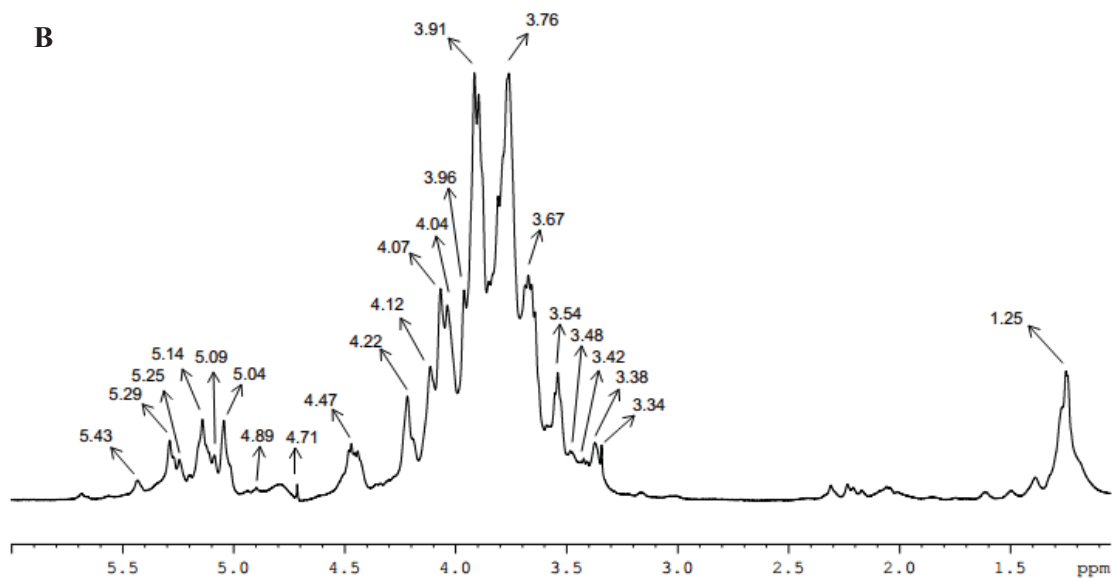
Vidal, S., Williams, P., Doco, T., Moutounet, M., Pellerin, P. (2003). The polysaccharides of red wine: Total fractionation and characterization. *Carbohydrate Polymers*, 54, 439-447. [https://doi.org/10.1016/S0144-8617\(03\)00152-8](https://doi.org/10.1016/S0144-8617(03)00152-8)

Supporting Information

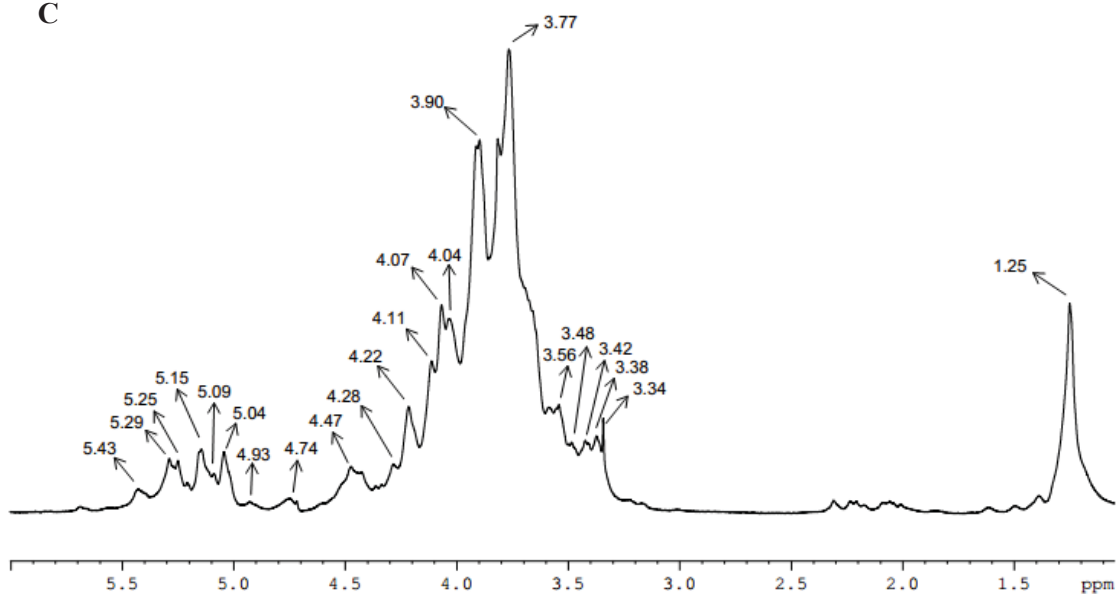
A



B



C



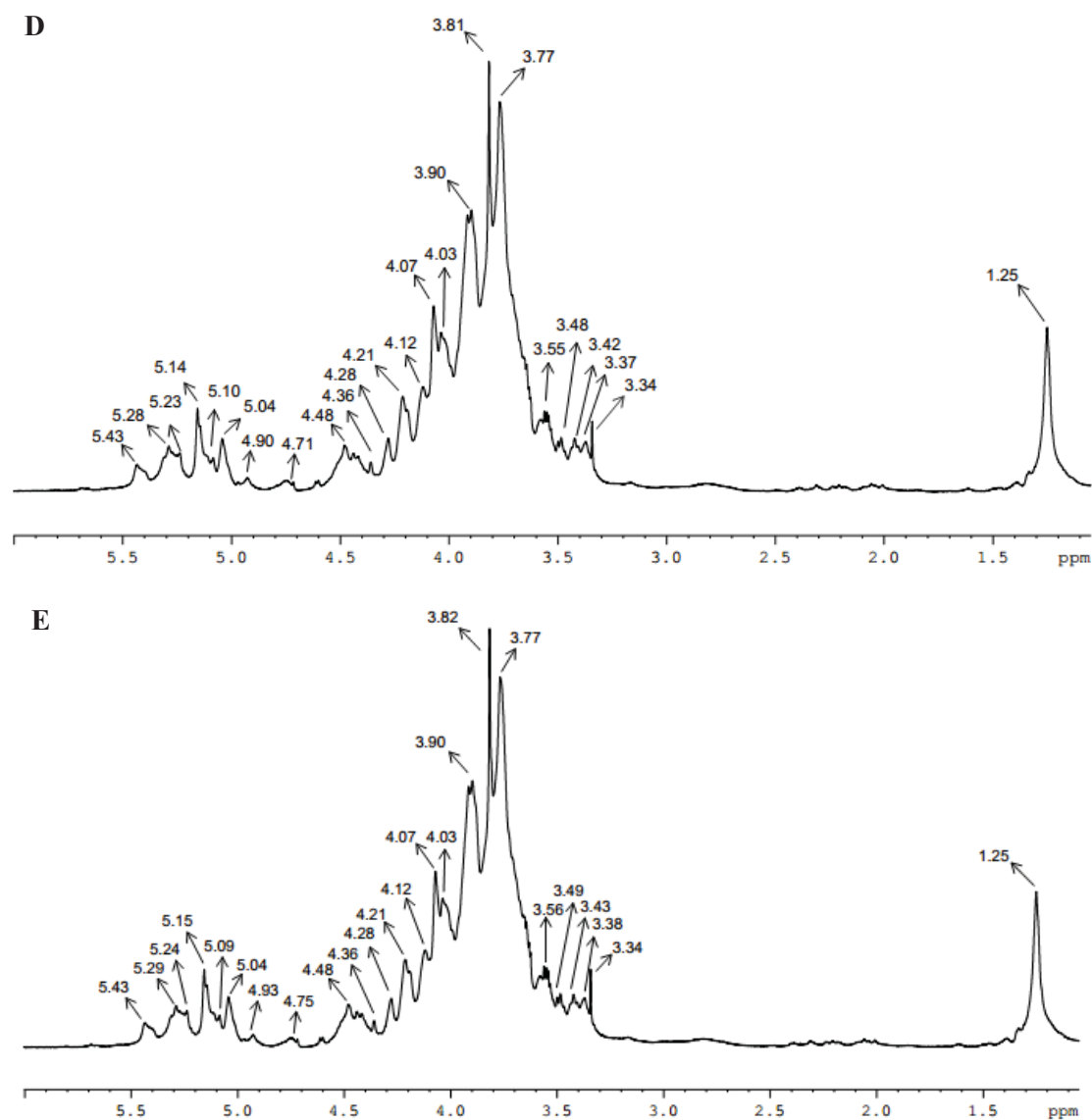


Figure S1: ^1H -NMR spectra of (A) CSCM. (B) CSJA. (C) CSVA. (D) CSFA and (E) TPFO in D_2O at 30°C (chemical shifts are expressed in δ ppm).

Table S1: Description of the samples used in the experiments

Sample	Grape variety	vintage	zone	pH	alcoholic content
CSCM	Cabernet	2017	Castilla la	3.29	13.5
	Sauvignon		Mancha		
CSVA	Cabernet	2017	Castilla y	3.30	13.5
	Sauvignon		León		
CSFA	Cabernet	2017	Castilla la	3.40	13.0
	Sauvignon		Mancha		
CSJA	Cabernet	2018	Castilla y	3.36	14.0
	Sauvignon		León		
TPHO	Tempranillo	2018	Castila y	3.39	14.5
			León		

Table S2: Procyanidins determined in Cabernet Sauvignon seed grapes

Procyanidins	FSE (%)
Monomers	32.91
Monomers Galloylated	0.97
Dimers	35.41
Dimers Galloylated	9.80
Dimers Digalloylated	0.51
Trimmers	12.05
Trimmers Galloylated	1.31
Trimmers Digalloylated	0.22
Tetramers	5.27
Tetramers Galloylated	0.79
Tetramers Digalloylated	0.00
Pentamers	0.72
Pentamers Galloylated	0.05
No Galloylated	86.36
Galloylated	13.64
mDP	2.21

The mean percentage was calculated from the individual percentage analyzed in triplicate.

Table S3: Pearson's Coefficients Correlation between polysaccharidic composition and molecular weight

Polysaccharides		Molecular weight			
		P1	P2	P3	P4
Mannan/ Mannoprotein	r	-0.883*	-0.783	0.975**	0.620
	<i>p</i> value	0.047	0.117	0.005	0.265
AGII	r	0.516	0.953*	-0.840	-0.703
	<i>p</i> value	0.373	0.012	0.075	0.185
RGs	r	0.942*	0.334	-0.702	-0.464
	<i>p</i> value	0.017	0.583	0.187	0.431
Xylan	r	-0.162	-0.642	0.324	0.744
	<i>p</i> value	0.795	0.243	0.595	0.150
Dextrin	r	-0.847	-0.943*	0.967**	0.893*
	<i>p</i> value	0.070	0.016	0.007	0.041

r: Pearson correlation coefficient. * and ** indicate the level of significance (no significant, $p < 0.05$, and $p < 0.01$, respectively, $n = 5$)

CAPITULO VI

Neste capítulo encontram-se as considerações finais, composta de resultados complementares, conclusão geral da tese e lista geral de referências.

1. RESULTADOS COMPLEMENTARES

1.1. Análise dos pigmentos antocianos em vinhos

Para a identificação e quantificação dos pigmentos antocianos, foram adicionados 2 mL de vinho a 2 mL de HCl 0,1N. Em seguida, 2 mL desta solução foram carregados em um cartucho SPE de troca catiônica de modo misto/fase reversa Oasis MCX (60 mg, 3 mL) da Waters (Milford, MA, EUA), previamente condicionada com 2 mL de metanol seguido por 2 mL água ultrapura. Em seguida foi lavado com 4 mL de água e as antocianinas foram eluídas com 8 mL de metanol. O eluato foi rotaevaporado a 30 °C e depois reconstituído em 500 µL de água ultrapura para obter o extrato de vinho de antocianinas. A quantificação foi realizada por HPLC - DAD - ESI / MS (García-Estévez, Alcalde-Eon & Escribano-Bailón, 2017). Para isso, 390 µL deste extrato foram misturados com 10 µL de ácido clorogênico (1 mg / mL), que foi utilizado como padrão interno, antes da injeção no sistema cromatográfico.

A análise por HPLC-MS dos compostos fenólicos nos vinhos mostrou maior concentração de antocianinas entre 141 e 364 mg/L para os vinhos Cabernet Sauvignon e 424,3 mg / L para o vinho Tempranillo (tabela 1). Os monoglicosídeos foram as antocianinas mais abundantes em todos os vinhos estudados, seu conteúdo variou entre 29 e 53% (tabela 1).

Tabela 1: Antocianinas determinadas nos vinhos Cabernet Sauvignon e Tempranillo

Amostras	Pigmentos antocianos (%)					Total
	Monoglicosídeos	Acetatos	Cumaratos	F-A+	Piranoantocianinas	mg/L
CSCM	53,01 ^a	18,39 ^a	4,10 ^c	7,65 ^c	16,85 ^b	165,43
CSJA	50,92 ^a	17,55 ^a	10,73 ^b	6,30 ^c	14,50 ^b	363,58
CSVA	36,16 ^b	19,06 ^a	12,00 ^{ab}	9,76 ^b	23,02 ^a	141,58
CSFA	51,32 ^a	13,73 ^{ab}	12,00 ^{ab}	9,97 ^b	12,98 ^c	172,69
CSMO	29,36 ^b	18,02 ^a	15,31 ^a	12,23 ^a	25,09 ^a	206,06
TPHO	60,73 ^a	8,59 ^b	11,09 ^b	7,65 ^c	11,95 ^c	424,35

A percentagem média foi calculada a partir da percentagem individual de cada composto analisado em triplicata. F-A+: Produtos de condensação direta de flavonol antocianina

1.2. Análises colorimétricas

Os espectros de absorção (190–770 nm) foram registrados no pH do vinho e após ajuste a pH 3,6 para todos os tipos de vinhos e em todas os pontos de amostragem em um espectrofotômetro Hewlett-Packard UV-vis HP 8453 (Agilent technologies, Waldbronn, Alemanha) com cubeta de quartzo de 2 mm de comprimento. A análise de cores foi feita a partir dos espectros visíveis (380–770 nm). A Cor CIELAB para os parâmetros (L^* , a^* , b^* , C^* , ab e hab) foram calculados utilizando o software CromaLab® (Heredia, Álvarez, González-Miret e Ramirez, 2004).

A tabela 2 mostra os principais parâmetros do CIELAB em todos os vinhos analisados. No parâmetro de luminosidade (L) é avaliada a claridade do vinho. Vinhos com valor alto de luminosidade são mais escuros e possuem grande quantidade de flavonoides. TPHO apresentou valor de luminosidade mais alto (64,63). O parâmetro cromaticidade está relacionado com a unificação das cores, vinhos com padrão de cor unificado apresentam C^* elevado, na tabela 1 é possível observar que todos os vinhos analisados apresentaram C^* entre 40 e 54, considerados valores aceitáveis para vinhos. O parâmetro hab está relacionado a cor do vinho, valores entre 0 e 90 são correspondentes as cores vermelho, laranja e amarelo, que são cores as cores encontradas nos vinhos. TPHO apresentou valor 7,02 que corresponde a cor vermelha e os outros vinhos que possuem valores mais elevados correspondem as cores vermelho-alaranjadas. O vinho TPHO apresentou valores dos parâmetros CIELAB elevados em comparação com os vinhos analisados, isso pode estar relacionado ao maior conteúdo de antocianos nesse vinho (424,35 mg/L) como mostrado na tabela 1 (Alcalde-Leon et al, 2019).

Tabela 2: Parâmetros CIELAB (L , Cab e Hab) dos vinhos Cabernet Sauvignon e Tempranillo

Amostras	L	Cab	Hab
CSCM	51,26	40,07	18,56
CSJA	57,67	47,25	25,10
CSVA	57,21	43,47	27,32
CSFA	59,48	41,02	21,55
CSMO	57,38	43,54	27,53
TPHO	64,63	53,71	7,02

Referências

Alcalde-Eon, C., Ferreras-Charroa, R., Ferrer-Gallegob, R., Riveroc, R. J., Herediad, Escribano-Bailón, M. T. Monitoring the effects and side-effects on wine colour and flavonoid composition of the combined post-fermentative additions of seeds and mannoproteins. *Food Research International*, 126. (2019)

García-Estévez, I., Alcalde-Eon, C., Escribano-Bailón, M. T. Flavanol Quantification of Grapes via Multiple Reaction Monitoring Mass Spectrometry. Application to Differentiation among Clones of *Vitis vinifera* L. cv. Rufete Grapes. *Journal of Agricultural and Food Chemistry* , 65 (31) , 6359-6368. (2017).

Heredia, F. J., Álvarez, C., González-Miret, M. L., & Ramirez, A. ChromaLab Análisis de Color; Registro General de la Propiedad Intelectual SE-1052-04: Sevilla, Spain. (2004).

CONCLUSÃO GERAL

Após a análise dos resultados obtidos nesta pesquisa, pode-se concluir que:

- Os polissacarídeos encontrados nos vinhos Cabernet Franc, Cabernet Sauvignon e Sauvignon Blanc foram: arabinogalactana do tipo II, ramnogalacturonana do tipo I e II, manana e dextrina. O polissacarídeo majoritário presente nos três vinhos é uma arabinogalactana tipo II. Ela é formada por uma cadeia principal de unidades de β -D-Galp (1 $\rightarrow$ 3)-ligadas, substituída em O-6 por cadeias laterais de β -D-Galp (1 $\rightarrow$ 6)-ligadas. As cadeias laterais são substituídas em O-3 por terminais não redutores de α -L-Araf. A ramnogalacturonana do tipo I encontrada é formada pela repetição da unidade dissacarídica [$\rightarrow$ 4)- α -D-GalpA-(1 $\rightarrow$ 2)- α -L-Rhap-(1 $\rightarrow$)]. A manana presente nos vinhos estudados é um polissacarídeo constituído por uma cadeia principal de unidades α -D-Manp (1 $\rightarrow$ 6) ligadas, substituída em O-2 por cadeias laterais curtas de α -D-Manp (1 $\rightarrow$ 2) ligadas.
- O estudo realizado com vinhos Cabernet Sauvignon da mesma vinícola em diferentes safras mostrou que a porcentagem de cada polissacarídeo e também o rendimento total de polissacarídeos permaneceram praticamente inalterados nas diferentes safras. A porcentagem semelhante na composição de monossacarídeos, bem como a quantidade de polissacarídeos, pode sugerir manutenção da qualidade do vinho, indicando que a vinícola mantém o mesmo padrão no processo de produção de vinho ao longo dos anos.
- O efeito anti-inflamatório das frações polissacarídicas extraídas de vinhos foi avaliada em células RAW 264.7. As frações reduziram marcadamente a produção de óxido nítrico (NO) e citocinas pró-inflamatórias (TNF- α e IL-1 β) em células estimuladas com LPS. Além disso, as análises realizadas *in vivo* em camundongos submetidos a CLP mostraram que a administração oral e subcutânea dos polissacarídeos apresentaram um aumento na sobrevivência dos camundongos com sepse, quando comparados com o controle. Levando também a diminuição dos níveis de citocinas pró-inflamatórias, bem como os níveis séricos de AST, ALT, bilirrubina, uréia e creatinina, com consequente proteção contra dano tissular. Além disso, foi observada a biodistribuição dos polissacarídeos marcados nos tecidos e órgãos após administração subcutânea em camundongos com sepse por CLP e a biodistribuição desse polímero pelo organismo aparentemente acontece através do sistema

imunológico. Os polissacarídeos demonstraram potencial anti-inflamatório *in vitro* e *in vivo*, o que pode indicar efeitos benéficos do consumo moderado de vinho para a saúde humana.

- As análises realizadas mostraram que os vinhos Cabernet Sauvignon e Tempranillo têm um padrão semelhante em sua composição flavânica, embora haja diferenças marcantes entre as porcentagens de cada família de flavanóis entre os diferentes vinhos. As análises estatísticas indicaram que uma maior afinidade de certas famílias de polissacarídeos, como mananas, manoproteínas, xilanas e dextrinas, em relação aos flavanóis, causando maior precipitação destes quando eles interagem. Isso pode explicar a relação inversa observada entre a concentração desses polissacarídeos e a dos flavanóis nos vinhos estudados, o que indica que uma quantidade maior de determinadas famílias de polissacarídeos em um vinho pode afetar o conteúdo dos flavanóis, o que teria consequências importantes para propriedades organolépticas do vinho. Além disso, a análise da composição pigmentos dos vinhos também mostrou uma variabilidade importante entre os vinhos estudados, o que se traduz em diferenças importantes nos parâmetros colorimétricos dos vinhos (principalmente clareza e tom) e, portanto, na cor destes.

CONCLUSIONES

Después de analizar los resultados obtenidos en esta investigación, se puede concluir que:

- Los polisacáridos encontrados en los vino Cabernet Fran, Cabernet Sauvignon y Sauvignon Blanc fueron: arabinogalactanos de tipo II, ramnogalacturonanos de tipo I y II, manano y dextrina. La familia de polisacáridos mayoritaria encontrada en los tres vinos fueron los arabinogalactanos de tipo II, los cuales están formados por una cadena principal de unidades de β -(1 $\rightarrow$ 3)-D-Galp que presenta ramificaciones en las posiciones O-6 formadas por cadenas laterales de β -(1 $\rightarrow$ 6)-D-Galp. A su vez, en las cadenas laterales aparecen sustituciones en las posiciones O-3 de monosacáridos no reductores de α -L-Araf. Los ramnogalacturonanos de tipo I encontrados estaban formados por la repetición de la unidad disacáridica [$\rightarrow$ 4)- α -D-GalpA-(1 $\rightarrow$ 2)- α -L-Rhap-(1 $\rightarrow$]. Por último, los mananos presentes en los vinos estudiados son polisacáridos constituídos por una cadena principal de unidades α -(1 $\rightarrow$ 6)- D-Manp que presenta ramificaciones en las posiciones O-2 formadas por pequeñas cadenas laterales de α -(1 $\rightarrow$ 2)-D-Manp.
- El estudio realizado con los vinos Cabernet Sauvignon elaborados por la misma bodega en diferentes vendimias mostró que el porcentaje de cada polisacárido, así como el contenido total de estos compuestos en el vino, no se ven modificados en las diferentes vendimias. La estabilidad en la composición de polisacáridos (tanto en su contenido total como en su composición porcentual), podría indicar una gran regularidad en la calidad del vino, mostrando así que la bodega mantiene el mismo patrón en el proceso de producción de los vinos a lo largo de los diferentes años.
- El efecto antiinflamatorio de las fracciones de polisacáridos extraídas de los vinos se estudió en células RAW 264.7. Los polisacáridos ensayados redujeron de forma importante la producción de óxido nítrico (NO) y citocinas proinflamatorias (TNF- α e IL-1 β) en las células estimuladas con LPS. Además, los análisis realizados *in vivo* con ratones sometidos a CLP mostraron que la administración oral y subcutánea de los polisacáridos obtenidos no solo generaba un aumento en la supervivencia de los ratones con sepsis en comparación con la población control, sino que también provocaba la disminución de los niveles de citocinas proinflamatorias, así como los niveles séricos de AST, ALT, bilirrubina, urea y creatinina, lo

que puede asociarse a una protección contra el daño celular. Por otro parte, se pudo observar una migración de los polisacáridos marcados en los tejidos y órganos después de la administración subcutánea en los ratones con sepsis por CLP. Esta biodistribución de los polisacáridos por los órganos ocurre, aparentemente, a través del sistema inmunológico. Los polisacáridos demostraron, por tanto, un potencial anti-inflamatorio *in vitro* e *in vivo*, lo que puede indicar un posible efecto beneficioso del consumo moderado de vino para la salud humana.

- Los análisis realizados mostraron que los vinos de Cabernet Sauvignon y Tempranillo tienen un patrón similar en su composición flavánica, aunque existen diferencias marcadas entre los porcentajes de cada familia de flavanoles entre los diferentes vinos. Los análisis estadísticos señalaron que una mayor afinidad de ciertas familias de polisacáridos, como mananos, manoproteínas, xilanos y dextrinas, hacia los flavanoles, provocando una mayor precipitación de éstos cuando interaccionan. Esto puede explicar la relación inversa observada entre la concentración de estos polisacáridos y la de flavanoles en los vinos estudiados, lo que indica que una mayor cantidad de ciertas familias de polisacáridos en un vino puede afectar al contenido de flavanoles, lo que tendría consecuencias importantes en las propiedades organolépticas del vino. Además, el análisis de la composición de pigmentos de los vinos mostró también una importante variabilidad entre los vinos estudiados, lo que se traduce en importantes diferencias en los parámetros colorimétricos de los vinos (principalmente claridad y tono) y, por tanto, en el color de éstos.

LISTA DE REFERÊNCIAS

ABAD, F. B.; PLASENCIA, J. M. B. Consumo moderado de bebidas alcohólicas: salud y civilización. Madrid: Inesiba, 135 p, 1993.

ABDEL-MOTTALEB, M. M., BEDUNEAU, A., PELLEQUER, Y. & LAMPRECHT, A. Stability of fluorescent labels in PLGA polymeric nanoparticles: quantum dots versus organic dyes. *Int. J. Pharm.*, 494 (1), 471-478, 2015.

ABRAHAM, E., GLAUSER, M. P., BUTLER, T., GARBINO, J., GELMONT, D., LATERRE, P. F., KUDSK, K., BRUINING, H. A, OTTO, C., TOBIN, E., ZWINGELSTEIN, C., LESSLAUER W. Leighton, p55 tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock. A randomized controlled multicenter trial. Ro 45-2081 study group, *JAMA*. 277, 1531–1538, 1987.

ADRIAN, M.; JEANDET, P.; BREUIL, A. C.; LEVITE, D.; DEBORD, S.; BESSIS, R. Assay of resveratrol and derivative stilbenes in wines by direct injection High Performance Liquid Chromatography. *American Journal of Enology & Viticulture*, Davis, v. 51, n. 1, p. 37-40, 2000.

AGARWAL, S., PIESCO, N. P., JOHNS, L. P., RICCELLI, A. E. Differential expression of IL-1 β , TNF- α , IL-6, and IL-8 in human monocytes in response to lipopolysaccharides from different microbes. *Journal of Dental Research*, 74, 1057–65, 1995.

AKIRA, S., TAKEDA, K. Toll-like receptor signalling. *Nature Reviews Immunology*. 4, 499– 511, 2004.

ALBERSHEIM, P.; DARVILL, A. G.; O'NEILL, M. A.; SCHOLS, H. A.; VORAGEN, A. G. J. An hypothesis: the same six polysaccharides are components of the primary cell walls of all higher plants. *Progress in biotechnology: Pectins and Pectinases*. Amsterdam: Elsevier, v. 14, p. 47-55, 1996.

ALCALDE-EON, C., ESCRIBANO-BAILÓN, M. T., SANTOS-BUELGA, C., RIVAS-GONZALO, J. C. Separation of pyranoanthocyanins from red wine by column chromatography. *Analytica Chimica Acta*, 513(1), 305–318, 2004.

Alcalde-Eon, C., Ferreras-Charroa, R., Ferrer-Gallegob, R., Riveroc, R. J., Herediad, Escribano-Bailón, M. T. Monitoring the effects and side-effects on wine colour and flavonoid composition of the combined post-fermentative additions of seeds and mannoproteins. *Food Research International*, 126, 2019.

AMERINE, M. A. Le vin dans l'alimentation humaine. Bulletin de l'O.I.V. Paris, número especial, p. 137-151, 1977.

ANGUS, D. C., LINDE-ZWIRBLE, W. T., LIDICKER, J., CLERMONT, G. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Critical Care Medicine*, 29, 1303–1310, 2001.

APOLINAR-VALIENTE, R., WILLIAMS, P., ROMERO-CASCALES, I., GOMEZ-PLAZA, E., LOPEZ-ROCA, J. M., ROS-GARCIA, J. M., et al. Polysaccharide composition of Monastrell red wines from four different Spanish Terroirs: Effect of wine-making techniques. *Journal of Agricultural and Food Chemistry*, 61(10), 2538–2547, 2013.

APOLINAR-VALIENTE, R., ROMERO-CASCALES, I., WILLIAMS, P., GÓMEZ-PLAZA, E., LÓPEZ-ROCA, J. M., ROS-GARCÍA, J. M., DOCO, T. Effect of winemaking techniques on polysaccharide composition of Cabernet Sauvignon, Syrah and Monastrell red wines. *Australian Journal of Grape and Wine Research*, 20, 62-71, 2014.

ASEHNOUNE, K., STRASSHEIM, D., MITRA, S., KIM, J.Y., ABRAHAM, E. Involvement of reactive oxygen species in toll-like receptor 4-dependent activation of NF- κ B. *The Journal of Immunology*, 172, 22–29, 2004.

ASPINAL, G. O. In: *Biogenesis of Plant Cell Wall Polysaccharides*. New York: Academic Press, p. 95-115, 1973.

ASPINAL, G. O. Chemistry of cell wall polysaccharides. In: STUMPF, P. K.; CONN, E. E. *The Biochemistry of Plants*. New York: Academic Press. v. 3, p. 473-500, 1980.

ASPINAL, G.O. Structural chemistry of the hemicelluloses. *Advances in Carbohydrate Chemistry*. New York, p. 429-430, 1959.

ATMODJO, M. A.; HAO, Z.; MOHNEN, D. Evolving views of pectin biosynthesis. *Annual Review of Plant Biology*, vol. 64, p. 747-749, 2013.

AVIGAD, G.; DEY, P. M. Carbohydrate metabolism: storage carbohydrates. In: DEY, P. M.; HARBORNE, J. B. *Plant Biochemistry*. Bristol: Academic Press, p.143, 1997.

AYESTARAN, B.; GUADALUPE, Z.; LEON, D. Quantification of major grape polysaccharides (Tremamillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta*, v. 513, p. 29-39, 2004.

AZH DARINIA, A., GHOSH, P., GHOSH, S., WILGANOWSKI, N. & SEVICK-MURACA, E. M. Dual-labeling strategies for nuclear and fluorescence molecular imaging: a review and analysis, *Mol. Imaging Biol.*, 14 (3), 261–276, 2012.

BADERSCHNEIDER, B., & WINTERHALTER, P. Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry*, 49(6), 2788–2798, 2001.

B. WEISS, B. et al. Nanoparticles made of fluorescence-labelled poly (L-lactide-co-glycolide): preparation, stability, and biocompatibility, *J. Nanosci. Nanotechnol.*, 6 (9–10), 3048-3056, 2006.

BAE, S. W., TAN, W. & HONG, J-I. Fluorescent dye-doped silica nanoparticles: new tools for bioapplications. *Chem. Commun.*, 48 (17), 2270-2282, 2012.

BALLOU, C.E. A study of the immunochemistry of three yeast mannans. *Journal of Biological Chemistry*, v.245, n.5, p.1197-1203, 1970.

BARÓN, R.; MAYÉN, M.; MÉRIDA, J.; MEDINA, M. Changes in phenolic compounds and browning during biological aging of SherryType Wine. *J. Agric. Food Chem.* 45: 1682- 1685. 1997.

BARRIO-GALÁN, DEL R., CÁCERES-MELLA, A., MEDEL-MARABOLÍ, M., PEÑA-NEIRA, A. Effect of selected *Saccharomyces cerevisiae* yeast strains and different aging techniques on the polysaccharide and polyphenolic composition and sensorial characteristics of Cabernet Sauvignon red wines, 2015.

BARRETO-BERGTER, E.; GORIN, P. A. J.; TRAVASSOS, L. R. Cell constituents of the mycelia and conidia of *A. fumigatus*. *Carbohydrates Letters*, 95 (1): 205-218, 1981.

BARRINGTON, R.; ZHANG, M., FISCHER, M., CARROLL, M.C. The role of complemente in inflammation and adaptative immunity. *Immunolo Rev.* 180, 5-15, 2001.

BARTNICKI-GARCIA, S; LIPPMAN, E. Fungal morphogenesis: Cell wall construction in *Muca rouxii*. *Scienc*, 1659(1): 302-304, 1969.

BELLEVILLE, M. P., BRILLOUET, J. M., TARODO DE LA FUENTE, B., MOUTOUNET, M. Polysaccharide effects on cross-flow microfiltration of two red wines with a microporous alumina membrane. *Journal Food Science*, 55, 1598-1602, 1991.

BAU, A. E., DURHAN, R., FAIST, E. Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): Are we winning the battle? *Shock*, 10, 79-89, 1998.

BEZERRA, I.L.; CAILLOT, A. R. C.; PALHARES, L. C. G. F.; SANTANA-FILHO, A. P.; CHAVANTE, S. F. GUILHERME LANZI SASSAKI, G. L. Structural characterization of polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines:

Anti-inflammatory activity in LPS stimulated RAW 264.7 cells. *Carbohydrate Polymers*, 186, 91-99, 2018.

BEZERRA, I.L., CAILLOT, A. R. C., OLIVEIRA, A. F., SANTANA-FILHO, A. P. & SASSAKI, G. L. Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice. *Carbohydrate Polymers*, 210, 254-263, 2019.

BHATIA M, MOOCHHALA S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *The Journal of Pathology*, 202(2), 145-56, 2004.

BINDON, K.A.; SMITH, P.A.; KENNEDY, J.A. Interaction between grape-derived proanthocyanidins and cell wall material. Effect on proanthocyanidin composition and molecular mass. *J. Agric. Food Chem*, 58, 2520–2528, 2010.

BONE, R. C., BALK, R. A, CERRA, F. B., DELLINGER, R. P., FEIN, A. M., KNAUS, W. A., SCHEIN, R. M., SIBBALD, W. J. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis, *Chest*. 101, 1644-1655, 1992.

BONE, R. C., BALK, R. A., FEIN, A. M., PERL, T. M., WENZEL, R. P. REINES, H. D., QUENZER, R.W., IBERTI, T. J., MACINTYRE, N., SCHEIN R. M. A second large controlled clinical study of E5, a monoclonal antibody to endotoxin: Results of a prospective, multicenter, randomized, controlled trial. The E5 Study Investigators. *Critical Care Medicine*, 23, 94–1006, 1995.

BOHN, J. A.; Be MILLER, J. N. 1 →3 β-D-glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers*, v. 28, p. 3-14, 1995.

BONE, R. C. et al. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest*, 101, 1644-1655, 1992.

BOURZEIX, M. Raisin, vin, nutrition et santé. Les catéchines et proanthocyanidols du raisin et du vin. *Progrès Agricole et Viticole*, Montpellier, v. 106, n. 22, p. 487-491, 1989.

BRERETON, R. G. Chemometrics: Data analysis for the laboratory and chemical plant. Chichester, West Sussex, England: J. Wiley, 2003.

BRETT, C.; WALDRON, K. Physiology and biochemistry of plant cell walls. London: Unwin Hyman, 194p, 1990.

BRILLOUET, J. M., BOSSO, C., MOUTOUNET, M. (1990). Isolation, purification, and characterization of an arabinogalactan from a red wine. *American Journal of Enology and Viticulture*, 41, 29-36, 1990.

BRITO, M. A., SILVA, R. F., BRITES, D. Bilirubin toxicity to human erythrocytes: a review, *Clinica Chimica Acta*. 374, 46-56, 2006.

BROSSAUD, F., CHEYNIER, V., & NOBLE, A. C. Bitterness and astringency of grape and wine polyphenols. *Australian Journal of Grape and Wine Research*, 7(1), 33–39, 2001.

CAILLOT, A. R. C.; BEZERRA, I.L.; PALHARES, L. C. G. F.; SANTANA-FILHO, A. P.; CHAVANTE, S.; SASSAKI, G. L. Structural characterization of blackberry wine polysaccharides and immunomodulatory effects on LPS-activated RAW 264.7 macrophages. *Food Chemistry*, 257, 143-159, 2018.

CABANIS, J. C.; CABANIS, M. T. Tablas de composición. Cap.8. In: FLANZY, C. *Enología: Fundamentos científicos y tecnológicos*. Madrid: Ediciones A. Madrid Vicente, Ediciones Mundi-Prensa. 1ª edición, 783p, 2000b.

CABIB, E. Molecular aspects of yeast morphogenesis. *Annual Review of Microbiology*, 29: 191-214, 1975.

CAPEK, P.; HRIBALOV£, V.; SVANDOV£, E.; EBRINGEROV£, A.; SASINKOV£, V., MASAROV£, J. Characterization of immunomodulatory polysaccharides from *Salvia officinalis* L. *International Journal of Biological Macromolecules*, v. 33, p. 113-119, 2003.

CARPITA, N. C.; GIBEAUT, D. M. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, v. 3, p. 1-30, 1993.

CARPITA, N.; McCANN, M. The cell wall. In: BUCHANAN, B. B.; GRUISSEM, W.; JONES, R. L. *Biochemistry & Molecular Biology of Plants*. USA: Courier. p. 52-108, 2000.

CASTILLO-MUÑOZ, N., WINTERHALTER, P., WEBER, F., GÓMEZ, M. V., GÓMEZ-ALONSO, S., GARCÍA-ROMERO, E. Structure elucidation of peonidin 3,7-O-β-diglucoside isolated from Garnacha Tintorera (*Vitis vinifera* L.) grapes. *Journal of Agricultural and Food Chemistry*, 58(20), 11105–11111, 2010.

CEJUDO-BASTANTE, M.J.; HERMOSÍN-GUTIERREZ, I.; CASTRO-VAZQUEZ, L.I.; PÉREZ-COELLO, M.S. Hyperoxygenation and bottle storage of Chardonnay white wines: Effects on color-related phenolics, volatile composition, and sensory characteristics. *J. Agric. Food Chem.* 59: 4171- 4182, 2011.

CHEN, J. K., WANG, W. C, ZANG, L., ZHAO, J., LI, W., JIANG, T. Repression of a chromatin modifier aggravates lipopolysaccharide-induced acute lung injury in mouse. *Biochemical and Biophysical Research Communications*. 471, 515-521, 2016.

CHEYNIER, V.; DUEÑAS-PATON, M.; SALAS, E.; MAURY, C.; SOUQUET, J. M.; SARNI-MANCHADO, P.; FULCRAND, H. Structure and properties of wine pigments and tannins. *Am. J. Enology Vitic.*, 57: 298-305, 2006.

CHIU-TSUN, T. P., NG Y-F., HO S., GYDA M., CHAN S-W. Resveratrol and cardiovascular health – promising therapeutic or hopeless illusion? *Pharmacological Research*, 90, 88–115, 2014.

CINEL I, OPAL SM. Molecular biology of inflammation and sepsis: a primer. *Crit Care Med*;37:291–304. 2009.

CHEYNIER, V., SCHNEIDER, R., SALMON, J. -M., & FULCRAND, H. Chemistry of wine. *Comprehensive Natural Products II*, 3, 1119–1172, 2010.

CIPRIANI, T. R.; MELLINGER, C. G.; SOUZA, L. M., BAGGIO, C. H., FREITAS, C. S. Polysaccharide from a tea (Infusion) of *Maytenus ilicifolia* leaves with anti-ulcer protective effects. *Journal of Natural Products*, v. 69, p. 1018-1021, 2006.

CIPRIANI, T.R., MELLINGER, C.G., SOUZA, L.M., BAGGIO, C.H., FREITAS, C.S., MARQUES, M.C.A., GORIN, P.A.J., SASSAKI, G.L., IACOMINI, M. Acidic heteroxylans from medicinal plants and their anti-ulcer activity. *Carbohydrate Polymers*, 74, 274-278, 2008.

CIPRIANI, T. R.; MELLINGER, C. G.; BERTOLINI, L. C.; BAGGIO, C. H.; FREITAS, C. S.; MARQUES, M. C. A.; GORIN, P. A. J; SASSAKI, G. L.; IACOMINI, M. Gastroprotective effect of a type I arabinogalactan from soybean meal, *Food Chemistry*. V115. p. 687-690, 2009a.

CIPRIANI, T. R.; MELLINGER, C. G.; SOUZA, L. M.; BAGGIO, C. H., FREITAS, C. S.; MARQUES, M. C. A.; GORIN, P. A. J.; SASSAKI, G. L. IACOMINI, M. Polygalacturonic acid: Another anti-ulcer polysaccharide from the medicinal plant *Maytenus ilicifolia*. *Carbohydrate Polymers*, v. 78, p.361-363, 2009b.

COHEN, J. The immunopathogenesis of sepsis. *Nature*. 420(6917), 885-91, 2002.

CORDEIRO, L. M., BEILKE, F., BETTIM, F. L., REINHARDT, V DE, F., RATTMANN, Y. D., IACOMINI, M. (1->2) and (1->6)-Linked beta-D-galactofuranan of microalga *Myrmecia biatorellae*, symbiotic partner of *Lobaria linita*. *Carbohydrate Polymers*. 90, 1779– 1785, 2012.

CORDEIRO CAILLOT, A. R.; BEZERRA, I. L.; PALHARES, L. C. G. F.; SANTANA-FILHO, A. P.; CHAVANTE, S.; SASSAKI, G. L. Structural characterization of blackberry wine polysaccharides and immunomodulatory effects on LPS-activated RAW 264.7 macrophages. *Food Chemistry*, 257, 143-159, 2018.

CIUCANU, I., KEREK, F., 1984. A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, 131, 209-217, 1984.

CRUZ, INÊZ. A *Vitis vinifera* e as demais *Vitis*. *Saúde de Viver*. p.63-95. 2006.

DARTORA, N.; SOUZA, L. M.; SIMONE M.M. PAIVA, S. M. M.; CAMILA T. SCOPARO, C. T.; MARCELLO IACOMINI, M.; GORIN, P. A. J.; YANNA D. RATTMANN, Y. D.; SASSAKI, G. L. Rhamnogalacturonan from *Ilex paraguariensis*: A potential adjuvant in sepsis treatment. *Carbohydrate Polymers*, 1776–1782, 2013.

DECENDIT, A., WAFFO-TEGUO, P., RICHARD, T., KRISA, S., VERCAUTEREN, J., MONTI, J. -P., et al. Galloylated catechins and stilbene diglucosides in *Vitis vinifera* cell suspensions cultures. *Phytochemistry*, 60(8), 795–798, 2002.

DEL BARRIO-GALAN, R., PÉREZ-MAGARINO, S., ORTEGA-HERAS, M., WILLIAMS, P., DOCO, T. Effect of aging on lees and of three different dry yeast derivative products on Verdejo white wine composition and sensorial characteristics. *Journal of Agricultural and Food Chemistry*, 59, 12433–12442, 2011.

DEL CARMEN JUÁREZ-VÁZQUEZ, M., ALONSO-CASTRO, A. J., GARCÍA-CARRANCÁ, A. Kaempferitrin induces immunostimulatory effects in vitro. *Journal of Ethnopharmacology*, 148, 337-340, 2013.

DELGOBO, C. L., GORIN, P. A. J., JONES, C., IACOMINI, M. Gum heteropolysaccharide and free reducing mono- and oligosaccharides of *Anadenanthera colubrina*. *Phytochemistry*, 47, 1207-1214, 1998.

DELGOBO, C. L., GORIN, P. A. J., TISCHER, C. A., IACOMINI, M. The free reducing oligosaccharides of angico branco (*Anadenanthera colubrina*) gum exudates: an aid for structural assignments in the heteropolysaccharide. *Carbohydrate Research*, 320, 167-175, 1999.

DEY, P.M.; BROWNLEADER, M.D.; HARBORNE, J.B. The plant, the cell and its molecular components. In: DEY, P. M.; HARBORNE, J. B. *Plant Biochemistry*. Bristol: Academic Press, p. 6-9. 1997.

DHAINAUT, J. F., MARIN, N., MIGNON, A., VINSONNEAU, C. Hepatic response to sepsis: interaction between coagulation and inflammatory processes. *Critical Care Medicine*, 29, 42-47, 2001.

DIAZ-GEREVINI, G. T., REPOSSI, G., DAIN, A., TARRES, M. C., DAS, U. N., EYNARD, A. R. Beneficial action of resveratrol: How and why?. *Nutrition*, 2, 174-178. 2016.

DOCO, T., WILLIAMS, P.; CHEYNIER, V. Effect of flash release and pectinolytic enzyme treatments on wine polysaccharide composition. *Journal Agricultural and Food Chemistry*, 55 (16), 6643–6649, 2007.

DOCO, T.; VUCHOT, P.; CHEYNIER, V.; MOUTOUNET, M. Structural modification of arabinogalactan-proteins during aging of red wines on lees. *American Journal of Enology and Viticulture*, 54, 150–157. 2003.

DOCO, T.; QUELLEC, N.; MOUTOUNET, M.; PELLERIN, P.; Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of nology and Viticulture*, v. 50, p. 28-32, 1999.

DOCO, T., BRILLOUET, J. M. Isolation and characterization of a rhamnogalacturonan II from red wine. *Carbohydrate Research*, 243, 333-343, 1993.

ESTREICHER, S. K., Ed. *Wine: From Neolithic Times to 21st Century*. Nova York: Algora, 1 ed. 2006.

DONG, S. & ROMAN, M. Fluorescently labeled cellulose nanocrystals for bioimaging applications. *J. Am. Chem. Soc.*, 129 (45), 13810-13811, 2007.

FARGHALI, H., KUTINOVA C. N., LEKIC N. Resveratrol and related compounds as antioxidants with an allosteric mechanism of action in epigenetic drug targets. *Physiological Research*, 62, 1-13, 20013.

FARKAŠ, V. Polysaccharide Metabolism. In: ROSE, A.H.; HARRISON, J.S. *The yeasts: metabolism and physiology of yeasts*, 2ed. London: Academic Press, Vol.3, cap. 9, p. 317-366, 1989.

FERRER-GALLEGO, R., GARCÍA-MARINO, M., HERNÁNDEZ-HIERRO, J. M., RIVAS-GONZALO, ESCRIBANO-BAILÓN, M. T. Statistical correlation between flavanolic composition, colour and sensorial parameters in grape seed during ripening. *Analytica Chimica Acta* 660, 22–28, 2010.

FENGEL, D.; WEGNER, G. *Wood: chemistry, ultrastructure, reactions*. Berlin: Gruyter, W. 613p, 1989.

FEVERY, J. Bilirubin in clinical practice: a review. *Liver International*. 28, 592-605, 2008.

FILISSETTI-COZZI, T. M. C. C., CARPITA, N. C. Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry*, 197, 157-162, 1991.

FLAMINI, R. Mass spectrometry in grape and wine chemistry. Part I: Polyphenols. *Mass Spectrometry Reviews*, 22(4), 218–250. (2003).

FOURNAIRON, C.; CAMARASA, C.; MOUTOUNET, M.; SALMON, J. M. New trends on yeast autolysis and wine ageing on lees. *Journal International de la Vigne et du Vin*, 36, 49–69, 2002.

FRANSEN, C. T. M., HASELEY, S. R., HUISMAN, M. M. H., SCHOLS, H. A., VORAGEN, A. G. J., KAMERLING, J. P., VLIEGENTHART, J. F. G. Studies on the structure of a lithium-treated soybean pectin: characteristics of the fragments and determination of the carbohydrate substituents of galacturonic acid. *Carbohydrate Research*, 328, 539-547, 2000.

GARCÍA-ESTÉVEZ, I., ALCALDE-EON, C., ESCRIBANO-BAILÓN, M. T. Flavanol Quantification of Grapes via Multiple Reaction Monitoring Mass Spectrometry. Application to Differentiation among Clones of *Vitis vinifera* L. cv. Rufete Grapes. *Journal of Agricultural and Food Chemistry*, 65 (31), 6359-6368, 2017.

GARCIA-MARINO, M., RIVAS-GONZALO, J. C., IBAÑEZ, E., GARCIA-MORENO, C. Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. *Analytica Chimica Acta*, 563, 44-50, 2006.

GARRIDO J., BORGES F. Wine and grape polyphenols. A chemical perspective. *Food Research International*; 54: 1844-1858, 2013.

GERBAUD, V.; GABAS, N.; BLOUNIN, J.; PELLERIN, P.; MOUTOUNET, M. Influence of wine polysaccharides and polyphenols on the crystallization of potassium hydrogen tartrate. *Journal International Science Vigne et du Vin*, 31, 65–83. 1997.

GERTZ M, NGUYEN GT, FISCHER F, SUENKEL B, SCHLICKER C, FRANZEL B, JANA TOMASCHEWSKI, J., ALADINI, F., BECKER, C., WOLTERS, D., STEEGBORN, C. A molecular mechanism for direct sirtuin activation by resveratrol. *PLoS ONE*, 7(11): e49761, 2012.

GOJKOVIC-BUKARICA, L., MARKOVIC-LIPKOVSKI, J., HEINLE, H., CIROVIC, S., JOVANA RAJKOVIC, J., DJOKIC, V., ZIVANOVIC, V., ANA BUKARICA, A., NOVAKOVIC, R. The red wine polyphenol resveratrol induced relaxation

of the isolated renal artery of diabetic rats: The role of potassium channels. *Journal of Functional Foods*, 52, 266–275. 2018.

GORIN, P. A. J., MAZUREK, M. Further studies on the assignment of signals in ^{13}C magnetic resonance spectra of aldoses and derived methyl glycosides. *Canadian Journal of Chemistry*, 53, 1212-1222, 1975.

GORIN, P. A. J., IACOMINI, M. Polysaccharides of the lichens *Cetraria islandica* and *Ramalina usnea*. *Carbohydrate Research*, 142, 119-132, 1984.

GRAINGER, K.; TATTERSALL, H., Eds. *Wine Production: Vine to Bottle*. Oxford: Blackwell, p.127, 1 ed. 2005.

GUADALUPE, Z.; AYESTARAN, B. Polysaccharide profile and content during the vinification and aging of Tempranillo red wines. *Journal of Agricultural and Food Chemistry*, v. 55, p. 10720-10728, 2007.

GUADALUPE, Z., AYESTARÁN, B. Effect of commercial mannoprotein addition on polysaccharide, polyphenolic, and color composition in red wines. *Journal of Agricultural and Food Chemistry*, 56, 9022–9029, 2008.

GUERRA, C. C. Conhecendo o Essencial sobre Uvas e Vinhos. 2009. Disponível em: <<http://www.cnpuv.embrapa.br/publica/documentos/doc048.pdf>>. Acesso em: 20/06/2018.

GUERRA, C. C. Maturação e Colheita. In: GUERRA, C. C. *UVA para processamento Pós-colheita*. Brasília: Embrapa, 2003.

HALÁSZ, A., LÁSZTITY, R. Use of yeast biomass in food production. New York, CRC press, 312p, 1991.

HAYASHIZAKI, Y., HUME, D. A. & SWEET, M. J. LPS regulates proinflammatory gene expression in macrophages by altering histone deacetylase expression. *FASEB J.*, 20 (9), 1315-1327, 2006.

HE, F., MU, L., YAN, G. -L., LIANG, N. -N., PAN, Q. -H., WANG, J. Biosynthesis of anthocyanins and their regulation in colored grapes. *Molecules*, 15(12), 9057–9091, 2010.

HEREDIA, F. J., ÁLVAREZ, C., GONZÁLEZ-MIRET, M. L., & RAMIREZ, A. *ChromaLab Análisis de Color*; Registro General de la Propiedad Intelectual SE-1052-04: Sevilla, Spain, 2004.

HOFFMAN, ALEXANDRE. Sistema de produção de destilado de vinho. EMBRAPA. 2009.

HOGG, J.C.; DORESCHUK, CM. Leukocyte traffic in the lung. *Annu. Rev. Physiol.* 57, 97-114, 1995.

HOOSHMAND, S., KUMAR, A., ZHANG, J. Y., JOHNSON, S. A., CHAI, S. C., & ARJMANDI, B. H. Evidence for anti-inflammatory and antioxidative properties of dried plum polyphenols in macrophage RAW 264.7 cells. *Food Funct*, 6(5), 1719-1725, 2015.

HOTCHKISS R.S., KARL, I. E. The pathophysiology and treatment of sepsis. *New England Journal of Medicine*, 348:138, 2003.

HOUGH, J. S. *Biotechnologia de la cerveza y de la malta*. Zaragoza, Acribia. 194.p, 1990.

HUANG, W. Value of neutrophil counts in predicting surgery-related acute kidney injury and the interaction of these counts with diabetes in chronic kidney disease patients with hypertension., *Medicine*, 94, 1-7, 2015.

INNGJERDINGEN, K. T., THÖLE, C., DIALLO, D., PAULSEN, B. S. HENSEL, A. Inhibition of *Helicobacter pylori* adhesion to human gastric adenocarcinoma epithelial cells by aqueous extracts and pectic polysaccharides from the roots of *Cochlospermum tinctorium* A. Rich. And *Vernonia kotschyana* Sch. Bip. Ex Walp. *Fitoterapia*, 95, 127–132, 2014.

JANEWAY, C. A. JR., MEDZHITOV, R. Innate immune recognition. *Annual Review of Immunology*, 20, 197-216, 2002.

JANNIN, B.; DELMAS, D.; CHERKAOUI MALKI, M.; LATRUFFE, N. Étude du mécanisme d'action du resvératrol, un polyphénol du vin, sur la prolifération cellulaire. *Bulletin de l'O.I.V.*, Paris, v. 74, n. 839-840, p. 54-60, 2001.

JONES, J. K. N., STOODLEY, R. J. Fractionation using copper complexes. *Methods in Carbohydrate Chemistry*, 5, 36–38, 1965.

JOHNSON, H. *Vintage: The Story of Wine* .Simon & Schuster. p.11-16.1989.

JUNG, C.H., KIM, J.H., PARK, S.J., KWEON, D.H., KIM, S.H., KO, S.G. Inhibitory effect of *Agrimonia pilosa Ledeb.* on inflammation by suppression of iNOS and ROS production. *Immunological Investigations*, 39, 159-70, 2010.

KAKURÁKOVÁ, M.; CAPEK, P.; WELLNER, N.; EBRINGEROVÁ, A. FT-IR study of plant cell model compounds: pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers*, v. 43, p. 195-203, 2000.

KAZARIAN, A. A. et al. Development of a novel fluorescent tag O-2-[aminoethyl] fluorescein for the electrophoretic separation of oligosaccharides, *Anal. Chim. Acta*, 662 (2), 206–213, 2010.

KENNEDY, J. A., FERRIER, J., HARBERTSON, J. F., & PEYROT DES GACHONS, C. Analysis of tannins in red wine using multiple methods: Correlation with perceived astringency. *American Journal of Enology and Viticulture*, 57(4), 481-485, 2006.

KIKUCHI, K. Design, synthesis and biological application of chemical probes for bio-imaging. *Chem. Soc. Rev.*, 39 (6), 2048-2053, 2010.

KOBAYASHI, H., WATANABE, M., KOMIDO, M., MATSUDA, K., IKEDA-HASEBE, K., SUZUKI, M., SHIBATA, N., HISAMICHI, K., SUZUKI, S. Assignment of ^1H and ^{13}C NMR chemical shifts of a D-mannan composed of α -(1 $\rightarrow$ 2) and α -(1 $\rightarrow$ 6) linkages obtained from *Candida kefyr* IFO 0586 strain. *Carbohydrate Research*, 267, 299-306, 1995.

KOMURA, D. L., RUTHES, A. C., CARBONERO, E. R., ALQUINI, G., ROSA, M. C. C., SASSAKI, G. L., IACOMINI, M. The origin of mannans found in submerged culture of basidiomycetes. *Carbohydrate Polymers*, 79, 1052–1056, 2010.

KOPONEN, J. M., HAPPONEN, A.M., MATTILA, P. H., & TÖRRÖNEN, A.R. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *Journal of Agricultural and Food Chemistry*, 55(4), 1612–1619, 2007.

KOSIR, I. J., & KIDRIC, J. Use of modern nuclear magnetic resonance spectroscopy in wine analysis: Determination of minor compounds. *Analytica Chimica Acta*, 458(1), 77–84, 2002.

KLIS, F. M.; MOL, P.; HELLINGWERF, K.; BRUL, S. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiology Reviews*, v. 26, p. 239-256, 2002.

LANDRY, D. W., OLIVER, J. A. The pathogenesis of vasodilatory shock. *The New England Journal of Medicine*, 345, 588–595, 2001.

LAPA, F. R., SILVA, M. D., ALMEIDA CABRINI, D., & SANTOS, A. R. Anti-inflammatory effects of purine nucleosides, adenosine and inosine, in a mouse model of pleurisy: Evidence for the role of adenosine A (2) receptors. *Purinergic Signal*, 8, 693–704, 2012.

LAKSHMINARASIMHAN M., CURTH U., MONIOT S., MOSALAGANTI S., RAUNSER S., STEEGBORN C. Molecular architecture of the human protein deacetylase Sirt1 and its regulation by AROS and resveratrol. *Bioscience Reports*, 33, e00037, 2013.

LAROUSSE DO VINHO. Edição brasileira atualizada. 2ª edição, 2007.

LASKY, L.A. Selectins: interpreters of cell-specific information during inflammation. *Science*. 258, 964-969, 1992.

LE BOURVELLEC, C., BOUCHET B., RENARD, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochimica et Biophysica Acta*, 1725,10-18, 2005.

LE BOURVELLEC, C., GUYOT, S., RENARD, C. M. G. C. Interactions between polyphenols and cell-walls: Modification of polysaccharide extractability. *Carbohydrate Polymers*, 75, 251-261, 2009.

LEI, X., ZHU, Y., WANG, X., ZHAO, P., LIU, P., ZHANG, Q., CHEN, T., YUAN, H., GUO, Y. Wine polysaccharides modulating astringency through the interference on interaction of flavan-3-ols and BSA in model wine. *International Journal of Biological Macromolecules*, 139, 896-903, 2019.

LEE, K. P., SUDJARWO, G.W., KIM, J.S., DIRGANTARA, S., MAENG, W.J., HONG, H. (2014). The anti-inflammatory effect of *Indonesian Areca* catechu leaf extract *in vitro* and *in vivo*. *Nutrition Research Practice*. 8, 267–271, 2014.

LEVI M, SCHULTZ M, VAN DER POLL T. Disseminated intravascular coagulation in infectious disease. *Semin Thromb Hemost.*;36:367–77. 2010.

LIM, D. S., BAE, K.G., JUNG, I. S., KIM, C.H, YUN, Y.S., SONG, J.Y. Anti-septicaemic effect of polysaccharide from *Panax ginseng* by macrophage activation, *Journal of Infection Prevention*, 45, 32–38, 2002.

LIN, Z., FISCHER, J., WICKER, L. Intermolecular binding of blueberry pectin-rich fractions and anthocyanin. *Food Chem.* 194, 986–993, 2016.

LINSKENS, H. F., & JACKSON, J. F. Phenolic composition of natural wine types — Wine analysis. Berlin: Springer-Verlag Press, 1988.

LIU, M.Y., CHENG, Y.J., CHEN, C.K., YANG. B.C. Coexposure of lead- and lipopolysaccharide-induced liver injury in rats: involvement of nitric oxide-initiated oxidative stress and TNF- α . *Shock*, 23, 360–64, 2005.

LONGHI-BALBINOT, D. T., LANZNASTER, D., BAGGIO, C. H., SILVA, M. D., CABRERA, C. H., FACUNDO, V. A., et al. Anti-inflammatory effect of triterpene 3 β , 6 β , 16 β - trihydroxylup-20(29)-ene obtained from *Combretum leprosum* Mart & Eich in mice. *Journal of Ethnopharmacology*, 26, 59–64, 2012.

LOWE, J.B. Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Curr. Opin. Cell Biol.* 15: 531–538, 2003.

MARIATHASAN S., MONACK, D. M. Inflamma some adaptors and sensors: intracellular regulators of infection and inflammation. *Nature Reviews Immunology*, 7, 31–40, 2007.

MATEUS, N., CARVALHO, E., LUÍS, C., DE FREITAS, V. Influence of the tannin structure on the disruption effect of carbohydrates on protein–tannin aggregates. *Anal. Chim. Acta*. 513, 135–140, 2004.

MARTÍNEZ-LAPUENTE, L.; GUADALUPE, Z.; AYESTARAN, B.; ORTEGA-HERAS, M.; PEREZ-MAGARINO, S. Changes in polisaccharide composition during sparkling wine marking and aging. *Journal Agricultural and Food Chemistry*, v. 61, p. 12362–12373, 2013.

MATTIVI, F., GUZZON, R., VRHOVSEK, U., STEFANINI, M., & VELASCO, R. Metabolite profiling of grape: Flavonols and anthocyanins. *Journal of Agricultural and Food Chemistry*, 54(20), 7692–7702, 2006.

MAZZA, G., FUKUMOTO, L., DELAQUIS, P., GIRARD, B., & EWERT, B. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir Wines from British Columbia. *Journal of Agricultural and Food Chemistry*, 47(10), 4009–4017, 1999.

MELLINGER, C. G., CIPRIANI, T. R., NOLETO, G. R., CARBONERO, E. R., OLIVEIRA, M. B. M., GORIN, P., IACOMINI, M. Chemical and immunological modifications of an arabinogalactan present in tea preparations of *Phyllanthus niruri* after treatment with gastric fluid. *International Journal of Biological Macromolecules*, 43, 115–120, 2008.

MOINE-LEDOUX, V., DUBOURDIEU, D. An invertase fragment responsible for improving the protein stability of dry white wines. *Journal of the Science of Food and Agriculture*, 79, 537–543, 1999.

MONAGAS, M., GOMEZ-CORDOVE, C., BARTOLOME', B., LAUREANO, O., RICARDO DA SILVA, J. M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem*, 51, 6475–6481, 2003.

MOSMANN, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65, 55–63, 1983.

MUELLER, E. A., & ANDERER, F. A. Synergistic action of a plant rhamnogalacturonan enhancing antitumor cytotoxicity of human natural killer and

lymphokine-activated killer cells: Chemical specificity of target cell recognition. *Cancer Research*, 50(12), 3646–3651. 1990.

MULLER, W.A. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunology*. 24: 327-334, 2003.

NAKAMURA, H. Netrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J. Clin Invest.* 89, 1478-1484, 1992.

NASCIMENTO, A. M., DE SOUZA, L. M., BAGGIO, C. H., WERNER, M. F. D. P., MARIA-FERREIRA, D., DA SILVA, L. M., CIPRIANI, T. R. Gastroprotective effect and structure of a rhamnogalacturonan from *Acmella oleracea*. *Phytochemistry*, 85, 137–142, 2013.

NDIAYE, M.; CHATAIGNEAU, M.; LOBYSHEVA, I.; CHATAIGNEAU, T.; SCHINI-KERTH, V.B. Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. *FASEB J.* 2005 Mar;19(3):455-7. Epub 2004.

NERGARD, C.S., DIALLO, D., INNGJERDINGEN, K., MICHAELSEN, T.E., MATSUMOTO, T., KIYOHARA, H., YAMADA, H., PAULSEN, B.S. Medicinal use of *Cochlospermum tinctorium* in Mali: anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology*, 96, 255–269, 2005.

NIEUWENHUIJZEN, G. A., DEITCH, E. A., GORIS, R. J. Infection, the gut and the development of the multiple organ dysfunction syndrome. *European Journal of Surgery*. 162, 259–273, 1996.

NORTHCOTE, D. H.; HORNE, R. W. The Chemical composition and structure of the yeast cell wall. *Biochemical Journal* 51: 232-236, 1952.

O'NEILL, M.; ALBERSHEIM, P.; DARVILL, A. The pectic polysaccharides os primary cell walls. In: DEY, D. M. *Methods in Plant Biochemistry*. London: Academic Press, p. 415-441, 1990.

OIV (Office International de la Vigne et du Vin. 2019 Statistical Report on World Vitiviniculture, 2019.

OSETE-ALCARAZ, A., BAUTISTA-ORTÍN, A. A., GÓMEZ-PLAZA. The Role of Soluble Polysaccharides in Tannin-Cell Wall Interactions in Model Solutions and in Wines. *Biomolecules*, 10, 36, 2019.

OSUMI, M. The ultrastructure of yeast: Cell wall formation and structure. *Micron*, v.29, n.2/3, p.207-233, 1998.

OTERO-ANTON, E., GONZALEZ-QUINTELA, A., LOPEZ-SOTO, A., LOPEZ-BEM, S., LLOVO, J., & PÉREZ, L. F. Cecal ligation and puncture as a model of sepsis in the rat: Influence of the puncture size on mortality, bacteremia, endotoxemia and tumor necrosis factor alpha levels. *European Surgical Research*, 33, 77–79, 2001.

OVERHAUS, M., TOGEL, S., PEZZONE, M. A., BAUER, A.J. Mechanisms of polymicrobial sepsis-induced ileus. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 287, G685–694, 2004.

OVODOVA, R. G., GOLOVCHENKO, V. V., POPOV, S. V., POPOVA, G. Y., PADERIN, N. M., SHASHKOV, A. S., OVODOV, Y. S. Chemical composition and anti-inflammatory activity of pectic polysaccharide isolated from celery stalks. *Food Chemistry*, 114, 610-615, 2009.

PACHER, P., BECKMAN, J.S., LIAUDET, L. Nitric oxide and peroxynitrite in health and disease. *Physiological Review*; 87, 315–424, 2007.

PAN, M. H., LIN-SHIAU, S. Y., LIN, J. K. Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of I kappa B kinase and NF kappa B activation in macrophages. *Biochemical Pharmacology* 60, 1665–1676, 2000.

PARISH, C.R. The role of Heparan sulphate in inflammation. *Nature Reviews Immunology*. 6: 633-643, 2006.

PARK, S. N., NOH, K. T., JEONG, Y.-I., JUNG, I. D., KANG, H. K., CHA, G. S., PARK, Y.-M. Rhamnogalacturonan II is a Toll-like receptor 4 agonist that inhibits tumor growth by activating dendritic cell-mediated CD8+ T cells. *Experimental & Molecular Medicine*, 45(2), e8, 2013.

PAULSEN, B. S. Plant polysaccharides with immunostimulatory activities. *Current Organic Chemistry*, vol. 5, p. 939-950, 2001.

PECK, G. M. F. Ativação de macrófagos e sua correlação com o desfecho de sepse. [S.l.]: Dissertação de mestrado. Universidade do Extremo Sul Catarinense, 2007.

PELLERIN, P.; VIDAL, S.; WILLIAMS, P.; BRILLOUET, J. M. Characterization of five type II arabinogalactan-protein fractions from red wine of increasing uronic acid content. *Carbohydrate Research* 277, 135- 143, 1995.

PELLERIN, P., DOCO, T., VIDAL, S., WILLIAMS, P., BRILLOUET, J. M., O'NEILL, M. A. Structural characterization of red wine rhamnogalacturonan II. *Carbohydrate Research*, 190, 183-197. 1996.

PELLERIN, P., CABANIS, J-C. Les glucides. In *nologie: fondements scientifiques et technologiques*. Lavoisier, Tech. & Doc, France: Paris, 40-92, 1998.

PÉREZ, S.; MAZEAU, K.; PENHOAT, H. The three-dimensional structures of the pectic polysaccharides. *Plant Physiology and Biochemistry*, vol. 32, n. 1/2, p. 37-55, 2000.

PRATT, D S. Liver chemistry and function tests. In: Feldman M, Friedman LS, Brandt LJ, editors. *Sleisenger and Fordtran's gastrointestinal and liver disease*. 9th ed. Philadelphia: Saunders. p. 1227–37. 2010.

PETTOLINO, F.A., WALSH, C., FINCHER, G. B., BACIC, A. Determining the polysaccharide composition of plant cell walls, *Nature Protocols*, 1590-1607, 2012.

POPOV, S. V., OVODOVA, R. G., GOLOVCHENKO, V. V., PUPOVA, G. Y., VIATYASEV, F. V., SHASHKOV, A. S., OVODOV, Y. S. Chemical composition and anti-inflammatory activity of a pectic polysaccharide isolated from sweet pepper using a simulated gastric medium. *Food Chemistry*, 124, 309-315, 2011.

PULS, J.; SCHUSEIL, J. Chemistry of hemicelluloses: relationship between hemicellulose structure and enzymes required for hydrolysis. In: *Hemicellulose and hemicellulases.*, London: Portland Press Research Monograph, p. 1-27, 1993.

QUIJADA-MORÍN, N., WILLIAMS, P., RIVAS-GONZALO, J. C., DOCO, T., ESCRIBANO-BAILÓN. M. T. Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. *Food Chemistry*, 154, 44–51, 2014.

RAMOS-PINEDA, A. M., GARCÍA-ESTÉVEZ, I., BRÁS, N. F., MARTÍN DEL VALLE, E. M., DUEÑAS, M., & ESCRIBANO BAILÓN, M. T. Molecular approach to the synergistic effect on astringency elicited by mixtures of flavanols. *Journal of Agricultural and Food Chemistry*, 65, 6425–6433, 2017.

REETTA HUTTUNEN, JANNE AITTONIEMI. New concepts in the pathogenesis, diagnosis and treatment of bacteremia and sepsis. *British infection association*. 63, 407 e 419. 2011.

REID, J. S. G. Carbohydrate metabolism: structural carbohydrates. In: DEY, P. M.; HARBORNE, J. B. *Plant Biochemistry*. Bristol: Academic Press, p. 205-236, 1997.

RENARD, C. M. G. C.; LAHAYE, M.; MUTTER, M.; VORAGEN, F. G. J.; THIBAUT, J. F. Isolation and structural characterisation of rhamnogalacturonan oligomers generated by controlled acid hydrolysis of sugar-beet pulp. *Carbohydrate Research*, v. 305, p. 271-280, 1998.

RENARD, C. M., BARON, A., GUYOT, S., DRILLEAU, J. F. Interactions between apple cell walls and native apple polyphenols: Quantification and some consequences. *Int. J. Biol. Macromol.*, 29, 115–125, 2001.

RENARD, M. G. C. C., WATRELOT, A. A., LE BOURVELLEC, A. Interactions between polyphenols and polysaccharides: Mechanisms and consequences in food processing and digestion. *Trends in Food Science & Technology*, 63, 43-51, 2017.

RUIZ-GARCÍA, Y., SMITH, P. A., BINDON, K. A. Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. *Carbohydr. Polym.* 114, 102–114, 2014.

REWA, O., BAGSHAW S. M. Acute kidney injury-epidemiology, outcomes and economics, *Nature Reviews Nephrology*. 10, 193–207, 2014.

RIBÉREAU-GAYON, P.; GLORIES, Y.; MAUJEAN, A.; DUBOURDIEU, D. *Handbook of enology - The chemistry of wine stabilization and treatments*, Wiley, West Sussex. vol 2. 2006.

RITTIRSCH, D., HUBER-LANG, M. S., FLIERL, M. A., WARD, P. A. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nature Protocols*. 4, 31–36. 2009.

RIOU, V., VERNHET, A., DOCO, T., MOUTOUNET, M. Aggregation of grape seed tannins in model wine: effect of wine polysaccharides. *Food Hydrocolloids*, 16, 17–23, 2002.

ROSADO A., "Evolução de Parâmetros Físicos, Químicos e Controlo Microbiológico em Vinhos Brancos e Tintos da Adega Cooperativa de Palmela", FCT-UNL, 2013.

ROSSOL, M., HEINE, H., MEUSCH, U., QUANDT, D., KLEIN, C., SWEET, M.J., HAUSCHILDT, S. LPS-induced cytokine production in human monocytes and macrophages. *Critical Reviews in Immunology*, 31, 379–446, 2011.

RUEDAS-RAMA, M. J., WALTERS, J. D., ORTE, A. & HALL, E. A. Fluorescent nanoparticles for intracellular sensing: a review. *Anal. Chim. Acta*, 751, 1–23, 2012.

RUTHES, A. C., KOMURA, D. L., CARBONERO, E. R., SASSAKI, G. L., GORIN, P. A. J., & IACOMINI, M. Structural characterization of the uncommon polysaccharides

obtained from *Peltigera canina* photobiont *Nostoc muscorum*. Carbohydrate Polymers, 81, 29–34, 2010.

RUTHES, A. C., RATTMANN, Y. D., CARBONERO, E. R., GORIN, A. J., & IACOMINI, M. Structural characterization and protective effect against murine sepsis of fucogalactans from *Agaricus bisporus* and *Lactarius rufus*. Carbohydrate Polymers, 87, 1620–1627, 2012.

SAEMAN, J. F., MOORE, W. E., MITCHELL, R. L., MILLET, M. A. Techniques for the determination of pulp constituents by quantitative paper chromatography. Technical Association of Pulp and Paper Industry, 37, 336-343, 1954.

SASSAKI, G. L., GORIN, P. A. J., SOUZA, L. M., CZELUSNIAK, P. A., IACOMINI, M. Rapid synthesis of partially *O*-methylated alditol acetate standards for GC-MS: some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation. Carbohydrate Research, 340, 731-739, 2005.

SASSAKI, G. L., SOUZA, L. M., CIPRIANI, T. R., IACOMINI, M. TLC of Carbohydrates in M. Waksmundzka-Hajnos, J. Sherma, T. Kowalska (Ed), Thin Layer Chromatography in Phytochemistry. CRC Press: Boca Raton, USA, 255-276, 2008a.

SASSAKI, G. L., GUERRINI, M., SERRATO, R. V., SANTANA-FILHO, A. P., CARLOTTO, J., SIMAS-TOSIN, F., CIPRIANI, T. R., IACOMINI, M., TORRI, G., & GORIN, P. A. J. Monosaccharide composition of glycans based on Q-HSQC NMR. Carbohydrate Polymers, 104, 34-41, 2014.

Schepetkin, I. A., & Quinn, M. T. (2006). Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. International Immunopharmacology, 6(3), 317–333.

SCALBERT, A. Phenolics in fruits and fruit products: Progress and prospects, polyphenolic phenomena. Paris: INRA Editions, 1993.

SCOPARO, C. T., SOUZA, L. M., RATTMANN Y. D., DARTORA, N., PAIVA, S. M. M., SASSAKI, G. L., GORIN, P. A. J., IACOMINI, M. Polysaccharides from green and black teas and their protective effect against murine sepsis. Food Research International, 53, 780–785. 2013.

SEELY, A. J. E.; PASCUAL, J. L.; CHRISTOU, N. V. Science review: cell membrane expression (connectivity) regulates neutrophil delivery, function and clearance. Critical Care, v. 7, p. 291-307, 2003.

SEMERARO N, AMMOLLO CT, SEMERARO F, COLUCCI M. Sepsis-associated disseminated intravascular coagulation and thromboembolic disease. *Mediterr J Hematol Infect Dis*;2(3):e2010024, 2010.

SCHEPETKIN, I. A., & QUINN, M. T. Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *International Immunopharmacology*, 6 (3), 317–333, 2006.

SHERWOOD, E. R.; TOLIVER-KINSKY, T. Mechanisms of the inflammatory response. *Best Pract Res Clin Anaesthesiol*, v. 18, n. 3, p. 385–405, Sep/2004.

SKIPSKI, V. P. Thin layer chromatography of neutral glycolipids. *Methods in Enzymology*, 35, 396–425, 1975.

SRIVASTAVA, R., KULSHVESHTHA, D.K. Bioactive polysaccharides from plants. *Phytochemistry* 28, 2877–2883, 1989.

SIEDLE, L. G.; GUSTAVSSOM, L.; JOHANSSON S.; MURILLO R.; CASTRO V.; BOHLIN L.; MERFORT I. The effect of sesquiterpene lactones on the release of human neutrophil elastase. *Biochem. Pharmacol.* 7559, 1-7, 2003.

SILVA, M. D., GUGINSKI, G., WERNER, M. F. P., BAGGIO, C. H., MARCON, R., & SANTOS, A. R. Involvement of interleukin-10 in the anti-inflammatory effect of sanyinjiao (SP6) acupuncture in a mouse model of peritonitis. *Evidence-Based and Complementary and Alternative Medicine*, 217946, 2011.

SIMAS-TOSIN, F. F.; ABUD, A.P.R.; DE OLIVEIRA, C.C.; GORIN, P.A.J.; SASSAKI, G.L.; BUCCHI, D.F; IACOMINI, M. Polysaccharides from peach pulp: Structure and effects on mouse peritoneal macrophages. *Food Chemistry*, 2257–2260, 2012.

SIMÕES, C. M. O.; SHENKEL, E. P.; GOSMANN, G.; MELLO, J. C. P.; MENTZ, L. A.; PETROVICK, P. R. *Farmacognosia: da planta ao medicamento*. 5.ed. Porto Alegre, UFRGS, Florianópolis, UFSC, p. 403, 498, 2003.

SIMON, S. I.; CHERAPANOV, V.; NADRA, I.; WADDELL, T.K.; SEO, S. M.; WANG, Q.; DOERSCHUK, C. M.; DOWNEY, G.P. Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and localization with CD18. *Journal Immunology*. 163, 2891-2901, 1999.

SIMON, S. I.; CHERAPANOV, V.; NADRA, I.; WADDELL, T.K.; SEO, S. M.; WANG, Q.; DOERSCHUK, C. M.; DOWNEY, G.P. Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and localization with CD18. *J. Immunol.* 163, 2891-2901, 1999.

SINGER, M., DEUTSCHMAN, C. S., SEYMOUR, C. W., SHANKAR-HARI, M., ANNANE, D., BAUER, M., BELLOMO, R., BERNARD, G. R., CHICHE, J. D., COOPERSMITH, C. M., HOTCHKISS, R. S., LEVY, M. M., MARSHALL, J. C., MARTIN, G. S., OPAL, S. M., RUBENFELD, G. D., POLL, T., VINCENT, J. L., ANGUS, D. C. The sepsis definitions task force the third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315, 801–810, 2016.

SRIVASTAVA, R.; KULSHVESHTHA, D. K. Bioactive polysaccharides from plants, *Phytochemistry*, v. 28, p. 2877-2883, 1989.

STEARNS-KUROSAWA, D. J., OSUCHOWSKI, M.F., VALENTINE, C., KUROSAWA, S., REMICK, D. G. The pathogenesis of sepsis. *Annual Review of Pathology*. 6, 19e48, 2011.

STEWART, T. S.; BALLOU, C. E. A comparison of yeast mannans and phosphomannans by actolysis. *Biochemistry*, 7: 1855-1863, 1968.

STIPP, M. C., BEZERRA, I.D. L., CORSO, C. R., DOS REIS LIVERO, F. A., LOMBA, L. A., CAILLOT, A. R. C., ZAMPRONIO, A. R., QUEIROZ-TELLES, J. E., KLASSEN, G., RAMOS, E. A. S., SASSAKI, G. L., ACCO, A. Necroptosis mediates the antineoplastic effects of the soluble fraction of polysaccharide from red wine in Walker-256 tumor-bearing rats. *Carbohydrate Polymers*, 160, 123–133, 2017.

STONE, C.W.; MILLS, D.V. Yeast products in the feed industry: a practical guide for feed professionals. 2007. Disponível em: <http://www.engormix.com/e_articles_view.asp?art=243&AREA=MAS>. Acesso em: 04 nov. 2017.

TAYLOR, R. L., CONRAD, H. E. Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry*, 11, 1383-1388, 1972.

TERAO J. Dietary flavonoids as antioxidants. *Forum of Nutrition*; 61, 87–94, 2009.

TISCHER, C. A., GORIN, P. A. J., IACOMINI, M. The free reducing oligosaccharides of gum arabic: aids for structural assignments in the polysaccharide. *Carbohydrate Polymers*, 47, 151-158, 2002.

TZIANABOS, A. O., KASPER, D. L., CISNEIROS, R. L., SMITH, R. S., ONDERDONK, A. B. Polysaccharide-mediated protection against abscess formation in experimental intra-abdominal sepsis. *Journal of Clinical Investigation*. 96, 2727–2731, 1995.

VARKI, A. Selectin ligands: will the real ones please stand up? *J. Clin Invest.*, 100, 31-35. 1997.

VENTER, G. et al. Glucose controls morphodynamics of LPS-stimulated macrophages. *PLoS One*, 9 (5), e96786, 2014.

VERNHET, A., PELLERIN, P., BELLEVILLE, M. P., PLANQUE, J., MOUTOUNET, M. Relative impact of major wine polysaccharides on the performances of an organic microfiltration membrane. *American Journal of Enology and Viticulture*, 50, 51–56, 1999.

VIDAL, S. et al. Structural characterization of the pectic polysaccharide rhamnogalacturonan II: Evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain. *Carbohydrate Research*, v. 326, p. 277-294, 2000.

VIDAL, S.; WILLIAMS, P.; DOCO, T.; MOUTOUNET, M.; PELLERIN, P. The polysaccharides of red wine: total fractionation and characterization. *Carbohydrate Polymers*, 54 439–447, 2003.

VITALITI, G., PAVONE, P., MAHMOOD, F., NUNNARI, G., FALSAPERLA, R. Targeting inflammation as a therapeutic strategy for drug-resistant epilepsies: an update of new immune-modulating, *Human Vaccines and Immunotherapeutics*. 10, 868–875, 2014.

VINOGRADOV E., PETERSEN B., BOCK K. Structural analysis of the intact polysaccharide mannan from *Saccharomyces cerevisiae* yeast using ^1H and ^{13}C NMR spectroscopy at 750 MHz. *Carbohydrate Research*, 307, 177-183, 1998.

VUKOVIC, R.; HUNDINA-DOMLADOVEC, M.; MRSA, V. Molecular organization of the *Saccharomyces cerevisiae* cell wall. *Prehrambeno-Tehnology Review*, 32: (2-3) 99-102. 1994.

WANG H, MA S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. *Am J Emerg Med*; 26:711–5. 2008.

WANG, L.; BROWN, J.R.; VARKI, A.; ESKO J.D. Heparin's anti-inflammatory effects require glucosamine 6-O-sulfation and are mediated by blockade of L- and P-selectins. *J Clin Invest*. 110: 127–136, 2002.

WOLFROM, M. L., THOMPSON, A. Acetylation. *Methods in Carbohydrate Chemistry*, 2, 211–215, 1963a.

WOLFROM, M. L., THOMPSON, A. Reduction with sodium borohydride. *Methods in Carbohydrate Chemistry*, 2, 65–67, 1963b.

XU J, ZHANG X, PELAYO R, MONESTIER M, AMMOLLO CT, SEMERARO F, et al. Extracellular histones are major mediators of death in sepsis. *Nat Med*;15:1318–21. 2009.

YADAV, R.; LARBI, K.Y.; YOUNG, R.E. & NOURSHARGH, S. Migration of leukocytes through the vessel wall and beyond. *Thrombosis Haemostasis*. 90: 598-606, 2003.

YAMADA, H. Pectic polysaccharides from Chinese herbs: structure and biological activity. *Carbohydrate Polymers*, v. 25, p. 269-276, 1994.

YANG, J.; MARTINSON, T. E.; LIU, R. H. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chemistry*. v. 116, n.1, p. 332-339. 2009.

YAMASHIRO, S., KAMOHARA, H., WANG, J. M., YANG, D., GONG, W. H., YOSHIMURA, T. Phenotypic and functional change of cytokine activated neutrophils: inflammatory neutrophils are heterogeneous and enhance adaptive immune response. *Journal of Leukocyte Biology*, 69, 698-704, 2001.

ZHANG, X., SUN, J., XIN, W., LI, Y., NI, L., MA, X., ZHANG, D., ZHANG, D., ZHANG, T., DU, G. Anti-inflammation effect of methyl salicylate 2-*O*-beta-D-lactoside on adjuvant induced-arthritis rats and lipopolysaccharide (LPS)-treated murine macrophages RAW264.7 cells, *International Immunopharmacology*, 25, 88–95, 2015.

ZIMMERMANN, M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, 16, 109–110, 1983.

ZOECKLEIN, B., FUGELSANG, K. C., GUMP, B. H., NURY, F. S. Wine analysis and production. New York: Chapman & Hall, 1995.