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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
MESTRADO EM CLÍNICA ODONTOLÓGICA
ÊNFASE EM ODONTOPEDIATRIA

AVALIAÇÃO PROTEÔMICA DA PELÍCULA ADQUIRIDA DO ESMALTE DE PROVADORES DE VINHO PROFISSIONAIS COM DESGASTE DENTÁRIO EROSIVO

NATÁLIA CALDEIRA SILVA

Porto Alegre
2019

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Linha de Pesquisa

Epidemiologia, etiopatogenia e repercussão das doenças da cavidade bucal e estruturas anexas

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, Nível Mestrado, Universidade Federal do Rio Grande do Sul, como pré-requisito parcial para obtenção do título de Mestre em Odontologia, Área de Concentração Clínica Odontológica – Odontopediatria.

Orientador: Prof. Dr. Jonas de Almeida Rodrigues

Porto Alegre

2019

Que nada nos limite, que nada nos defina, que nada nos sujeite. Que a liberdade seja nossa própria substância, já que viver é ser livre. Porque alguém disse e eu concordo que o tempo cura, que a mágoa passa, que decepção não mata. E que a vida sempre, sempre continua.

Simone de Beauvoir

RESUMO

O desgaste dentário erosivo (DDE) é definido como um processo químico-mecânico que leva a perda cumulativa de tecido dental duro sem o envolvimento de bactérias. O DDE é multifatorial, sendo a exposição a ácidos a sua maior causa. Os provadores de vinho profissionais (PVP) são muito suscetíveis ao DDE devido à alta frequência de exposição da superfície do esmalte dentário ao vinho. Alguns fatores protetores podem estar presentes em indivíduos que não têm DDE ou o têm em menor severidade. Foi sugerido que isso pode ser explicado pelas diferenças no meio bucal ou no esmalte. Assim sendo, a película adquirida do esmalte (PAE) pode ter um papel importante neste processo, por formar uma interface entre a superfície dentária e a cavidade bucal, reduzindo fricção e abrasão, também agindo como uma barreira semipermeável, a qual interfere no processo de mineralização/desmineralização, modulando a precipitação mineral e a aderência de microorganismos a superfície dentária.
O objetivo deste estudo *in vivo* foi comparar o perfil proteico da película adquirida do esmalte (PAE) formada em PVP com desgaste dentário erosivo leve e moderado e de pacientes não PVP, sem desgaste dentário erosivo. Vinte e dois voluntários (3 GWL, 9 GWH e 10 GC) participaram do estudo e foram avaliados clinicamente de acordo com o Basic Erosive Wear Examination (BEWE). Os voluntários foram divididos em 3 grupos: 1) Grupo Vinho Leve (GWL) – 3 voluntários, PVP com BEWE ≤8); 2) Grupo Vinho Alto (GWH) – 9 voluntários, PVP com BEWE ≥ 9; 3) GC - Grupo controle – 10 voluntários, sem DDE, não PVP. Após profilaxia, foi permitido que a PAE se formasse por 120 min, a qual foi coletada com papéis filtro de eletrodos mergulhados em ácido cítrico a 3%. As amostras armazenadas em criotubos de 2 mL e mantidas a -80°C até o processamento. Após, foram processadas para análise proteômica em um espectrômetro de massas (nLC-ESI-MS/MS). Um total de 412 proteínas foram identificadas, sendo que 44 eram comuns a todos os grupos. O perfil proteômico da PAE foi diferente entre os grupos. O número de proteínas exclusivamente encontradas em GWL, GWH e C foram 101, 105 e 126, respectivamente. Das exclusivamente encontradas em GWL, a maioria foram sobretudo proteínas de membrana. Na análise quantitativa, quando comparado GWL com GWH, as proteínas mais expressas foram Squalene monooxygenase e Neutrophil defensins 1 e 3, enquanto as menos expressas foram Haptoglobin, seguida de Hemoglobin subunit alpha, Immunoglobulin heavy constant gamma 1, Hemoglobin subunit beta, delta, epsilon and gama-2, Immunoglobulin kappa constant, Albumin isoform CRA k, Serum albumin e Serotransferrin que também estavam diminuídas, porem em menores taxas. Concluiu-se que grandes alterações no perfil proteico da PAE foram identificadas no grupo GWL quando comparado ao GWH. Além disso, foi a primeira vez que a Squalene monooxygenase foi identificada na PAE, e esteve 26 vezes aumentada no grupo GWL quando comparado ao GWH. Estes achados podem revelar um papel na resistência ao DDE observada no GWL.

Descritores: Desgaste dentário erosivo; Erosão dentária; Diagnóstico; Prevenção; Película Adquirida do Esmalte, Vinho.

ABSTRACT

Erosive tooth wear (ETW) is defined as a chemical-mechanical process leading to the cumulative loss of hard dental tissue without the involvement of bacteria. It is multifactorial, with exposure to acids as the major cause. Professional wine tasters (PWT) are very susceptible to ETW due to the frequency of wine exposure to the enamel surfaces. Some protective factors might be present among individuals who do not present ETW or present it at low severity. It has been suggested that it could be explained by differences in the oral environment or in the enamel. The acquired enamel pellicle (AEP) could play an important role on this process, as it forms a protective interface between the tooth surface and the oral cavity, reducing friction and abrasion, also acting as a semi-permeable barrier, which interferes in the mineralization/demineralization processes, modulating mineral precipitation and adherence of microorganisms on the dental surface. The objective of this in vivo study was to compare the protein profile of the acquired enamel pellicle (AEP) in PWT with low and mild ETW and in volunteers not PWT, without ETW. Twenty-two subjects participated in the study and were clinically evaluated according to the Basic Erosive Wear Examination (BEWE). The volunteers were divided in 3 groups: 1) Group Wine Low (GWL): 3 volunteers, PWT with BEWE ≤8); 2) Group Wine High (GWH): 9 volunteers, PWT with BEWE ≥ 9; 3) Group Control (GC): 10 volunteers, not PWT and without ETW. After prophylaxis, the AEP was allowed to form for 120 min, when it was collected with electrode filter paper soaked in 3% citric acid. The wick filters were placed in 2 mL cryotubes and stored at -80°C until processing. The samples were processed for proteomic analysis in nano Liquid Chromatagraphy Electron Spray Ionization Tandem Mass Spectrometric (nLC-ESI-MS/MS). The PLGS software was used to compare the proteomic profiles of the distinct groups. In total, 412 proteins were identified, among which fourty-four proteins were common to all the groups. The proteomic profile of the AEP was quite different among the distinct groups. The numbers of proteins exclusively found in the GWL, GWH and C groups were 101, 105 and 126, respectively. Most of the proteins exclusively identified in the GWL group are mainly membrane proteins. In the quantitative analyses, when GWL was compared with GWH, the proteins with the highest increases were Squalene monooxygenase and Neutrophil defensins 1 and 3, while those with the highest decreases were Haptoglobin, followed by Hemoglobin subunit alpha, Immunoglobulin heavy constant gamma 1, Hemoglobin subunit beta, delta, epsilon and gama-2, Immunoglobulin kappa constant, Albumin isoform CRA k, Serum albumin and Serotransferrin also decreased, but in lower rates. It was concluded that profound alterations in the proteomic profile of the AEP were seen in GWL compared with GWH volunteers. Also, it was the first time Squalene monooxygenase was identified in the AEP. It was increased (up to 26-fold) at GWL compared to GWH. These findings might play a role in the resistance to ETW seen in GWL.

Key words: Erosive Tooth Wear, Dental Erosion, Diagnosis, Prevention, Acquired enamel pellicle, Wine.

LISTA DE ABREVIATURAS

AEP	Acquired Enamel Pellicle
BEWE	Basic Erosive Wear Examination
DDE	Desgaste Dentário Erosivo
ETW	Erosive Tooth Wear
GC	Group Control
GERD	Gastroesophageal Reflux Disease
GWH	Group Wine High
GWL	Group Wine Low
PAE	Película Adquirida do Esmalte
PVP	Provadores de Vinho Profissionais
PWT	Professional Wine Tasters
DTT	Dithiothreitol
IAA	Iodoacetamide
nLC-ESI-MS/MS	Nano Liquid Chromatagraphy Electron Spray Ionization Tandem Mass Spectrometric
PLGS	ProteinLynx Global Server

SUMÁRIO

1 ANTECEDENTES E JUSTIFICATIVA

A partir de 1960 houve um decréscimo na prevalência da doença cárie, o que fez com que a preocupação com a perda dos dentes tenha se voltado para outras causas, como o desgaste dentário erosivo (DDE) (ATTIN et al., 1998). Dentre os diferentes tipos de desgaste dentário, o DDE tem sido bastante documentado (MOSS, 1998; SMITH; KNIGHT, 1984). Trata-se de um processo químico-mecânico, que leva à perda cumulativa de tecidos dentais duros, devido à exposição aos ácidos de origem não bacteriana (CARVALHO et al., 2015; LUSSI; CARVALHO, 2014). O DDE deve ser diferenciado de outras lesões não cariosas, tais como abrasão, que ocorre devido a forças mecânicas sobre os dentes; atrição, a qual se deve ao contato dente a dente e abfração, que ocorre devido à forças que atuam na região cervical dos dentes (MISTRY; GRENBY, 1993; TEN CATE; IMFELD, 1996). Difere ainda da cárie dentária por não haver envolvimento bacteriano na perda do tecido dentário (MOSS, 1998; NUNN, 1996; TEN CATE; IMFELD, 1996).

A dissolução do esmalte ocorre na interface esmalte/ácido, bem como dentro de uma parte fina, amolecida e desmineralizada de esmalte, em um processo chamado "desmineralização perto da superfície" (SHELLIS et al., 2013), levando a perda de minerais e consequentemente perda de estrutura dentária. Clinicamente, o DDE é caracterizado pela perda da morfologia e contorno natural, com superfícies brilhantes, de seda acetinada, mas às vezes amorfo, superfícies excessivamente lisas e com ausência de *perikymata*. Além disso, as lesões podem ser localizadas, generalizadas ou assimétricas, dependendo da sua etiologia (CARVALHO et al., 2015; LUSSI et al., 2011; GANSS; LUSSI, 2014). Como demonstrado por Jaeggi e Lussi (2014), é difícil fazer comparações entre estudos de prevalência de DDE devido aos diferentes índices utilizados e às diferentes dentições avaliadas nas amostras. Desta forma, a prevalência do DDE reportadas tem variado de 1% a 79% em pré-escolares, 10% a mais de 80% em crianças, 7% a 100% em adolescentes e de 4% a 100% em adultos (TAJI; SEOW, 2010; JAEGGI; LUSSI, 2014)

O DDE é uma ocorrência multifatorial, sendo a exposição aos ácidos a sua maior causa (CARVALHO et al., 2015). A etiologia pode ser classificada de acordo com sua origem em extrínseca ou intrínseca (IMFELD, 1996; LINNETT; SEOW, 2001; LUSSI, 1996; TEN CATE; IMFELD, 1996). As causas intrínsecas compreendem a ação de ácidos endógenos provenientes do refluxo gastroesofágico, regurgitação crônica, alcoolismo, gravidez ou distúrbios provenientes do sistema nervoso, tais como anorexia e/ou bulimia (IMFELD, 1996). É decorrente da atuação crônica do ácido gástrico sobre a superfície dentária por um

longo período e de forma regular (MEURMAN; TEN CATE, 1996; SCHEUTZEL, 1996). As causas extrínsecas compreendem os efeitos de ácidos exógenos, provenientes, por exemplo, dos ácidos contidos na dieta, bem como nas formulações medicamentosas (LUSSI, 1996). A principal causa extrínseca do DDE é proveniente da dieta (comidas e bebidas ácidas) (AINE et al., 1993; IMFELD, 1996; ZERO, 1996; LUSSI; JAEGGI; ZERO, 2004; BARTLETT, 2006). A maioria dos alimentos e bebidas de baixo pH (abaixo de 4,5) apresenta potencial para causar a desgaste dentário erosivo, uma vez que nesta faixa de pH existe uma subsaturação dos fluidos bucais em relação à hidroxiapatita e à fluorapatita (MAGALHÃES et al., 2009; ZERO, 1996). A exposição a ácidos extrínsecos também pode ser identificada em ambiente ocupacional devido a vapores químicos (fábricas de baterias, munição e galvanização) e líquidos ácidos (nadadores e provadores de vinho profissionais - PVP) (CARVALHO et al., 2015; MANDEL et. al., 2005).

Dentre as bebidas ácidas, o vinho apresenta potencial erosivo resultante de seu conteúdo ácido, o qual é derivado das frutas, sendo os ácidos tartárico e málico os mais presentes (FERGUSON et al., 1996; MOK et al., 2001; MANDEL et al., 2005). O baixo pH do vinho, relatado entre 3,0 e 3,8, indica que exposições dos dentes por longo tempo pode resultar em severo desgaste dentário (CHIKTE et al., 2005; GRAY et al., 1998; WIKTORSSON et al., 1997; MANDEL et al., 2005), já que o pH crítico reportado para dissolução do esmalte está entre 5,0 e 5,7 (FERGUSON et al., 1996).

Alguns trabalhos na literatura têm reportado uma maior prevalência de DDE em PVP, devido à alta frequência de contato com a bebida (CHIKTE et al., 2005; GEORGE et al., 2014; MULIC et al., 2011; WIKTORSSON et al., 1997; MANDEL, 2005). Lussi e Jaeggi (2006) reportaram que PVP suecos provam de 20 a 50 diferentes tipos de vinho, trabalhando aproximadamente 5 dias por semana. Durante as provas, o vinho é sorvido, rodado ou balançado pela boca em um período de 30 a 60 segundos, o que aumenta o risco de DDE do esmalte ou da dentina (MOK et al., 2001). Na Noruega, um estudo realizado com PVP revelou que 50% deles tinham DDE, em comparação a 20% dos indivíduos controle (não PVP). Além disso, a severidade das lesões era maior entre os provadores de vinho, uma vez que 39% deles apresentaram envolvimento de dentina, enquanto no grupo controle, apenas 7%. O padrão de localização das lesões foi distinto, visto que nos PVP as lesões acometiam principalmente as superfícies oclusais de primeiros molares inferiores, enquanto nos indivíduos controle, as superfícies palatinas dos incisivos superiores eram as mais afetadas (MULIC et al., 2011).

É notório o fato de, apesar da alta frequência de exposição ao vinho pelos PVP e de todos eles apresentarem padrões similares de utilização devido à sua atividade profissional, apenas 50% deles apresentaram desgaste dentário erosivo (MULIC et al., 2011). Este dado sugere que algum fator protetor deva existir dentre aqueles que não apresentam ou têm DDE em graus leves. Tem sido sugerido que a diferente susceptibilidade ao desgaste seja devido a fatores ligados à resistência do esmalte e a fatores relacionados ao ambiente bucal (UHLEN et al., 2016). Saliva, película adquirida do esmalte (PAE), hábitos alimentares e desafios mecânicos são importantes fatores relacionados ao ambiente bucal e que influenciam a formação e a progressão do DDE (ZWIER et al., 2013). Entre estes fatores, a PAE pode ter um papel importante neste processo.

Todas as superfícies sólidas expostas na cavidade bucal são cobertas por uma camada proteinácea chamada de película adquirida (HANNIG et al., 2005; HANNIG; BALZ, 1999; HANNIG; JOINER, 2006; LENDENMANN et al., 2000). A PAE é uma camada orgânica livre de bactérias formada in vivo como resultado da adsorção seletiva de proteínas salivares na superfície do esmalte (DAWES; JENKINS; TONGUE, 1963). Os principais componentes identificados na PAE são proteínas e glicoproteínas, mas carboidratos, lipídios neutros, fosfolipídios e glicolipídios também são encontrados (HANNIG; JOINER, 2006; SIQUEIRA; CUSTODIO; MCDONALD, 2012). Já foram identificadas mais de 450 proteínas diferentes na PAE (MARTINI et al., 2018). Assim, a presença de proteínas recobrindo o esmalte ou a dentina, envolvidas na lubrificação, nas capacidades tampão e remineralizante, torna a PAE um fator importante na ocorrência do DDE (HANNIG; BALZ, 1999). Cada proteína tem um papel específico na PAE e é de grande importância entender o papel desta película orgânica (HANNIG; JOINER, 2006; SIQUEIRA; CUSTODIO; MCDONALD, 2012; BUZALAF; HANNAS; KATO, 2012; VITORINO et al., 2007). Devido a sua composição, a PAE forma uma interface protetora entre a superfície dentária e a cavidade oral, reduzindo a fricção e a abrasão. A PAE também age como uma barreira semipermeável, que modula os processos de mineralização/desmineralização, a precipitação mineral e a aderência de micro-organismos à superfície dentária (HANNIG; JOINER, 2006; BUZALAF; HANNAS; KATO, 2012; HARA; ZERO, 2010; VUKOSAVLJEVIC et al., 2014). As proteínas da PAE são derivadas de secreções glandulares (glândulas maiores e menores), mas também do fluido crevicular, mucosa oral e micro-organismos (SIQUEIRA; CUSTODIO; MCDONALD, 2012). Nesse sentido, a saliva é a maior contribuinte da composição proteica da PAE (HANNIG; JOINER, 2006).

Vários estudos descrevem o impacto protetor da PAE formada in situ sobre a superfície do esmalte (HANNIG; BALZ, 1999; HANNIG et al., 2003; HANNIG et al., 2004). Recentemente, a formação e a composição da PAE também foram estudadas in vivo (LEE at al., 2013; DELECRODE et al., 2015; VENTURA et al., 2017; MARTINI et al., 2018). Martini e colaboradores (2018), avaliando voluntários com refluxo gastroesofágico, mostraram o papel da PAE na proteção do desgaste dentário erosivo. Diante disso, torna-se importante avaliar outros grupos de risco de DDE e, neste sentido, nenhum estudo avaliou o papel das proteínas da PAE em PVP. Em virtude do exposto, é possível que o perfil proteico da PAE de PVP que possuam um grau leve de desgaste dentário erosivo, seja diferente daquela que possuem desgaste dentário erosivo moderado.

2 OBJETIVO

O objetivo deste estudo foi avaliar o perfil proteico da película adquirida do esmalte (PAE) formada in vivo de PVP com desgaste dentário erosivo leve e moderado e de pacientes não PVP, sem desgaste dentário erosivo.

As hipóteses nulas a serem testadas foram: 1) não há diferença no perfil proteico da PAE de provadores profissionais de vinho com desgaste dentário erosivo leve comparado aqueles com desgaste dentário erosivo moderado; 2) não há diferença no perfil proteico da PAE de provadores profissionais de vinho comparado a controles não provadores profissionais de vinho e sem desgaste dentário erosivo.

3 ARTIGO CIENTÍFICO

Proteomic profile of the acquired enamel pellicle of professional wine tasters with erosive tooth wear

Silva NC[1], Ventura TMS[2], Oliveira BP[1], Santos NM[1], Pela VT[2], Buzalaf MAR[2], Rodrigues JA[1]

[1] Department of Surgery and Orthopedics, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

[2] Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil.

Corresponding Author:

Jonas Almeida Rodrigues

Pediatric Dentistry Division, School of Dentistry

Federal University of Rio Grande do Sul

Rua Ramiro Barcelos, 2492 – Rio Branco - 90035-003

+55 (51) 3308-5026 – Porto Alegre, RS, Brazil

E-mail: jorodrigues@ufrgs.br

***To be submitted to Journal of Dentistry**

ABSTRACT

Objectives: This in vivo study compared the protein profile of the acquired enamel pellicle (AEP) in professional wine tasters (PWT) with mild (GWL) and moderate (GWH) erosive tooth wear (ETW) and in volunteers not PWT, without ETW (GC).
Methods: 22 subjects participated (3 GWL/ 9 GWH/ 10 GC) and were clinically evaluated according to the Basic Erosive Wear Examination (BEWE). After prophylaxis, the AEP was allowed to form for 120 min, when it was collected with electrode filter paper soaked in 3% citric acid. Samples were processed for proteomic analysis (nLC-ESI-MS/MS). The PLGS software was used to compare the proteomics profiles of groups.
Results: In total, 412 proteins were identified, among which, 44 were common to all groups. The proteomic profile of the AEP was quite different among the distinct groups. The numbers of proteins exclusively found in the GWL, GWH and C groups were 101, 105 and 126, respectively. Most of the proteins exclusively identified in the GWL group are mainly membrane proteins. In the quantitative analyses, when GWL was compared with GWH, the proteins with the highest increases were Squalene monooxygenase and Neutrophil defensins 1 and 3, while those with the highest decreases were Haptoglobin and subunits of Hemoglobin.
Conclusion: Profound alterations in the proteomic profile of the AEP were seen in GWL x GWH. Also, it was the first time Squalene monooxygenase was identified in the AEP. These findings might play a role in the resistance to ETW seen in GWL.

Clinical significance: This pioneer study compared the proteomic profile of the AEP of PWT with low and moderate ETW. Increased proteins in those with low ETW might be protective and are good candidates to be added to dental products to protect PWT against extrinsic ETW.

Key words: Erosive Tooth Wear, Dental Erosion, Diagnosis, Prevention, Acquired enamel pellicle.

INTRODUCTION

Erosive tooth wear (ETW) is defined as a chemical-mechanical process that leads to the cumulative loss of dental hard tissue without the involvement of bacteria [1, 2]. Enamel dissolution occurs both at the enamel/acid interface, as well as within a partly demineralized thin softened layer of enamel, in a process called near-surface demineralization [3], leading to loss of minerals, and consequently, loss of tooth substance. Clinically, ETW is characterized as loss of the natural surface morphology and contour, with shiny, silky-glazed, but sometimes dull, excessively smooth tooth surfaces, with the absence of perikymata. Moreover, lesions can be localized, generalized or asymmetric, depending on the aetiology [1, 4, 5]. The prevalence of ETW has been reported to range from 10% to over 80% in children and 4% to 82% in adults [6]. As Jaeggi and Lussi (2014) showed, it is difficult to compare ETW prevalence between studies due to different indices used and also due to the different teeth assessed in the samples. Thus, the prevalence of ETW reported has ranged between 1% to 79% in preschool children, 10% to over 80% in schoolchildren, 7% to 100% in adolescents and from 4% to 100% in adults [6; 7, 8]

ETW is multifactorial, with acids as the major cause [1]. The most important sources are dietary (acidic foods and drinks) and gastric acids (regurgitation and reflux disorders) [7,9]. Acid exposure might also be identified in occupational environment due to acidic vapours and chemicals (e.g. battery, ammunition and galvanizing factories) and acidic liquids (e.g. professional swimmers and professional wine tasters - PWT) [1,11].

The potential of wine to cause ETW is a result of its fruit-acid content, with tartaric and malic acids being the most abundant, and lower concentrations of citric and succinic acids [11,12,13]. The acidity of wine (pH 3.0 to 3.8) indicates that long-term exposure of the teeth can result in serious tooth erosion [11], since the critical point at which enamel dissolves is reported to be between 5.0 and 5.7 pH [12]. Lussi and Jaeggi [13] reported that full-time PWT taste on average 20–50 different wines, working nearly 5 days a week in Sweden. During wine tasting, wine is sipped, swirled, or swished around the mouth for approximately 30 to 60 seconds, increasing the risk of enamel and dentine wear [14]. Mandel et al. [11] reported that PWT are very susceptible to ETW due to the frequency of wine exposure to the enamel surfaces. However, an investigation on the prevalence and severity of ETW among PWT revealed that half of them did not show any sign of ETW, despite extensive exposure to acidic drinks [15]. This data suggests that some protective factors might be present among individuals who do not present ETW or present it in a low severity. It has been suggested that

this susceptibility could be explained by differences in the oral environment or in the enamel [16]. Saliva, the acquired enamel pellicle (AEP), dietary habits and mechanical challenges are important factors related to the oral environment and that influence the formation and progression of ETW [17]. Therefore, between these factors, the AEP could play an important role on this process.

The AEP is a bacteria-free organic layer formed in vivo as a result of selective adsorption of salivary proteins on the surface of the enamel [18]. The main components identified in the AEP are proteins and glycoproteins, but carbohydrates, neutral lipids, phospholipids and glycolipids are also found [19, 20]. Each protein has a specific role in the pellicle and is of great importance to understand the role of this organic film [19, 20, 21, 22]. Due to its composition, the AEP forms a protective interface between the tooth surface and the oral cavity, reducing friction and abrasion. AEP also acts as a semi-permeable barrier, which interferes in the mineralization/demineralization processes, modulating mineral precipitation and adherence of microorganisms to the dental surface [19, 21, 23, 24]. The proteins of the AEP are derived mainly from salivary glandular secretions (major glands as well as minor glands) but also originate from the crevicular fluid, oral mucosa and microorganisms [20]. Saliva is the major contributor for the protein composition of the AEP [19].

Recently, it was shown that gastroesophageal reflux disease (GERD) patients without ETW have a distinct protein profile within the AEP when compared with those with GERD and ETW. Hemoglobin was increased in the AEP of GERD patients without ETW. Since this protein has a high affinity to hydroxyapatite, it was suggested as a probably protective protein, with potential to be included in dental products [25]. The same rationale was applied in the present study, in which we compared the protein profile of the AEP of PWT with mild ETW with that of PWT with moderate ETW, volunteers that were not PWT and did not present any sign of ETW were included as controls.

The null hypotheses tested were: 1) There is no difference in the protein composition of the AEP of PWT with mild ETW compared to that of PWT with moderate ETW; 2) There is no difference in the protein composition of the AEP of PWT compared to controls, not PWT, without ETW.

MATERIALS AND METHODS

Study design and sample

The protocol of the study was approved by the local Research and Ethics Human Committee (CAAE 58440116.7.0000.5347). Written informed consent was acquired from all volunteers prior to the beginning of the study.

PWT that are members of the Brazilian Society of Enology and were attending the "25th National Wine Evaluation – 2017 Harvest", were invited to participate in the study. Twelve PWT accepted to participate, met the inclusion criteria and were clinically evaluated according to the Basic Erosive Wear Examination (BEWE). According to BEWE index, the buccal/facial, occlusal and lingual/palatal surfaces are evaluated, and then the highest score are recorded. The surface more severely affected in each sextant is recorded with a four level score and the sum of the sextant's score are classified and combined to risk levels which guide the management of the condition [26]. Ten volunteers that did not work as PWT and did not present ETW that agreed to participate and met the inclusion criteria, composed the control group. To be included in the test sample, PWT should work for at least 5 years as wine tasters, from both genders, non-smokers, in good general and oral health (no gingivitis, periodontitis or any other condition which could interfere in the oral fluids). Group control (GC) could not work as PWT and do not present ETW, from both genders, non-smokers, in good general and oral health (no gingivitis, periodontitis or any other condition that could interfere in the oral fluids).

PWT volunteers were further divided into 2 groups: Group Wine Low (GWL), comprising 3 PWT with a BEWE index lower or equal than 8 and Group Wine High (GWH), comprising 9 PWT with a BEWE index higher or equal than 9.

Training and calibration

Clinical examination was performed by two properly trained and calibrated dentists (NCS and NMS). The training of visual-tactile and BEWE index examination was done through photographs with a discussion of doubtful points until consensus was reached. The calibration was done with 38 photographs, with interval of one week, as a previous study [25]. The intra- and interexaminer reproducibility was calculated using the Cohen Kappa Coefficient, achieving a Kappa value of 0.83 (minimum value required 0.70).

Clinical exams

The two previously trained and calibrated dentists performed the clinical examinations and the AEP collection under artificial light and using air compressor, suction, clinical mirror and probe, in a dental clinic at Bento Gonçalves, southern Brazil, city where the event "25th

National Wine Evaluation – 2017 Harvest" was held. The subjects underwent a dental prophylaxis performed with rubber cop and coarse pumice containing no additives. ETW was recorded based on the BEWE index [26]. The volunteers also answered a questionnaire for personal data collection.

AEP formation and collection

For the collection of acquired enamel pellicle, dental prophylaxis with pumice containing no additives was performed and after prophylaxis, the subjects waited 120 minutes, deprived of foods and beverages consumption, to allow the formation of the AEP and to avoid possible bacterial aggregation [27]. After, each quadrant of the mouth was rinsed with deionized water and dried with compressed air twice and isolated with cotton rolls. The pellicle was then collected with the aid of 5X10 mm electrode filter papers (filter paper wick electrode, Bio-Rad, Hercules, CA) pre-dipped in 3% citric acid (pH 2.5; Sigma-Aldrich, USA). The filter papers were rubbed (without pressure) on the coronal two-thirds (to avoid contamination from the gingival margin) of the surfaces (vestibular and lingual) of all teeth with tweezers [27]. The experiment began in the morning to avoid circadian effects on the composition of the AEP [28, 29]. The wick filters were placed in 2 mL cryotubes and initially stored at -20ºC, prior to final storage at -80°C until used for proteomic analysis. The filters collected from each group were pooled, resulting in 3 pools (one for each group).

Preparation of the AEP samples

The protocol of protein extraction was based in a previous study [30]. For the extraction, the papers were cut into small pieces and were put together in another cryotube, constituting a pool for each group. To the tubes containing the cut papers a solution containing 6 M urea, 2 M thiourea in 50 mM NH_4HCO_3 pH 7.8 was added, vortexed for 10 minutes at 4°C, sonicated for 5 minutes and centrifuged for 10 minutes at 14,000 *g* at 4°C and the supernatant was collected. This step was repeated twice. Thus, the papers were placed in tube filters (Corning Costar®Spin-X® Plastic Centrifuge Tube Filters, Sigma-Aldrich, New York, USA) and centrifuged at 14,000 *g* for 10 minutes at 4°C. The supernatant was recovered and added to that previously collected. Then, 50 mM NH_4HCO_3 (volume corresponding to 1.5 X the sample volume) was added and the samples were concentrated to 150 µL in Falcon Amicon tubes (Amicon Ultra – 15 Centrifugal Filter Units - Merk Millipore®, Tallagreen, Irland). Samples were then reduced by adding 5 mM dithiothreitol (DTT) and then alkylated by adding 10 mM iodoacetamide (IAA). The samples were then

digested for 14 h at 37ºC by adding 2% (p/p) trypsin (Promega, Madison, USA). After this time lapsed, 10 μL of 5% formic acid was added to stop the action of trypsin. Samples were then desalted and purified using C18 Spin columns (Thermo Scientific, United States). Then, an aliquot of 1 μL from each sample was removed and protein quantification was performed using the Bradford method (Bio-Rad Bradford Assays, United States). The samples were resuspended in a solution containing 3% acetonitrile and 0.1% formic acid to be submitted to nano Liquid Chromatagraphy Electron Spray Ionization Tandem Mass Spectrometric (nLC-ESI-MS/MS).

Shotgun Label-free Quantitative Proteomic Analysis

Peptides identification was performed on a nanoACQUITY UPLC-Xevo QTof MS system (Waters, Manchester, UK), exactly as previously described [30]. ProteinLynx Global Server (PLGS) software (Waters Co., Manchester, UK) version 3.0 was used to process and search the continuum LC-MSE data. Proteins were identified with the embedded ion accounting algorithm in the software and a search of the Homosapiens database (reviewed only, UniProtKB/Swiss-Prot) downloaded on February 2017 from UniProtKB (http://www.uniprot.org/). The use of the human database excludes the identification of bacterial proteins that could be present in the AEP. The identified proteins were classified and assigned by biological function [26, 28], origin and molecular interaction (http://www.uniprot.org/).

For label-free quantitative proteome, three MS raw files from each pooled group were analysed using the PLGS software. All the proteins identified with a score with confidence greater than that 95% were included in the quantitative statistical analysis embedded in the PLGS software. Identical peptides from each triplicate by sample were grouped based on mass accuracy (<10 ppm) and on time of retention tolerance <0.25 min, using the clustering software embedded in the PLGS. Difference in expression among the groups was calculated using Monte-Carlo algorithm and expressed as $p < 0.05$ for proteins present in lower abundance and $1- p > 0.95$ for proteins present in higher abundance, when one group was compared to another. The following relevant comparisons were: GWLxGC, GWHxGC, GWLxGWH.

RESULTS

Data were collected between June 2017 and December 2017. All the 22 volunteers completed the study. Table 1 shows the characterization of the volunteers according to gender, age, time of work as PWT and BEWE score. The mean age was higher at group GWH. The mean time working as PWT was similar between GWL and GWH groups. Volunteers in GWL and GWH groups had mean BEWE score of 8.0 and 12.0, respectively.

Table 1. Characterization of the volunteers according to gender, age, time of work as professional wine taster (PWT) and BEWE score.

Group*	Gender	Mean age (Min - Max)	**Mean time (±SD) as PWT****	**Mean BEWE (± SD)**
GWL	1F; 2M	31.0 (28.0 - 38.0)	12.0 (±1.1)	12.0 (±4.8)
GWH	4F; 5M	41.0 (28.0 – 76.0)	15.0 (±16.2)	8.0 (±0)
GC	10F	26.6 (21.0 – 31.0)	-	-

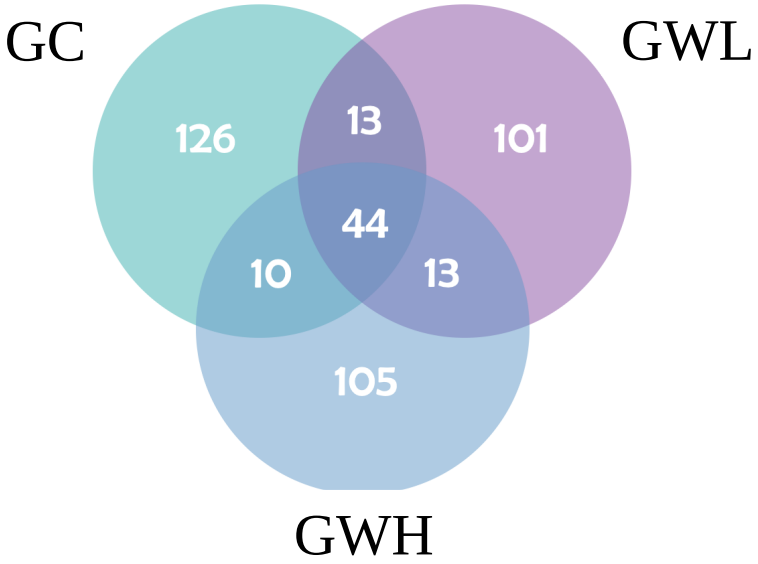
* GWL - PWT with a BEWE index ≤ 8; GWH - PWT with a BEWE index ≥ 9; GC – group control, no ETW, not PWT; F – female; M – male.

** *Time in years working as PWT.*

The total amount of protein obtained was 37, 66 and 88 µg for GWL, GWH and GC, respectively.

A total of 412 proteins were identified, among which 42 were common to all groups (Figure 1). Among them are proteins usually found in the AEP, such as isoforms of cystatin, neutrophil defensins, actin, protein S100-A, proline-rich proteins , albumin, immunoglobulin, besides lactotransferrin, lysozyme, lysozyme C, sthaterin, myeloperoxidase, mucins and histatins.

Figure 1. Venn diagram of the proteins identified in the acquired enamel pellicle of the groups: GWL - professional wine tasters (PWT) with a BEWE index lower or equal than 8; GWH - PWT with a BEWE index higher or equal than 9 (GWH); and GC - Group control, not PWT and without ETW.



The proteomic profile of the AEP was considerably different among the groups. The number of proteins exclusively found in the GWL, GWH and GC groups were 101, 105 and 126, respectively (Table 2). Regarding the proteins identified exclusively in one or two of the groups, some findings must be highlighted: a) Squalene Monooxygenase and Myeloperoxidase were not found in GC group; b) Mucin 21 and Mucin 7 were found only in GWL group; c) Histatin-1 and Histatin-3 were only present in GWL.

Regarding quantitative analysis (Table 3), for the comparison GWL vs. GC group, 33 proteins were significantly increased, and 13 proteins were significantly decreased in the first. Among the increased proteins are Neutrophil defensin 1 and 3, besides Zinc finger homeobox protein 2, Serum albumin, Lysozyme C, Lysozyme, and Statherin. On the other hand, Spectrin alpha chain non-erythrocytic 1, Carboxylic ester hydrolase and Acetylcholinesterase were decreased. When GWH were compared to GC, 19 and 17 proteins were increased and decreased, respectively, in the first. Among the increased ones are Hemoglobin subunits beta, delta, epsilon, gamma-1 and gama-2, as well as Albumin isoform CRA k, Serum albumin and Haptoglobin. Proteins with the greatest decreases were neutrophil defensins, Lysozyme C and Statherin.

The most relevant comparison is GWL vs. GWH. In this case, 17 proteins were increased and 20 were decreased in the first. Remarkably, the protein with the highest rate of increase (close to 26-fold) was Squalene monooxygenase, followed by Neutrophil defensin 1 and 3 (close to 8-fold). Other increased proteins, despite in lower rates, were Lysozyme C, Lysozyme, Statherin and Myeloperoxidase. The proteins with the highest decreases were Haptoglobin, followed by Hemoglobin subunit alpha, Immunoglobulin heavy constant gamma 1, Hemoglobin subunit beta, delta, epsilon and gama-2, Immunoglobulin kappa constant, Albumin isoform CRA k, Serum albumin, Serotransferrin also decreased, but in lower rates.

Table 2. Proteins identified in the acquired enamel pellicle in only one of the groups: GWL - professional wine tasters (PWT) with a BEWE index lower or equal than 8; GWH - PWT with a BEWE index higher or equal than 9 (GWH); and GC – Group control, not PWT and without ETW.

Access number*	Protein name	*PLGS Score*	Unique
Q9NUB1	Acetyl-coenzyme A synthetase 2-like_ mitochondrial	459.04	GC**
Q96M93	Adenosine deaminase domain-containing protein 1	168.08	GC
Q9Y653	Adhesion G-protein coupled receptor G1	382.45	GC
O43572	A-kinase anchor protein 10_ mitochondrial	132.31	GC
E7EQT3	Anion exchange protein	338.85	GC
Q96PS8	Aquaporin-10	160.05	GC
Q8WWZ7	ATP-binding cassette sub-family A member 5	152.05	GC
Q676U5	Autophagy-related protein 16-1	186.64	GC
Q9HB09	Bcl-2-like protein 12	255.66	GC
Q8TDL5	BPI fold-containing family B member 1	224.35	GC
Q3B891	BRCA1 protein (Fragment)	431.63	GC
P38398	Breast cancer type 1 susceptibility protein	447.2	GC
Q9UI42	Carboxypeptidase A4	203.78	GC
P55211	Caspase-9	296.32	GC
Q8N163	Cell cycle and apoptosis regulator protein 2	217.18	GC
A0A3B3IRL2	Cellular repressor of E1A-stimulated genes 1_ isoform CRA_a	216.21	GC
Q9BV73	Centrosome-associated protein CEP250	124.49	GC
Q8TD26	Chromodomain-helicase-DNA-binding protein 6	181.4	GC
A5D8W1	Cilia- and flagella-associated protein 69	151.66	GC
A0A140TA43	COL11A2	141.18	GC
P13942	Collagen alpha-2(XI) chain	141.18	GC
Q6UXH8	Collagen and calcium-binding EGF domain-containing protein 1	360.5	GC
Q6ZS62	Colorectal cancer-associated protein 1	313.76	GC
Q15131	Cyclin-dependent kinase 10	222.55	GC
Q9UBD3	Cytokine SCM-1 beta	219.29	GC
O75907	Diacylglycerol O-acyltransferase 1	352.1	GC
Q15700	Disks large homolog 2	124.57	GC

B8ZZZ7	DNA polymerase-transactivated protein 6_ isoform CRA_b1	214.38	GC
H7BY40	DNA-directed RNA polymerase III subunit RPC9	432.67	GC
Q8IXS2	Dynein regulatory complex subunit 2	201.73	GC
Q9BY07	Electrogenic sodium bicarbonate cotransporter 4	338.85	GC
Q7L2H7	Eukaryotic translation initiation factor 3 subunit M	348.07	GC
Q5TF85	Family with sequence similarity 46_ member A_ isoform CRA_a	302.5	GC
P49327	Fatty acid synthase	144.04	GC
P85037	Forkhead box protein K1	159.49	GC
P05062	Fructose-bisphosphate aldolase B	127.89	GC
H7C358	Gamma-tubulin complex component (Fragment)	166.23	GC
Q9BQ67	Glutamate-rich WD repeat-containing protein 1	167.93	GC
B5MC36	Glutathione hydrolase 1 proenzyme	204.35	GC
Q14390	Glutathione hydrolase light chain 2	204.35	GC
Q14789	Golgin subfamily B member 1	192.5	GC
P16260	Graves disease carrier protein	1144.07	GC
Q02108	Guanylate cyclase soluble subunit alpha-1	92.38	GC
E7ES21	Hephaestin	774.47	GC
P0C5Y9	Histone H2A-Bbd type 1	150.03	GC
P0C5Z0	Histone H2A-Bbd type 2/3	150.03	GC
Q03164	Histone-lysine N-methyltransferase 2A	191.36	GC
Q9UBX0	Homeobox expressed in ES cells 1	215.9	GC
Q4G0P3	Hydrocephalus-inducing protein homolog	245	GC
P01742	Immunoglobulin heavy variable 1-69	283.74	GC
P04433	Immunoglobulin kappa variable 3-11	190.6	GC
A0A0A0MRZ8	Immunoglobulin kappa variable 3D-11	190.6	GC
Q14571	Inositol 1_4_5-trisphosphate receptor type 2	398.43	GC
P53708	Integrin alpha-8	90.44	GC
Q9H079	KATNB1-like protein 1	182.41	GC
B9EIJ4	KCNK3 protein	279.07	GC
O76011	Keratin_ type I cuticular Ha4	151.08	GC
P13647	Keratin_ type II cytoskeletal 5	122.47	GC
P02538	Keratin_ type II cytoskeletal 6A	193.12	GC
P04259	Keratin_ type II cytoskeletal 6B	180.62	GC
P48668	Keratin_ type II cytoskeletal 6C	193.12	GC
Q14894	Ketimine reductase mu-crystallin	284.45	GC
Q8IVT5	Kinase suppressor of Ras 1	225.56	GC
O60333	Kinesin-like protein KIF1B	116.56	GC
F8W6J0	LETM1 domain-containing protein 1	208.78	GC
Q13136	Liprin-alpha-1	174.07	GC
P47992	Lymphotactin	147.34	GC
A0JLT2	Mediator of RNA polymerase II transcription subunit 19	228.2	GC
E9PD25	Metalloendopeptidase	299.17	GC
F6WRY4	Msx2-interacting protein (Fragment)	140.68	GC
H0YGQ3	Multiple PDZ domain protein (Fragment)	214.43	GC
Q8N0W4	Neuroligin-4_ X-linked	176.09	GC
Q5JPE7	Nodal modulator 2	285.7	GC

H7C498	Nuclear factor erythroid 2-related factor 2 (Fragment)	457.16	GC
F6X8W2	Nuclear factor of-activated T-cells 5	461.81	GC
Q92508	Piezo-type mechanosensitive ion channel component 1	199.71	GC
H0YEQ7	Polycystic kidney disease protein 1-like 2 (Fragment)	263.56	GC
O14649	Potassium channel subfamily K member 3	279.07	GC
Q6ZPD9	Probable C-mannosyltransferase DPY19L3	211.89	GC
Q2NL68	Proline and serine-rich protein 3	144.2	GC
O75629	Protein CREG1	216.21	GC
Q96MY7	Protein FAM161B	132.13	GC
Q5TBA9	Protein furry homolog	217.17	GC
Q13438	Protein OS-9	244.32	GC
Q9BVV6	Protein TALPID3	269.52	GC
E5RJ77	Protein-tyrosine kinase 2-beta (Fragment)	194.84	GC
A0A087X1P2	Protocadherin gamma-C3 (Fragment)	134.19	GC
Q3KPI9	PTPRD protein	207.2	GC
Q2PPJ7	Ral GTPase-activating protein subunit alpha-2	262.57	GC
P23468	Receptor-type tyrosine-protein phosphatase delta	211.49	GC
Q2NKQ5	Reticulon	129.27	GC
Q16799	Reticulon-1	129.27	GC
Q13017	Rho GTPase-activating protein 5	223.2	GC
Q15633	RISC-loading complex subunit TARBP2	191.75	GC
Q7Z7L1	Schlafen family member 11	517.44	GC
Q68D06	Schlafen family member 13	502.88	GC
Q96BR1	Serine/threonine-protein kinase Sgk3	191.88	GC
Q8TAD8	Smad nuclear-interacting protein 1	506.67	GC
A0A087X1R1	Smoothelin	179.4	GC
Q9NUQ6	SPATS2-like protein	215.59	GC
H0YAC0	Sperm flagellar protein 2 (Fragment)	259.03	GC
Q86XZ4	Spermatogenesis-associated serine-rich protein 2	102.85	GC
K7EJ32	Sphingosine kinase 1 (Fragment)	304.66	GC
Q9Y2I9	TBC1 domain family member 30	442.3	GC
Q96IP4	Terminal nucleotidyltransferase 5A	302.5	GC
B4E171	Tetraspanin	168.56	GC
O43897	Tolloid-like protein 1	299.17	GC
O75674	TOM1-like protein 1	156.14	GC
Q9P1P5	Trace amine-associated receptor 2	151.56	GC
Q9UPV9	Trafficking kinesin-binding protein 1	69.33	GC
P48553	Trafficking protein particle complex subunit 10	149.55	GC
Q7Z6M4	Transcription termination factor 4_ mitochondrial	160.92	GC
Q2TAA8	Translin-associated factor X-interacting protein 1	94.68	GC
O14668	Transmembrane gamma-carboxyglutamic acid protein 1	168.56	GC
P02766	Transthyretin	144.13	GC
Q14679	Tubulin polyglutamylase TTLL4	156.01	GC
E9PIN5	Tumor protein p53-inducible protein 11 (Fragment)	319.64	GC
P35236	Tyrosine-protein phosphatase non-receptor type 7	280.14	GC
O14562	Ubiquitin domain-containing protein UBFD1	327.32	GC

A0A140TA62	Uncharacterized protein	151.08	GC
B2RTY4	Unconventional myosin-Ixa	197.47	GC
P54727	UV excision repair protein RAD23 homolog B	194.06	GC
Q68DQ2	Very large A-kinase anchor protein	146.95	GC
A2RRD8	Zinc finger protein 320	186.02	GC
Q96MU6	Zinc finger protein 778	269.41	GC
Q96DA0	Zymogen granule protein 16 homolog B	153.51	GC
P04217	Alpha-1B-glycoprotein	56.51	GWL**
Q8N944	APC membrane recruitment protein 3	130.19	GWL
Q9NUQ8	ATP-binding cassette sub-family F member 3	280.96	GWL
Q7Z589	BRCA2-interacting transcriptional repressor EMSY	113.36	GWL
Q8N5Z5	BTB/POZ domain-containing protein KCTD17	280.54	GWL
Q96HY3	CALM1 protein [m, m, t, v]	497.91	GWL
P0DP23	Calmodulin-1	497.91	GWL
P0DP24	Calmodulin-2	497.91	GWL
P0DP25	Calmodulin-3	497.91	GWL
Q6ZRH7	Cation channel sperm-associated protein subunit gamma	141.62	GWL
B7Z2K3	cDNA FLJ54631_ highly similar to Protein FAM13C1	100.1	GWL
A0A0B4J1Z0	COBL-like 1_ isoform CRA_a	202.51	GWL
A0A0J9YY35	Coiled-coil and C2 domain-containing protein 2A (Fragment)	185.24	GWL
Q8NCX0	Coiled-coil domain-containing protein 150	215.22	GWL
Q6IBW4	Condensin-2 complex subunit H2	92.92	GWL
Q53SF7	Cordon-bleu protein-like 1	214.09	GWL
P78396	Cyclin-A1	131.83	GWL
Q96N67	Dedicator of cytokinesis protein 7	53.84	GWL
Q8NF50	Dedicator of cytokinesis protein 8	142.63	GWL
P54886	Delta-1-pyrroline-5-carboxylate synthase	92.95	GWL
B7Z647	Discs_ large homolog 4 (Drosophila)_ isoform CRA_b	126.13	GWL
O14909	Discs_ large homolog 4 (Drosophila)_ isoform CRA_d	126.13	GWL
P78352	Disks large homolog 4	126.13	GWL
B9EGL1	DLG4 protein	126.13	GWL
Q9NXL9	DNA helicase MCM9	140.79	GWL
Q9UNA4	DNA polymerase iota	207.32	GWL
Q8NEP3	Dynein assembly factor 1_ axonemal	206.16	GWL
Q7Z6J0	E3 ubiquitin-protein ligase SH3RF1	88.58	GWL
O43921	Ephrin-A2	149.52	GWL
Q9H6T0	Epithelial splicing regulatory protein 2	87	GWL
Q96RT1	Erbin	202.56	GWL
A0A087X216	Family with sequence similarity 13_ member C1_ isoform CRA_g	100.1	GWL
Q96AE4	Far upstream element-binding protein 1	133.85	GWL
Q969Z0	FAST kinase domain-containing protein 4	85.18	GWL
Q9Y2G5	GDP-fucose protein O-fucosyltransferase 2	99.71	GWL
H3BU37	General transcription factor 3C polypeptide 1 (Fragment)	100.02	GWL
P04406	Glyceraldehyde-3-phosphate dehydrogenase	179.9	GWL
P30968	Gonadotropin-releasing hormone receptor	104.53	GWL
C9J4A3	HCG2018530_ isoform CRA_c	119.84	GWL

Q15477	Helicase SKI2W	113.47	GWL
P15515	Histatin-1	507.22	GWL
P15516	Histatin-3	1834.82	GWL
Q9UPP1	Histone lysine demethylase PHF8	104.65	GWL
Q9NR48	Histone-lysine N-methyltransferase ASH1L	195.26	GWL
A9QM74	Importin subunit alpha-8	73.32	GWL
Q14974	Importin subunit beta-1	127.47	GWL
Q9H0H0	Integrator complex subunit 2	133.4	GWL
Q7Z4S6	Kinesin-like protein KIF21A	118.58	GWL
P12318	Low affinity immunoglobulin gamma Fc region receptor II-a	182	GWL
H0Y8A3	Mitogen-activated protein kinase kinase kinase 6 (Fragment)	260.76	GWL
Q9Y4K4	Mitogen-activated protein kinase kinase kinase kinase 5	129.58	GWL
Q5SSG8	Mucin-21	147.14	GWL
Q8TAX7	Mucin-7	351.26	GWL
U3KQ59	Mucosal addressin cell adhesion molecule 1	139.92	GWL
K7EMK2	NACHT_ LRR and PYD domains-containing protein 2 (Fragment)	381.63	GWL
Q86UW6	NEDD4-binding protein 2	104.2	GWL
Q99574	Neuroserpin	346.63	GWL
Q9Y314	Nitric oxide synthase-interacting protein	152.98	GWL
Q86WB0	Nuclear-interacting partner of ALK	238.23	GWL
A0A087X0P9	Probable RNA polymerase II nuclear localization protein SLC7A6OS (Fragment)	108.61	GWL
Q92530	Proteasome inhibitor PI31 subunit	108.37	GWL
Q8NE31	Protein FAM13C	100.1	GWL
U3KQR7	Protein FAM47E (Fragment)	300.47	GWL
Q08174	Protocadherin-1	145.24	GWL
Q9BZA8	Protocadherin-11 Y-linked	160.08	GWL
Q8NHS7	PTPRS protein	208.79	GWL
A0A3B3IRI8	Putative aldo-keto reductase family 1 member C8	110.73	GWL
A6ND91	Putative L-aspartate dehydrogenase	316.77	GWL
P0C7V0	Putative uncharacterized protein encoded by LINC00271	146.73	GWL
P52306	Rap1 GTPase-GDP dissociation stimulator 1	122.84	GWL
Q9UN86	Ras GTPase-activating protein-binding protein 2	220.29	GWL
P61018	Ras-related protein Rab-4B	118.48	GWL
Q13332	Receptor-type tyrosine-protein phosphatase S	209.98	GWL
P49796	Regulator of G-protein signaling 3	432.69	GWL
E5RJ23	Regulator of G-protein-signaling 22 (Fragment)	342.62	GWL
Q6XE24	RNA-binding motif_ single-stranded-interacting protein 3	110.84	GWL
A0A087WV26	Sjoegren syndrome nuclear autoantigen 1	145.47	GWL
Q8IVB4	Sodium/hydrogen exchanger 9	96.61	GWL
Q99624	Sodium-coupled neutral amino acid transporter 3	140.45	GWL
Q695T7	Sodium-dependent neutral amino acid transporter B(0)AT1	53.55	GWL
Q8WWT9	Solute carrier family 13 member 3	119.84	GWL
Q96BD8	Spindle and kinetochore-associated protein 1	127.04	GWL
A0A087WU33	SRC kinase-signaling inhibitor 1 (Fragment)	86.11	GWL
P13686	Tartrate-resistant acid phosphatase type 5	112.09	GWL

Q96DN5	TBC1 domain family member 31	189.49	GWL
Q9BZW7	Testis-specific gene 10 protein	98.69	GWL
P07996	Thrombospondin-1	218.43	GWL
Q13009	T-lymphoma invasion and metastasis-inducing protein 1	81.33	GWL
Q99990	Transcription cofactor vestigial-like protein 1	272.46	GWL
O43934	UNC93-like protein MFSD11	93.75	GWL
A0A1W2PNV4	Uncharacterized protein	83.51	GWL
Q6P1W5	Uncharacterized protein C1orf94	177.82	GWL
P0DPF5	Uncharacterized protein C2orf27A	117.85	GWL
P0DPF6	Uncharacterized protein C2orf27B	117.85	GWL
P46939	Utrophin	168.5	GWL
P08670	Vimentin	151.23	GWL
Q6VVX0	Vitamin D 25-hydroxylase	75.77	GWL
A0A1B0GVB2	Voltage-dependent L-type calcium channel subunit beta-4	287.24	GWL
Q502W6	von Willebrand factor A domain-containing protein 3B	55.8	GWL
Q05481	Zinc finger protein 91	315.43	GWL
H7BZ52	Zinc finger SWIM domain-containing protein 8	64.44	GWL
Q8TDZ2	[F-actin]-monooxygenase MICAL1	157.98	GWH**
P82673	28S ribosomal protein S35_ mitochondrial	196.81	GWH
P04035	3-hydroxy-3-methylglutaryl-coenzyme A reductase	452.23	GWH
D6RHJ2	3-hydroxymethyl-3-methylglutaryl-CoA lyase_ cytoplasmic	405.78	GWH
O00763	Acetyl-CoA carboxylase 2	211.7	GWH
Q96PE1	Adhesion G protein-coupled receptor A2	154.88	GWH
A0A1W2PR51	Adhesion G-protein-coupled receptor V1 (Fragment)	466.28	GWH
Q99996	A-kinase anchor protein 9	192.23	GWH
A0A1B0GU55	Aminomethyltransferase_ mitochondrial	776.28	GWH
Q9H1A4	Anaphase-promoting complex subunit 1	299.25	GWH
H0Y8F8	Angiogenic factor with G patch and FHA domains 1 (Fragment)	636.35	GWH
P12821	Angiotensin-converting enzyme	292.83	GWH
P16157	Ankyrin-1	186.23	GWH
A0A075B752	Annexin	185.05	GWH
Q5VT79	Annexin A8-like protein 1	185.05	GWH
P41181	Aquaporin-2	189.99	GWH
A0A0A0MRE5	Arf-GAP with SH3 domain_ ANK repeat and PH domain-containing protein 1	559.26	GWH
Q4G0X4	BTB/POZ domain-containing protein KCTD21	219.18	GWH
J3KTG8	Cadherin-8 (Fragment)	142.82	GWH
Q8N187	Calcium-responsive transcription factor	869.09	GWH
H0YD38	Centrosome-associated protein 350 (Fragment)	710.13	GWH
Q9HC52	Chromobox protein homolog 8	266.09	GWH
Q6GPI1	Chymotrypsinogen B2	230.68	GWH
Q8IYR0	Cilia- and flagella-associated protein 206	110.26	GWH
P53621	Coatomer subunit alpha	200.41	GWH
P01024	Complement C3 [b,e,j,o,u,w]	254.28	GWH
Q8WZ74	Cortactin-binding protein 2	203.9	GWH
Q2VPK5	Cytoplasmic tRNA 2-thiolation protein 2	309.65	GWH

Q5JSL3	Dedicator of cytokinesis protein 11	259.38	GWH
Q9NP87	DNA-directed DNA/RNA polymerase mu	174.55	GWH
O43812	Double homeobox protein 1	167.94	GWH
H0YGZ2	Dynein heavy chain 10_ axonemal (Fragment)	145.17	GWH
Q9Y282	Endoplasmic reticulum-Golgi intermediate compartment protein 3	265.81	GWH
A6PVJ2	ERGIC and golgi 3 (Fragment)	253.8	GWH
Q9HAV4	Exportin-5	147.23	GWH
Q9BSJ8	Extended synaptotagmin-1	240.87	GWH
O75369	Filamin-B	186.21	GWH
H3BQN4	Fructose-bisphosphate aldolase	144.02	GWH
P04075	Fructose-bisphosphate aldolase A	144.02	GWH
Q14687	Genetic suppressor element 1	96.86	GWH
Q3V6T2	Girdin	317.47	GWH
P47897	Glutamine--tRNA ligase	203.03	GWH
E7ETN1	Glutathione hydrolase 1 proenzyme (Fragment)	385.88	GWH
H3BMS9	HCG2029558_ isoform CRA_b	157.14	GWH
K7ELA5	HEAT repeat-containing protein 6 (Fragment)	260.01	GWH
Q5SSJ5	Heterochromatin protein 1-binding protein 3	204.97	GWH
A5PLL3	Histone acetyltransferase	171.8	GWH
Q92794	Histone acetyltransferase KAT6A	171.8	GWH
Q92800	Histone-lysine N-methyltransferase EZH1	279.12	GWH
S4R460	Immunoglobulin heavy variable 3/OR16-9 (non-functional)	291.46	GWH
Q9Y471	Inactive cytidine monophosphate-N-acetylneuraminic acid hydroxylase	139.96	GWH
A0AVF1	Intraflagellar transport protein 56	139.21	GWH
Q96Q89	Kinesin-like protein KIF20B	177.25	GWH
Q9UGL1	Lysine-specific demethylase 5B	194.05	GWH
Q9BUT9	MAPK regulated corepressor interacting protein 2	184.55	GWH
P50539	Max-interacting protein 1	155.37	GWH
O00255	Menin	133.58	GWH
Q10713	Mitochondrial-processing peptidase subunit alpha	168.86	GWH
Q9Y5R4	MTRF1L release factor glutamine methyltransferase	295.71	GWH
P18615	Negative elongation factor E	136.42	GWH
F5H6Y5	Nuclear mitotic apparatus protein 1 (Fragment)	309.98	GWH
P42356	Phosphatidylinositol 4-kinase alpha	246.69	GWH
H7C4H9	Polyhomeotic-like protein 3 (Fragment)	216.4	GWH
P40426	Pre-B-cell leukemia transcription factor 3	178.8	GWH
M0QXP2	Pregnancy-specific beta-1-glycoprotein 3	314.6	GWH
Q86UW9	Probable E3 ubiquitin-protein ligase DTX2	192.11	GWH
A0A0A0MT31	Proline-rich protein 4	1751.55	GWH
Q5U5X8	Protein FAM222A	128.24	GWH
Q8N9W8	Protein FAM71D	193.57	GWH
Q86WI3	Protein NLRC5	210.07	GWH
H0YNY1	Protein PAT1 homolog 2 (Fragment)	169.83	GWH
H7C3T6	Protein tweety homolog (Fragment)	349.27	GWH
Q9C0H2	Protein tweety homolog 3	349.27	GWH

Q9Y5F8	Protocadherin gamma-B7	150.18	GWH
P35247	Pulmonary surfactant-associated protein D	281.81	GWH
Q9H0H9	Putative cytochrome P450 family member 4F30	173.17	GWH
B4DNK4	Pyruvate kinase	707.24	GWH
P14618	Pyruvate kinase PKM	707.24	GWH
Q12913	Receptor-type tyrosine-protein phosphatase eta	150.62	GWH
Q16849	Receptor-type tyrosine-protein phosphatase-like N	169.26	GWH
Q8N122	Regulatory-associated protein of mTOR	200.08	GWH
Q8N264	Rho GTPase-activating protein 24	186.44	GWH
P02810	Salivary acidic proline-rich phosphoprotein 1/2	1751.55	GWH
P16615	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	383.35	GWH
P15056	Serine/threonine-protein kinase B-raf	234.64	GWH
Q13315	Serine-protein kinase ATM	288.04	GWH
Q96GZ6	Solute carrier family 41 member 3	165.59	GWH
Q96JI7	Spatacsin	191.01	GWH
Q7KZF4	Staphylococcal nuclease domain-containing protein 1	155.95	GWH
Q9BPZ7	Target of rapamycin complex 2 subunit MAPKAP1	335.67	GWH
Q99973	Telomerase protein component 1	218.26	GWH
Q96BF3	Transmembrane and immunoglobulin domain-containing protein 2	104.42	GWH
Q14694	Ubiquitin carboxyl-terminal hydrolase 10	294.17	GWH
Q9H8M7	Ubiquitin carboxyl-terminal hydrolase MINDY-3	126.95	GWH
A0A087WZY1	Uncharacterized protein	1352.39	GWH
Q5THJ4	Vacuolar protein sorting-associated protein 13D	400.76	GWH
Q2NL98	Vimentin-type intermediate filament-associated coiled-coil protein	197.16	GWH
P21281	V-type proton ATPase subunit B_ brain isoform	566.35	GWH
Q9BV38	WD repeat-containing protein 18	183.47	GWH
Q9HCL3	Zinc finger protein 14 homolog	20.96	GWH
Q9NYT6	Zinc finger protein 226	331.2	GWH
Q8TAQ5	Zinc finger protein 420	388.22	GWH
M0R1P0	Zinc finger protein 530 (Fragment)	430.67	GWH
Q7Z3V5	Zinc finger protein 571	222.6	GWH

*Identification is based on protein ID from UniProt protein database, reviewed only (http://wwwwww.uniprot.org/).
**Indicates unique proteins in alphabetical order.

Table 3. Classification and relative quantification of proteins identified in the acquired enamel pellicle collected from volunteers: GWL - professional wine tasters (PWT) with a BEWE index lower or equal than 8, GWH - PWT with a BEWE index higher or equal than 9 and GC – Group control, not PWT and without ETW.

Accession number*	Protein name	PLGS Score	Ratio GWL:GC**
A2RRC6	*ZFHX2 protein*	*386.51*	*28.50*
Q9C0A1	*Zinc finger homeobox protein 2*	*389.95*	*28.50*
P59666	*Neutrophil defensin 3*	*2297.19*	*3.86*
P59665	*Neutrophil defensin 1*	*2297.19*	*3.82*
P02768	*Serum albumin*	*4139.94*	*3.78*
C9JKR2	*Albumin_ isoform CRA_k*	*1995.86*	*2.51*

F8VV32	*Lysozyme*	*699.52*	*2.39*
P61626	*Lysozyme C*	*849.44*	*1.99*
P02808	*Statherin*	*8736.19*	*1.72*
Q14568	Heat shock protein HSP 90-alpha A2	76.22	1.55
Q15772	Striated muscle preferentially expressed protein kinase	261.36	1.54
Q562R1	Beta-actin-like protein 2	338.01	1.46
P05109	Protein S100-A8	1746.65	1.45
P0CG39	POTE ankyrin domain family member J	641.07	1.40
P60709	Actin_ cytoplasmic 1	5751.01	1.36
P63261	Actin_ cytoplasmic 2	5776.82	1.36
P01857	Immunoglobulin heavy constant gamma 1	544.92	1.34
Q9BYX7	Putative beta-actin-like protein 3	174.64	1.30
P68871	Hemoglobin subunit beta	2850.27	1.28
P02042	Hemoglobin subunit delta	896.02	1.28
P69892	Hemoglobin subunit gamma-2	896.02	1.28
P02100	Hemoglobin subunit epsilon	896.02	1.27
P69891	Hemoglobin subunit gamma-1	896.02	1.27
A0A2R8Y7X9	Uncharacterized protein	896.02	1.27
P01859	Immunoglobulin heavy constant gamma 2	272.04	1.26
P62736	Actin_ aortic smooth muscle	2871.92	1.23
P63267	Actin_ gamma-enteric smooth muscle	2871.92	1.23
P68032	Actin_ alpha cardiac muscle 1	2871.92	1.22
P68133	Actin_ alpha skeletal muscle	2871.92	1.21
P19961	Alpha-amylase 2B	1003.3	1.20
Q5T3N0	Annexin (Fragment)	1159.02	1.15
P06702	Protein S100-A9	10407.64	1.07
Q6S8J3	POTE ankyrin domain family member E	1017.45	0.90
A5A3E0	POTE ankyrin domain family member F	1017.45	0.90
P01036	Cystatin-S	1221.53	0.84
P01037	Cystatin-SN	1585.84	0.84
P09228	Cystatin-SA	768.56	0.84
P0CG38	POTE ankyrin domain family member I	842.81	0.79
P03973	Antileukoproteinase	296.65	0.77
P02812	Basic salivary proline-rich protein 2	166.03	0.58
Q9UP83	Conserved oligomeric Golgi complex subunit 5	155.48	0.58
P13646	Keratin_ type I cytoskeletal 13	176.19	0.51
Q13813	*Spectrin alpha chain_ non-erythrocytic 1*	*181.62*	*0.38*
F8WD68	*Carboxylic ester hydrolase*	*350.27*	*0.27*
P22303	*Acetylcholinesterase*	*350.27*	*0.26*

Accession number*	**Protein name**	**PLGS Score**	**Ratio GWH:GC****
C9JKR2	*Albumin_ isoform CRA_k*	*3847.73*	*4.44*
O75376	*Nuclear receptor corepressor 1*	*325.32*	*4.22*
P02768	*Serum albumin*	*6532.33*	*4.10*
P00738	*Haptoglobin*	*764.85*	*3.19*
G5E9F7	*Pregnancy specific beta-1-glycoprotein 1_ isoform CRA_a*	*231.47*	*3.16*

M0R235	*Pregnancy specific beta-1-glycoprotein 1_ isoform CRA_e*	*231.47*	*3.13*
P11464	*Pregnancy-specific beta-1-glycoprotein 1*	*231.47*	*3.06*
P68871	*Hemoglobin subunit beta*	*3974.06*	*2.27*
A0A2R8Y7X9	*Uncharacterized protein*	*719.15*	*2.23*
P02042	*Hemoglobin subunit delta*	*719.15*	*2.20*
P02100	*Hemoglobin subunit epsilon*	*719.15*	*2.20*
P69891	*Hemoglobin subunit gamma-1*	*719.15*	*2.20*
P69892	*Hemoglobin subunit gamma-2*	*719.15*	*2.20*
Q9BYX7	Putative beta-actin-like protein 3	247.53	1.75
Q15772	Striated muscle preferentially expressed protein kinase	204.87	1.65
P01857	Immunoglobulin heavy constant gamma 1	570.39	1.46
P01876	Immunoglobulin heavy constant alpha 1	881.42	1.34
P01877	Immunoglobulin heavy constant alpha 2	603.69	1.30
P05109	Protein S100-A8	2575.06	1.20
P63261	Actin_ cytoplasmic 2	5038.12	0.85
P60709	Actin_ cytoplasmic 1	5038.12	0.84
P68032	Actin_ alpha cardiac muscle 1	3128.95	0.79
P68133	Actin_ alpha skeletal muscle	3152.77	0.79
P62736	Actin_ aortic smooth muscle	3128.95	0.79
P63267	Actin_ gamma-enteric smooth muscle	3128.95	0.79
P02808	Statherin	9488.73	0.77
Q6S8J3	POTE ankyrin domain family member E	1080.32	0.76
P04083	Annexin A1	875.98	0.75
A5A3E0	POTE ankyrin domain family member F	1080.32	0.74
P06702	Protein S100-A9	13277.33	0.70
P61626	Lysozyme C	787.93	0.70
P0CG38	POTE ankyrin domain family member I	832.79	0.63
P04746	Pancreatic alpha-amylase	493.13	0.61
P59665	Neutrophil defensin 1	877.34	0.53
P59666	Neutrophil defensin 3	877.34	0.53
Q6UWP8	*Suprabasin*	*177.99*	*0.28*

Accession number*	**Protein name**	**PLGS Score**	**Ratio GWL:GWH****
Q14534	*Squalene monooxygenase*	*257.23*	*26.05*
P59666	*Neutrophil defensin 3*	*877.34*	*7.54*
P59665	*Neutrophil defensin 1*	*877.34*	*7.46*
P61626	*Lysozyme C*	*787.93*	*3.29*
F8VV32	*Lysozyme*	*726.35*	*3.16*
P02808	*Statherin*	*9488.73*	*2.41*
P05164	*Myeloperoxidase*	*152.67*	*2.12*
P0CG39	POTE ankyrin domain family member J	421.78	1.82
P07478	Trypsin-2	183.84	1.75
A6XMV9	Protease serine 2 preproprotein	183.84	1.65
P04746	Pancreatic alpha-amylase	493.13	1.63
P06702	Protein S100-A9	13277.33	1.63
H7C319	AF4/FMR2 family member 4 (Fragment)	261.79	1.58

P0CG38	POTE ankyrin domain family member I	832.79	1.49
P04083	Annexin A1	875.98	1.46
P19961	Alpha-amylase 2B	784.21	1.42
A5A3E0	POTE ankyrin domain family member F	1080.32	1.32
P68032	Actin_ alpha cardiac muscle 1	3128.95	1.31
Q6S8J3	POTE ankyrin domain family member E	1080.32	1.31
P05109	Protein S100-A8	2575.06	1.31
P01859	Immunoglobulin heavy constant gamma 2	209.24	1.31
P68133	Actin_ alpha skeletal muscle	3152.77	1.30
P62736	Actin_ aortic smooth muscle	3128.95	1.30
P63267	Actin_ gamma-enteric smooth muscle	3128.95	1.30
P63261	Actin_ cytoplasmic 2	5038.12	1.19
P60709	Actin_ cytoplasmic 1	5038.12	1.17
P02788	Lactotransferrin	351.05	1.14
P02814	Submaxillary gland androgen-regulated protein 3B	2361.12	0.89
P01877	Immunoglobulin heavy constant alpha 2	603.69	0.79
P01876	Immunoglobulin heavy constant alpha 1	881.42	0.78
P02787	Serotransferrin	810.08	0.69
P02812	Basic salivary proline-rich protein 2	1228.83	0.67
P02768	Serum albumin	6532.33	0.65
P01860	Immunoglobulin heavy constant gamma 3	258.23	0.63
P69891	Hemoglobin subunit gamma-1	719.15	0.63
P01861	Immunoglobulin heavy constant gamma 4	340.18	0.62
C9JKR2	Albumin_ isoform CRA_k	3847.73	0.62
P02100	Hemoglobin subunit epsilon	719.15	0.62
A0A2R8Y7X9	Uncharacterized protein	719.15	0.62
P02042	Hemoglobin subunit delta	719.15	0.61
P69892	Hemoglobin subunit gamma-2	719.15	0.61
P68871	Hemoglobin subunit beta	3974.06	0.58
P01834	Immunoglobulin kappa constant	724.3	0.57
G3V1N2	HCG1745306_ isoform CRA_a	1537.2	0.56
P01857	Immunoglobulin heavy constant gamma 1	570.39	0.55
P69905	Hemoglobin subunit alpha	3152.2	0.54
P00738	*Haptoglobin*	*764.85*	*0.34*

*Identification is based on protein ID from UniProt protein database, reviewed only (http://wwwwww.uniprot.org/).
**Proteins with expression significantly altered are organized according to the ratio.
Proteins highlighted in italic are increased or decreased more than 2-fold.

DISCUSSION

This is the first study that compared the proteomic profile of AEP of PWT with ETW, with low and moderate BEWE index. A control group constituted of volunteers with no ETW and that were not PWT, allowing the detection of changes in the proteomic profile of the AEP in function of being a PWT. The results revealed many alterations in the protein profile of the

AEP from groups GWL and GWH, as well as between PWT groups and control volunteers, which led us to reject both null hypotheses of the study.

The characteristics of the volunteers included in the study were similar in terms of age in the groups GWL and GC, while GWH volunteers were slightly older. Despite ETW increases with age, the age range was not substantial. Moreover, the inclusion criteria for the PWT with ETW that volunteered assured that all of them worked as PWT for at least 5 years, enabling enough time for ETW to occur. Moreover, the median time of working as PWT was similar for GWL and GWH groups. Regarding gender, since this was a convenience sample, GC group had only females, unlike GWL and GWH groups, that had similar numbers of male and female volunteers. The mean BEWE score of the volunteers of GWH group was 12.0, which denotes moderate ETW. For GWL, it was 8.0, corresponding to mild ETW [31] (Table 1).

The protocol of protein extraction and proteomic analysis followed a recently developed methodology that increases the identification of proteins in the AEP samples [30]. It is important to highlight that even with only 3 volunteers at the GWL group, we were able to obtain 37 μg of protein, which was enough for proper proteomic analysis, as previously reported [32]. Accordingly, the number of proteins that were identified was 412, which is one of the highest numbers ever reported in studies involving in vivo analysis of AEP. The proteomic profiles of the AEPs collected in the 3 groups was shown to be different, as can be described from the high numbers of unique proteins in each group (all higher than 100). Most of the proteins identified in all groups were proteins typically found in the AEP (Table S1) that presented differences in expression among the groups (Table 3). This means that being a PWT with ETW has a great impact on the proteomic profile of the AEP that also changes remarkably in PWT volunteers with low or moderate ETW.

Among the proteins exclusively identified in the GWL group are Histatins 1 and 3, which are phosphorylated and negatively charged proteins, with antibacterial and antifungal activities, as well as Statherin, regarded as, an acid-resistant protein [32]. Both proteins have strong affinity to hydroxyapatite and are included among the precursor proteins of the AEP, constituting the basal layer of this integument [34] that concedes most of the protection against demineralization, since it is not removed after erosive challenges [35, 36].

Another interesting finding related to the exclusive proteins was the fact that Mucin 7 and Mucin 21 were only identified in the GWL group. Mucins are potentially protective proteins, that alone or combined with other proteins, when adhered to the enamel surface,

have the capacity to inhibit enamel demineralization caused by erosive challenge [37, 38]. Besides, they are pellicle precursor proteins, such as Histatins and Statherin, and also form the basal layer of the AEP [39, 40]. Therefore, it is possible that the greater number of Histatins and Mucins might help to protect against ETW. In addition, Statherin is able to maintain a state of saturation with respect to calcium and phosphate in the oral cavity by inhibiting their precipitation at neutral pH and releasing these ions following acidic attack and during demineralization [40]. This is important to highlight because Statherin was increased in the GWL group, compared to GWH, and could also be responsible, at least in part, for the protection against ETW.

Concerning to the expression analyses, the first two compare the PWT groups with the control one. These comparisons likely reflect the proteins that have their rates of expression changed in function of being a PWT. It is noteworthy that proteins higher in GWL compared with GC were lower when GWH was compared with GC, such as Lysozyme C and Statherin, as well as isoforms of neutrophil defensin. The opposite was not found, which is in disagreement with the findings of Martini et al. [25]. This could be explained by the different types of acids involved in both studies: intrinsic (gastric acids) for Martini et al. [25] and extrinsic (malic and tartaric acids) for the present study. It is also noteworthy that Statherin, a calcium-binding protein, was lower in the GWH group compared with GC, which is consistent with recent reports of lower rates of this protein in patients with erosion [25, 41, 42]. When the groups GWL and GC were compared, Statherin was higher in the first, suggesting that at lower rates of ETW, different results are found.

It is also interesting to highlight the increase in distinct subunits of Hemoglobins in the GWH group compared with GC, and in lower increase in the GWL group compared to GC. Hemoglobins were recently identified in the AEP collected from the posterior region [30]. Hydroxyapatite columns are used to purify hemoglobin and nanostructured hydroxyapatite polyhedral were developed to deliver this protein in a controlled manner, showing that Hemoglobin has strong affinity to hydroxyapatite [42]. Interestingly, the adsorption rate of hemoglobin to hydroxyapatite increases as pH decreases [43]. PWT have an oral pH lower during wine tasting due to wine acidity (pH 3.0 to 3.8) [11] than what is found in non PWT, which might increase the chance of hemoglobin adsorption onto the dental surfaces. This finding disagrees with Martini et al. [25] that suggested that Hemoglobin might have protective role against dental erosion caused by intrinsic acids, as the group with lower rates of ETW (GWL) had lower rates of Hemoglobin when compared to GWH. An explanation of

this could also be the different origin of the acids causing erosion: intrinsic at Martini et al. [25] and extrinsic, tartaric and malic acids in the present study.

The most interesting comparison, considering the main aim of this study, that is to find proteins in the GWL group that might be associated with protection against ETW, is between GWL and GWH groups. Among the proteins with the lower rates of expression (about 3- fold reduction) in GWL group compared with GWH group is Haptoglobin. Among the proteins with the higher rates of expression (up to 3- fold increase) are Lysozyme C and Lysozyme. Lysozyme C was recently reported as an acid-resistant protein, since it was higher in the AEP after challenge with 1% citric acid [32]. Lysozyme present protein-protein interaction features with other salivary proteins [44, 45]. This can justify the increase in the level of this protein in the last stage of AEP formation, where for example it can link to other proteins such as Histatin 1 [34]. Two isoforms of Neutrophil defensins (1 and 3) were also increased (up to 7-fold). They have antibiotic, fungicide, antiviral and antimicrobial activities. Also, Neutrophil defensins are thought to kill microbes by permeabilizing their plasma membrane [46]. Myeloperoxidase also presented increased at GWL group, (up to 2-fold). It has been shown that by incubating saliva with enamel powder, peroxidase is capable of binding irreversibly to human enamel in an enzymatically active conformation [47, 48]. An interesting finding of the present study was the identification, for the first time, of Squalene Monooxygenase (or Squalene Epoxidase) in AEP analysis. It was observed a higher level of Squalene Monooxygenase (up to 27- fold) in the GWL group compared to GWH group. Moreover, this protein was not found in the GC group. It has a strong affinity to hydroxyapatite. Hydroxyapatite columns have been already used to purify Squalene Monooxygenase [49]. This finding indicates that this protein might have protective role against ETW, mainly for tartaric and malic acids, which needs to be investigated in future studies.

Concluding, profound alterations in the proteomic profile of the AEP were seen in group GWL compared to group GWH. Also, it was the first time Squalene monooxygenase was identified in the AEP. These findings might play a role in the resistance to ETW seen in GWL. This pioneer study compared the proteomic profile of the AEP of PWT with low and moderate ETW. Increased proteins in those with low ETW might be protective and are good candidates to be added to dental products to protect PWT against extrinsic ETW.

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Table S1. Classification of identified proteins from the acquired enamel pellicle collected from volunteers: GWL - professional wine tasters (PWT) with a BEWE index lower or equal than 8, GWH - PWT with a BEWE index higher or equal than 9 (GWH) and GC – Group control, not PWT and without ETW.

Access number*	**Protein name**	***PLGS Score***	**GC**	**GWL**	**GWH**
Q8TDZ2	[F-actin]-monooxygenase MICAL1	157.98	-	-	Yes
P82673	28S ribosomal protein S35_ mitochondrial	196.81	-	-	Yes
P04035	3-hydroxy-3-methylglutaryl-coenzyme A reductase	452.23	-	-	Yes

D6RHJ2	3-hydroxymethyl-3-methylglutaryl-CoA lyase_ cytoplasmic	405.78	-	-	Yes
P22303	Acetylcholinesterase	350.27	Yes	Yes	-
O00763	Acetyl-CoA carboxylase 2	211.7	-	-	Yes
Q9NUB1	Acetyl-coenzyme A synthetase 2-like_ mitochondrial	459.04	Yes	-	-
P68032	Actin_ alpha cardiac muscle 1	2871.92	Yes	Yes	Yes
P68133	Actin_ alpha skeletal muscle	3152.77	Yes	Yes	Yes
P62736	Actin_ aortic smooth muscle	3128.95	Yes	Yes	Yes
P60709	Actin_ cytoplasmic 1	5751.01	Yes	Yes	Yes
P63261	Actin_ cytoplasmic 2	5776.82	Yes	Yes	Yes
P63267	Actin_ gamma-enteric smooth muscle	3128.95	Yes	Yes	Yes
Q96M93	Adenosine deaminase domain-containing protein 1	168.08	Yes	-	-
Q96PE1	Adhesion G protein-coupled receptor A2	154.88	-	-	Yes
Q9Y653	Adhesion G-protein coupled receptor G1	382.45	Yes	-	-
A0A1W2PR51	Adhesion G-protein-coupled receptor V1 (Fragment)	466.28	-	-	Yes
H7C319	AF4/FMR2 family member 4 (Fragment)	261.79	-	Yes	Yes
O43572	A-kinase anchor protein 10_ mitochondrial	132.31	Yes	-	-
Q99996	A-kinase anchor protein 9	192.23	-	-	Yes
C9JKR2	Albumin_ isoform CRA_k	3847.73	Yes	Yes	Yes
P04217	Alpha-1B-glycoprotein	56.51	-	Yes	-
P04745	Alpha-amylase 1	1478.19	Yes	Yes	Yes
P19961	Alpha-amylase 2B	1003.3	Yes	Yes	Yes
A0A1B0GU55	Aminomethyltransferase_ mitochondrial	776.28	-	-	Yes
Q9H1A4	Anaphase-promoting complex subunit 1	299.25	-	-	Yes
H0Y8F8	Angiogenic factor with G patch and FHA domains 1 (Fragment)	636.35	-	-	Yes
P12821	Angiotensin-converting enzyme	292.83	-	-	Yes
E7EQT3	Anion exchange protein	338.85	Yes	-	-
P16157	Ankyrin-1	186.23	-	-	Yes
A0A075B752	Annexin	185.05	-	-	Yes
Q5T3N0	Annexin (Fragment)	1159.02	Yes	Yes	-
P04083	Annexin A1	1262.4	Yes	Yes	Yes
Q5VT79	Annexin A8-like protein 1	185.05	-	-	Yes
P03973	Antileukoproteinase	296.65	Yes	Yes	-
Q8N944	APC membrane recruitment protein 3	130.19	-	Yes	-
Q96PS8	Aquaporin-10	160.05	Yes	-	-
P41181	Aquaporin-2	189.99	-	-	Yes
A0A0A0MRE5	Arf-GAP with SH3 domain_ ANK repeat and PH domain-containing protein 1	559.26	-	-	Yes
Q8WWZ7	ATP-binding cassette sub-family A member 5	152.05	Yes	-	-
Q9NUQ8	ATP-binding cassette sub-family F member 3	280.96	-	Yes	-
Q676U5	Autophagy-related protein 16-1	186.64	Yes	-	-
P04280	Basic salivary proline-rich protein 1	1034.83	Yes	-	Yes
P02812	Basic salivary proline-rich protein 2	1228.83	Yes	Yes	Yes

Q9HB09	Bcl-2-like protein 12	255.66	Yes	-	-
Q562R1	Beta-actin-like protein 2	848.06	Yes	Yes	Yes
Q8TDL5	BPI fold-containing family B member 1	224.35	Yes	-	-
Q3B891	BRCA1 protein (Fragment)	431.63	Yes	-	-
Q7Z589	BRCA2-interacting transcriptional repressor EMSY	113.36	-	Yes	-
P38398	Breast cancer type 1 susceptibility protein	447.2	Yes	-	-
Q8N5Z5	BTB/POZ domain-containing protein KCTD17	280.54	-	Yes	-
Q4G0X4	BTB/POZ domain-containing protein KCTD21	219.18	-	-	Yes
Q13634	Cadherin-18	171.95	-	Yes	Yes
J3KTG8	Cadherin-8 (Fragment)	142.82	-	-	Yes
Q8N187	Calcium-responsive transcription factor	869.09	-	-	Yes
Q96HY3	CALM1 protein	497.91	-	Yes	-
P0DP23	Calmodulin-1	497.91	-	Yes	-
P0DP24	Calmodulin-2	497.91	-	Yes	-
P0DP25	Calmodulin-3	497.91	-	Yes	-
F8WD68	Carboxylic ester hydrolase	350.27	Yes	Yes	-
Q9UI42	Carboxypeptidase A4	203.78	Yes	-	-
P55211	Caspase-9	296.32	Yes	-	-
Q6ZRH7	Cation channel sperm-associated protein subunit gamma	141.62	-	Yes	-
Q15762	CD226 antigen	200.9	Yes	Yes	-
B7Z2K3	cDNA FLJ54631_ highly similar to Protein FAM13C1	100.1	-	Yes	-
Q8N163	Cell cycle and apoptosis regulator protein 2	217.18	Yes	-	-
A0A3B3IRL2	Cellular repressor of E1A-stimulated genes 1_ isoform CRA_a	216.21	Yes	-	-
H0YD38	Centrosome-associated protein 350 (Fragment)	710.13	-	-	Yes
Q9BV73	Centrosome-associated protein CEP250	124.49	Yes	-	-
Q9HC52	Chromobox protein homolog 8	266.09	-	-	Yes
Q8TD26	Chromodomain-helicase-DNA-binding protein 6	181.4	Yes	-	-
Q6GPI1	Chymotrypsinogen B2	230.68	-	-	Yes
Q8IYR0	Cilia- and flagella-associated protein 206	110.26	-	-	Yes
A5D8W1	Cilia- and flagella-associated protein 69	151.66	Yes	-	-
P53621	Coatomer subunit alpha	200.41	-	-	Yes
A0A0B4J1Z0	COBL-like 1_ isoform CRA_a	202.51	-	Yes	
A0A0J9YY35	Coiled-coil and C2 domain-containing protein 2A (Fragment)	185.24	-	Yes	-
Q8NCX0	Coiled-coil domain-containing protein 150	215.22	-	Yes	-
A0A140TA43	COL11A2	141.18	Yes	-	-
P13942	Collagen alpha-2(XI) chain	141.18	Yes	-	-
Q6UXH8	Collagen and calcium-binding EGF domain-containing protein 1	360.5	Yes	-	-
Q6ZS62	Colorectal cancer-associated protein 1	313.76	Yes	-	-
P01024	Complement C3	254.28	-	-	Yes
Q6IBW4	Condensin-2 complex subunit H2	92.92	-	Yes	-

Q9UP83	Conserved oligomeric Golgi complex subunit 5	324.62	Yes	Yes	-
Q53SF7	Cordon-bleu protein-like 1	214.09	-	Yes	-
Q8WZ74	Cortactin-binding protein 2	203.9	-	-	Yes
P78396	Cyclin-A1	131.83	-	Yes	-
Q15131	Cyclin-dependent kinase 10	222.55	Yes	-	-
P04080	Cystatin-B	533.66	Yes	Yes	-
P01036	Cystatin-S	1838.96	Yes	Yes	Yes
P01037	Cystatin-SN	2300.5	Yes	Yes	Yes
Q9UBD3	Cytokine SCM-1 beta	219.29	Yes	-	-
Q2VPK5	Cytoplasmic tRNA 2-thiolation protein 2	309.65	-	-	Yes
Q5JSL3	Dedicator of cytokinesis protein 11	259.38	-	-	Yes
Q96N67	Dedicator of cytokinesis protein 7	53.84	-	Yes	-
Q8NF50	Dedicator of cytokinesis protein 8	142.63	-	Yes	-
P54886	Delta-1-pyrroline-5-carboxylate synthase	92.95	-	Yes	-
Accession	Description	Score	Yes	Yes	-
O75907	Diacylglycerol O-acyltransferase 1	352.1	Yes	-	-
B7Z647	Discs_ large homolog 4 (Drosophila)_ isoform CRA_b	126.13	-	Yes	-
O14909	Discs_ large homolog 4 (Drosophila)_ isoform CRA_d	126.13	-	Yes	-
Q15700	Disks large homolog 2	124.57	Yes	-	-
P78352	Disks large homolog 4	126.13	-	Yes	-
B9EGL1	DLG4 protein	126.13	-	Yes	-
Q9NXL9	DNA helicase MCM9	140.79	-	Yes	-
Q9UNA4	DNA polymerase iota	207.32	-	Yes	-
B8ZZZ7	DNA polymerase-transactivated protein 6_ isoform CRA_b1	214.38	Yes	-	-
Q9NP87	DNA-directed DNA/RNA polymerase mu	174.55	-	-	Yes
H7BY40	DNA-directed RNA polymerase III subunit RPC9	432.67	Yes	-	-
O43812	Double homeobox protein 1	167.94	-	-	Yes
Q8NEP3	Dynein assembly factor 1_ axonemal	206.16	-	Yes	-
H0YGZ2	Dynein heavy chain 10_ axonemal (Fragment)	145.17	-	-	Yes
Q8IXS2	Dynein regulatory complex subunit 2	201.73	Yes	-	-
Q7Z6J0	E3 ubiquitin-protein ligase SH3RF1	88.58	-	Yes	-
Q9BY07	Electrogenic sodium bicarbonate cotransporter 4	338.85	Yes	-	-
Q9Y282	Endoplasmic reticulum-Golgi intermediate compartment protein 3	265.81	-	-	Yes
O43921	Ephrin-A2	149.52	-	Yes	-
Q9H6T0	Epithelial splicing regulatory protein 2	87	-	Yes	-
Q96RT1	Erbin	202.56	-	Yes	-
A6PVJ2	ERGIC and golgi 3 (Fragment)	253.8	-	-	Yes
Q7L2H7	Eukaryotic translation initiation factor 3 subunit M	348.07	Yes	-	-
Q9HAV4	Exportin-5	147.23	-	-	Yes
Q9BSJ8	Extended synaptotagmin-1	240.87	-	-	Yes
A0A087X216	Family with sequence similarity 13_ member C1_ isoform CRA_g	100.1	-	Yes	-
Q5TF85	Family with sequence similarity 46_ member A_ isoform CRA_a	302.5	Yes	-	-

Q96AE4	Far upstream element-binding protein 1	133.85	-	Yes	-
Q969Z0	FAST kinase domain-containing protein 4	85.18	-	Yes	-
P49327	Fatty acid synthase	144.04	Yes	-	-
O75369	Filamin-B	186.21	-	-	Yes
P85037	Forkhead box protein K1	159.49	Yes	-	-
H3BQN4	Fructose-bisphosphate aldolase	144.02	-	-	Yes
P04075	Fructose-bisphosphate aldolase A	144.02	-	-	Yes
P05062	Fructose-bisphosphate aldolase B	127.89	Yes	-	-
H7C358	Gamma-tubulin complex component (Fragment)	166.23	Yes	-	-
Q9Y2G5	GDP-fucose protein O-fucosyltransferase 2	99.71	-	Yes	-
H3BU37	General transcription factor 3C polypeptide 1 (Fragment)	100.02	-	Yes	-
Q14687	Genetic suppressor element 1	96.86	-	-	Yes
Q3V6T2	Girdin	317.47	-	-	Yes
Q9BQ67	Glutamate-rich WD repeat-containing protein 1	167.93	Yes	-	-
P47897	Glutamine--tRNA ligase	203.03	-	-	Yes
B5MC36	Glutathione hydrolase 1 proenzyme	204.35	Yes	-	-
E7ETN1	Glutathione hydrolase 1 proenzyme (Fragment)	385.88	-	-	Yes
Q14390	Glutathione hydrolase light chain 2	204.35	Yes	-	-
P04406	Glyceraldehyde-3-phosphate dehydrogenase	179.9	-	Yes	-
Q14789	Golgin subfamily B member 1	192.5	Yes	-	-
P30968	Gonadotropin-releasing hormone receptor	104.53	-	Yes	-
P16260	Graves disease carrier protein	1144.07	Yes	-	-
Q02108	Guanylate cyclase soluble subunit alpha-1	92.38	Yes	-	-
P00738	Haptoglobin	764.85	Yes	Yes	Yes
G3V1N2	HCG1745306_ isoform CRA_a	396.31	-	Yes	Yes
C9J4A3	HCG2018530_ isoform CRA_c	119.84	-	Yes	-
H3BMS9	HCG2029558_ isoform CRA_b	157.14	-	-	Yes
K7ELA5	HEAT repeat-containing protein 6 (Fragment)	260.01	-	-	Yes
Q14568	Heat shock protein HSP 90-alpha A2	159.44	Yes	Yes	-
Q15477	Helicase SKI2W	113.47	-	Yes	-
P69905	Hemoglobin subunit alpha	929.89	-	Yes	Yes
P68871	Hemoglobin subunit beta	3974.06	Yes	Yes	Yes
P02042	Hemoglobin subunit delta	896.02	Yes	Yes	Yes
P02100	Hemoglobin subunit epsilon	896.02	Yes	Yes	Yes
P69891	Hemoglobin subunit gamma-1	896.02	Yes	Yes	Yes
P69892	Hemoglobin subunit gamma-2	896.02	Yes	Yes	Yes
E7ES21	Hephaestin	774.47	Yes	-	-
Q5SSJ5	Heterochromatin protein 1-binding protein 3	204.97	-	-	Yes
P15515	Histatin-1	507.22	-	Yes	-
P15516	Histatin-3	1834.82	-	Yes	-
A5PLL3	Histone acetyltransferase	171.8	-	-	Yes
Q92794	Histone acetyltransferase KAT6A	171.8	-	-	Yes
P0C5Y9	Histone H2A-Bbd type 1	150.03	Yes	-	-
P0C5Z0	Histone H2A-Bbd type 2/3	150.03	Yes	-	-
Q9UPP1	Histone lysine demethylase PHF8	104.65	-	Yes	-

Q03164	Histone-lysine N-methyltransferase 2A	191.36	Yes	-	-
Q9NR48	Histone-lysine N-methyltransferase ASH1L	195.26	-	Yes	-
Q92800	Histone-lysine N-methyltransferase EZH1	279.12	-	-	Yes
Q9UBX0	Homeobox expressed in ES cells 1	215.9	Yes	-	-
Q4G0P3	Hydrocephalus-inducing protein homolog	245	Yes	-	-
P01876	Immunoglobulin heavy constant alpha 1	881.42	Yes	Yes	Yes
P01877	Immunoglobulin heavy constant alpha 2	603.69	Yes	Yes	Yes
P01857	Immunoglobulin heavy constant gamma 1	570.39	Yes	Yes	Yes
P01859	Immunoglobulin heavy constant gamma 2	272.04	Yes	Yes	Yes
P01860	Immunoglobulin heavy constant gamma 3	258.23	-	Yes	Yes
P01861	Immunoglobulin heavy constant gamma 4	340.18	-	Yes	Yes
P01742	Immunoglobulin heavy variable 1-69	283.74	Yes	-	-
S4R460	Immunoglobulin heavy variable 3/OR16-9 (non-functional)	291.46	-	-	Yes
P01834	Immunoglobulin kappa constant	724.3	-	Yes	Yes
P04433	Immunoglobulin kappa variable 3-11	190.6	Yes	-	-
A0A0A0MRZ8	Immunoglobulin kappa variable 3D-11	190.6	Yes	-	-
A9QM74	Importin subunit alpha-8	73.32	-	Yes	-
Q14974	Importin subunit beta-1	127.47	-	Yes	-
Q9Y471	Inactive cytidine monophosphate-N-acetylneuraminic acid hydroxylase	139.96	-	-	Yes
Q14571	Inositol 1_4_5-trisphosphate receptor type 2	398.43	Yes	-	-
Q9H0H0	Integrator complex subunit 2	133.4	-	Yes	-
P53708	Integrin alpha-8	90.44	Yes	-	-
Q9NZM3	Intersectin-2	194.47	Yes	Yes	-
A0AVF1	Intraflagellar transport protein 56	139.21	-	-	Yes
Q9H079	KATNB1-like protein 1	182.41	Yes	-	-
B9EIJ4	KCNK3 protein	279.07	Yes	-	-
O76011	Keratin_ type I cuticular Ha4	151.08	Yes	-	-
P13646	Keratin_ type I cytoskeletal 13	286.55	Yes	Yes	-
P13647	Keratin_ type II cytoskeletal 5	122.47	Yes	-	-
P02538	Keratin_ type II cytoskeletal 6A	193.12	Yes	-	
P04259	Keratin_ type II cytoskeletal 6B	180.62	Yes	-	-
P48668	Keratin_ type II cytoskeletal 6C	193.12	Yes	-	-
Q14894	Ketimine reductase mu-crystallin	284.45	Yes	-	-
Q8IVT5	Kinase suppressor of Ras 1	225.56	Yes	-	-
O60333	Kinesin-like protein KIF1B	116.56	Yes	-	-
Q96Q89	Kinesin-like protein KIF20B	177.25	-	-	Yes
Q7Z4S6	Kinesin-like protein KIF21A	118.58	-	Yes	-
P02788	Lactotransferrin	351.05	Yes	Yes	Yes
F8W6J0	LETM1 domain-containing protein 1	208.78	Yes	-	-
Q13136	Liprin-alpha-1	174.07	Yes	-	-
P12318	Low affinity immunoglobulin gamma Fc region receptor II-a	182	-	Yes	-
P47992	Lymphotactin	147.34	Yes	-	-
P29375	Lysine-specific demethylase 5A	445.37	Yes	-	Yes

Q9UGL1	Lysine-specific demethylase 5B	194.05	-	-	Yes
A0A087X0R0	Lysine-specific demethylase 6A	130.38	Yes	Yes	-
F8VV32	Lysozyme	726.35	Yes	Yes	Yes
P61626	Lysozyme C	849.44	Yes	Yes	Yes
Q9BUT9	MAPK regulated corepressor interacting protein 2	184.55	-	-	Yes
P08493	Matrix Gla protein	348.64	Yes	Yes	-
P50539	Max-interacting protein 1	155.37	-	-	Yes
A0JLT2	Mediator of RNA polymerase II transcription subunit 19	228.2	Yes	-	-
O00255	Menin	133.58	-	-	Yes
E9PD25	Metalloendopeptidase	299.17	Yes	-	
Q10713	Mitochondrial-processing peptidase subunit alpha	168.86	-	-	Yes
H0Y8A3	Mitogen-activated protein kinase kinase kinase 6 (Fragment)	260.76	-	Yes	-
Q9Y4K4	Mitogen-activated protein kinase kinase kinase kinase 5	129.58	-	Yes	-
F6WRY4	Msx2-interacting protein (Fragment)	140.68	Yes	-	-
Q9Y5R4	MTRF1L release factor glutamine methyltransferase	295.71	-	-	Yes
Q5SSG8	Mucin-21	147.14	-	Yes	-
Q8TAX7	Mucin-7	351.26	-	Yes	-
U3KQ59	Mucosal addressin cell adhesion molecule 1	139.92	-	Yes	-
H0YGQ3	Multiple PDZ domain protein (Fragment)	214.43	Yes	-	-
P05164	Myeloperoxidase	152.67	-	Yes	Yes
K7EMK2	NACHT_ LRR and PYD domains-containing protein 2 (Fragment)	381.63	-	Yes	-
Q86UW6	NEDD4-binding protein 2	104.2	-	Yes	-
P18615	Negative elongation factor E	136.42	-	-	Yes
Q8N0W4	Neuroligin-4_ X-linked	176.09	Yes	-	-
Q99574	Neuroserpin	346.63	-	Yes	-
P59665	Neutrophil defensin 1	2297.19	Yes	Yes	Yes
P59666	Neutrophil defensin 3	2297.19	Yes	Yes	Yes
Q9Y314	Nitric oxide synthase-interacting protein	152.98	-	Yes	-
Q5JPE7	Nodal modulator 2	285.7	Yes	-	-
H7C498	Nuclear factor erythroid 2-related factor 2 (Fragment)	457.16	Yes	-	-
F6X8W2	Nuclear factor of-activated T-cells 5	461.81	Yes	-	-
F5H6Y5	Nuclear mitotic apparatus protein 1 (Fragment)	309.98	-	-	Yes
O75376	Nuclear receptor corepressor 1	325.32	Yes	-	Yes
Q86WB0	Nuclear-interacting partner of ALK	238.23	-	Yes	-
P04746	Pancreatic alpha-amylase	556.45	Yes	Yes	Yes
P42356	Phosphatidylinositol 4-kinase alpha	246.69	-	-	Yes
Q92508	Piezo-type mechanosensitive ion channel component 1	199.71	Yes	-	-
H0YEQ7	Polycystic kidney disease protein 1-like 2 (Fragment)	263.56	Yes	-	-
H7C4H9	Polyhomeotic-like protein 3 (Fragment)	216.4	-	-	Yes
O14649	Potassium channel subfamily K member 3	279.07	Yes	-	-

Q6S8J3	POTE ankyrin domain family member E	1080.32	Yes	Yes	Yes
A5A3E0	POTE ankyrin domain family member F	1080.32	Yes	Yes	Yes
P0CG38	POTE ankyrin domain family member I	832.79	Yes	Yes	Yes
P0CG39	POTE ankyrin domain family member J	641.07	Yes	Yes	Yes
P40424	Pre-B-cell leukemia transcription factor 1	492.95	Yes	-	Yes
P40426	Pre-B-cell leukemia transcription factor 3	178.8	-	-	Yes
G5E9F7	Pregnancy specific beta-1-glycoprotein 1_ isoform CRA_a	413.84	Yes	-	Yes
M0R235	Pregnancy specific beta-1-glycoprotein 1_ isoform CRA_e	231.47	Yes	-	Yes
P11464	Pregnancy-specific beta-1-glycoprotein 1	413.84	Yes	-	Yes
M0QXP2	Pregnancy-specific beta-1-glycoprotein 3	314.6	-	-	Yes
Q6ZPD9	Probable C-mannosyltransferase DPY19L3	211.89	Yes	-	-
Q86UW9	Probable E3 ubiquitin-protein ligase DTX2	192.11	-	-	Yes
A0A087X0P9	Probable RNA polymerase II nuclear localization protein SLC7A6OS (Fragment)	108.61	-	Yes	-
Q2NL68	Proline and serine-rich protein 3	144.2	Yes	-	-
A0A0A0MT31	Proline-rich protein 4	1751.55	-	-	Yes
A6XMV9	Protease serine 2 preproprotein	183.84	-	Yes	Yes
Q92530	Proteasome inhibitor PI31 subunit	108.37	-	Yes	-
O75629	Protein CREG1	216.21	Yes	-	-
Q8NE31	Protein FAM13C	100.1	-	Yes	-
Q96MY7	Protein FAM161B	132.13	Yes	-	-
Q5U5X8	Protein FAM222A	128.24	-	-	Yes
U3KQR7	Protein FAM47E (Fragment)	300.47	-	Yes	-
Q8N9W8	Protein FAM71D	193.57	-	-	Yes
Q5TBA9	Protein furry homolog	217.17	Yes	-	-
Q86WI3	Protein NLRC5	210.07	-	-	Yes
Q13438	Protein OS-9	244.32	Yes	-	-
H0YNY1	Protein PAT1 homolog 2 (Fragment)	169.83	-	-	Yes
P05109	Protein S100-A8	2575.06	Yes	Yes	Yes
P06702	Protein S100-A9	13277.33	Yes	Yes	Yes
Q9BVV6	Protein TALPID3	269.52	Yes	-	-
H7C3T6	Protein tweety homolog (Fragment)	349.27	-	-	Yes
Q9C0H2	Protein tweety homolog 3	349.27	-	-	Yes
E5RJ77	Protein-tyrosine kinase 2-beta (Fragment)	194.84	Yes	-	-
Q9Y5F8	Protocadherin gamma-B7	150.18	-	-	Yes
A0A087X1P2	Protocadherin gamma-C3 (Fragment)	134.19	Yes	-	-
Q08174	Protocadherin-1	145.24	-	Yes	-
Q9BZA8	Protocadherin-11 Y-linked	160.08	-	Yes	-
Q3KPI9	PTPRD protein	207.2	Yes	-	-
Q8NHS7	PTPRS protein	208.79	-	Yes	-
P35247	Pulmonary surfactant-associated protein D	281.81	-	-	Yes
A0A3B3IRI8	Putative aldo-keto reductase family 1 member C8	110.73	-	Yes	-

Q9BYX7	Putative beta-actin-like protein 3	247.53	Yes	Yes	Yes
Q9H0H9	Putative cytochrome P450 family member 4F30	173.17	-	-	Yes
A6ND91	Putative L-aspartate dehydrogenase	316.77	-	Yes	-
P0C7V0	Putative uncharacterized protein encoded by LINC00271	146.73	-	Yes	-
B4DNK4	Pyruvate kinase	707.24	-	-	Yes
P14618	Pyruvate kinase PKM	707.24	-	-	Yes
Q2PPJ7	Ral GTPase-activating protein subunit alpha-2	262.57	Yes	-	-
P52306	Rap1 GTPase-GDP dissociation stimulator 1	122.84	-	Yes	-
Q9UN86	Ras GTPase-activating protein-binding protein 2	220.29	-	Yes	-
P61018	Ras-related protein Rab-4B	118.48	-	Yes	-
P23468	Receptor-type tyrosine-protein phosphatase delta	211.49	Yes	-	-
Q12913	Receptor-type tyrosine-protein phosphatase eta	150.62	-	-	Yes
Q13332	Receptor-type tyrosine-protein phosphatase S	209.98	-	Yes	-
Q16849	Receptor-type tyrosine-protein phosphatase-like N	169.26	-	-	Yes
P49796	Regulator of G-protein signaling 3	432.69	-	Yes	-
E5RJ23	Regulator of G-protein-signaling 22 (Fragment)	342.62	-	Yes	-
Q8N122	Regulatory-associated protein of mTOR	200.08	-	-	Yes
Q2NKQ5	Reticulon	129.27	Yes	-	-
Q16799	Reticulon-1	129.27	Yes	-	-
Q8N264	Rho GTPase-activating protein 24	186.44	-	-	Yes
Q13017	Rho GTPase-activating protein 5	223.2	Yes	-	-
Q15633	RISC-loading complex subunit TARBP2	191.75	Yes	-	-
Q6XE24	RNA-binding motif_ single-stranded-interacting protein 3	110.84	-	Yes	-
P02810	Salivary acidic proline-rich phosphoprotein 1/2	1751.55	-	-	Yes
P16615	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	383.35	-	-	Yes
Q7Z7L1	Schlafen family member 11	517.44	Yes	-	-
Q68D06	Schlafen family member 13	502.88	Yes	-	-
P15056	Serine/threonine-protein kinase B-raf	234.64	-	-	Yes
Q96BR1	Serine/threonine-protein kinase Sgk3	191.88	Yes	-	-
Q13315	Serine-protein kinase ATM	288.04	-	-	Yes
P02787	Serotransferrin	810.08	-	Yes	Yes
P02768	Serum albumin	6532.33	Yes	Yes	Yes
A0A087WV26	Sjoegren syndrome nuclear autoantigen 1	145.47	-	Yes	-
Q8TAD8	Smad nuclear-interacting protein 1	506.67	Yes	-	-
A0A087X1R1	Smoothelin	179.4	Yes	-	-
Q8IVB4	Sodium/hydrogen exchanger 9	96.61	-	Yes	-
Q99624	Sodium-coupled neutral amino acid transporter 3	140.45	-	Yes	-
Q695T7	Sodium-dependent neutral amino acid transporter B(0)AT1	53.55	-	Yes	-
Q8WWT9	Solute carrier family 13 member 3	119.84	-	Yes	-
Q96GZ6	Solute carrier family 41 member 3	165.59	-	-	Yes
Q96JI7	Spatacsin	191.01	-	-	Yes
Q9NUQ6	SPATS2-like protein	215.59	Yes	-	-

Q13813	Spectrin alpha chain_ non-erythrocytic 1	335.1	Yes	Yes	-
H0YAC0	Sperm flagellar protein 2 (Fragment)	259.03	Yes	-	-
Q86XZ4	Spermatogenesis-associated serine-rich protein 2	102.85	Yes	-	-
K7EJ32	Sphingosine kinase 1 (Fragment)	304.66	Yes	-	-
Q96BD8	Spindle and kinetochore-associated protein 1	127.04	-	Yes	-
Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1	238.09	Yes	-	Yes
Q14534	Squalene monooxygenase	257.23	-	Yes	Yes
A0A087WU33	SRC kinase-signaling inhibitor 1 (Fragment)	86.11	-	Yes	-
Q7KZF4	Staphylococcal nuclease domain-containing protein 1	155.95	-	-	Yes
P02808	Statherin	9488.73	Yes	Yes	Yes
Q15772	Striated muscle preferentially expressed protein kinase	261.36	Yes	Yes	Yes
P02814	Submaxillary gland androgen-regulated protein 3B	2361.12	-	Yes	Yes
Q6UWP8	Suprabasin	191.43	Yes	-	Yes
Q9BPZ7	Target of rapamycin complex 2 subunit MAPKAP1	335.67	-	-	Yes
P13686	Tartrate-resistant acid phosphatase type 5	112.09	-	Yes	-
Q9Y2I9	TBC1 domain family member 30	442.3	Yes	-	-
Q96DN5	TBC1 domain family member 31	189.49	-	Yes	-
Q99973	Telomerase protein component 1	218.26	-	-	Yes
Q96IP4	Terminal nucleotidyltransferase 5A	302.5	Yes	-	-
Q9BZW7	Testis-specific gene 10 protein	98.69	-	Yes	-
B4E171	Tetraspanin	168.56	Yes	-	-
P07996	Thrombospondin-1	218.43	-	Yes	-
Q13009	T-lymphoma invasion and metastasis-inducing protein 1	81.33	-	Yes	-
O43897	Tolloid-like protein 1	299.17	Yes	-	-
O75674	TOM1-like protein 1	156.14	Yes	-	-
Q9P1P5	Trace amine-associated receptor 2	151.56	Yes	-	-
Q9UPV9	Trafficking kinesin-binding protein 1	69.33	Yes	-	-
P48553	Trafficking protein particle complex subunit 10	149.55	Yes	-	-
Q99990	Transcription cofactor vestigial-like protein 1	272.46	-	Yes	-
Q7Z6M4	Transcription termination factor 4_ mitochondrial	160.92	Yes	-	-
Q2TAA8	Translin-associated factor X-interacting protein 1	94.68	Yes	-	-
Q96BF3	Transmembrane and immunoglobulin domain-containing protein 2	104.42	-	-	Yes
O14668	Transmembrane gamma-carboxyglutamic acid protein 1	168.56	Yes	-	-
P02766	Transthyretin	144.13	Yes	-	-
P07478	Trypsin-2	183.84	-	Yes	Yes
Q14679	Tubulin polyglutamylase TTLL4	156.01	Yes	-	-
E9PIN5	Tumor protein p53-inducible protein 11 (Fragment)	319.64	Yes	-	-
P35236	Tyrosine-protein phosphatase non-receptor type 7	280.14	Yes	-	-
Q14694	Ubiquitin carboxyl-terminal hydrolase 10	294.17	-	-	Yes
Q9H8M7	Ubiquitin carboxyl-terminal hydrolase MINDY-3	126.95	-	-	Yes

O14562	Ubiquitin domain-containing protein UBFD1	327.32	Yes	-	-
O43934	UNC93-like protein MFSD11	93.75	-	Yes	-
A0A140TA62	Uncharacterized protein	151.08	Yes	-	-
A0A1W2PNV4	Uncharacterized protein	83.51	-	Yes	-
A0A087WZY1	Uncharacterized protein	1352.39	-	-	Yes
A0A2R8Y7X9	Uncharacterized protein	896.02	Yes	Yes	Yes
Q6P1W5	Uncharacterized protein C1orf94	177.82	-	Yes	-
P0DPF5	Uncharacterized protein C2orf27A	117.85	-	Yes	-
P0DPF6	Uncharacterized protein C2orf27B	117.85	-	Yes	-
B2RTY4	Unconventional myosin-Ixa	197.47	Yes	-	-
P46939	Utrophin	168.5	-	Yes	-
P54727	UV excision repair protein RAD23 homolog B	194.06	Yes	-	-
Q5THJ4	Vacuolar protein sorting-associated protein 13D	400.76	-	-	Yes
Q68DQ2	Very large A-kinase anchor protein	146.95	Yes	-	-
P08670	Vimentin	151.23	-	Yes	-
Q2NL98	Vimentin-type intermediate filament-associated coiled-coil protein	197.16	-	-	Yes
Q6VVX0	Vitamin D 25-hydroxylase	75.77	-	Yes	-
A0A1B0GVB2	Voltage-dependent L-type calcium channel subunit beta-4	287.24	-	Yes	-
Q502W6	von Willebrand factor A domain-containing protein 3B	55.8	-	Yes	-
P21281	V-type proton ATPase subunit B_ brain isoform	566.35	-	-	Yes
Q9BV38	WD repeat-containing protein 18	183.47	-	-	Yes
A2RRC6	ZFHX2 protein	386.51	Yes	Yes	Yes
Q86VM9	Zinc finger CCCH domain-containing protein 18	382.66	Yes	-	Yes
Q9C0A1	Zinc finger homeobox protein 2	394.88	Yes	Yes	Yes
Q9HCL3	Zinc finger protein 14 homolog	20.96	-	-	Yes
Q9NYT6	Zinc finger protein 226	331.2	-	-	Yes
A2RRD8	Zinc finger protein 320	186.02	Yes	-	-
Q8TAQ5	Zinc finger protein 420	388.22	-	-	Yes
M0R1P0	Zinc finger protein 530 (Fragment)	430.67	-	-	Yes
Q7Z3V5	Zinc finger protein 571	222.6	-	-	Yes
Q96MU6	Zinc finger protein 778	269.41	Yes	-	-
Q05481	Zinc finger protein 91	315.43	-	Yes	-
H7BZ52	Zinc finger SWIM domain-containing protein 8	64.44	-	Yes	-
P25311	Zinc-alpha-2-glycoprotein	400.61	Yes	Yes	Yes
Q96DA0	Zymogen granule protein 16 homolog B	153.51	Yes	-	-

*Identification is based on protein ID from UniProt protein database, reviewed only (http://wwwwww.uniprot.org/).

4 CONSIDERAÇÕES FINAIS

O presente estudo demonstrou que ser um PVP altera a composição proteica da PAE, e que além disso existe diferença no perfil proteico da PAE de PVP com desgaste dentário erosivo leve comparado aqueles com desgaste dentário erosivo moderado. O achado mais relevante do presente estudo foi a identificação da proteína Squalene monooxygenase, sendo a primeira vez que esta proteína foi encontrada na PAE. Ela apareceu aumentada no grupo GWL comparado ao grupo GWH (26 vezes), indicando que essa proteína deva ter um papel protetor contra o desgaste dentário erosivo.

O principal benefício obtido a partir dos resultados do presente estudo é, portanto, a identificação de proteínas da película capazes de conferir resistência ao esmalte à dissolução causada por ácidos não bacterianos. Assim, estas proteínas poderão ser, no futuro, incorporadas a produtos odontológicos, como dentifrícios, soluções para bochecho ou ainda géis para aplicação tópica, visando o fortalecimento da película adquirida, aumentando assim o potencial protetor desta película contra ácidos não bacterianos.

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ANEXO A – Parecer da Comissão de Pesquisa (COMPESQ-UFRGS)

UFRGS

Universidade Federal do Rio Grande do Sul — Faculdade de Odontologia

PARECER CONSUBSTÂNCIADO DA COMISSÃO DE PESQUISA

Parecer aprovado em reunião do dia 08 de julho de 2016

ATA nº 07/2016.

A Comissão de Pesquisa da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul após análise aprovou o projeto abaixo citado com o seguinte parecer:

31310 - Diferenças na composição da película adquirida e saliva em provadores de vinho profissionais com e sem erosão dentária: estudo proteô

Prezado Pesquisador Jonas Rodrigues

O objetivo deste trabalho será comparar o perfil protéico em películas adquiridas do esmalte (PAE) formadas in vivo e saliva, em provadores de vinho profissionais com lesões de erosão dentária, provadores de vinho sem lesões de erosão dentária e pacientes controle (não provadores de vinho e que não tenham erosão dentária). Trata-se de um estudo composto por 3 braços paralelos, no qual 30 voluntários serão divididos em 3 grupos, a saber: a) provadores de vinho com erosão dentária (BEWE ≥ 3); b) provadores de vinho sem erosão dentária (BEWE = 0); c) pacientes controle (não provadores de vinho e BEWE = 0). Uma hora antes da coleta de saliva, os voluntários (n=10 por grupo) escovarão os dentes com pasta de dentes não abrasiva e então não ingerirão nenhum alimento ou bebida. Quinze minutos antes da coleta, farão um bochecho com água deionizada. Depois de 120 min e após a formação PAE, os dentes serão isolados com rolos de algodão a película será removida com um papel de filtro umedecido em ácido cítrico a 3%. Será feito um "pool' com os papeis de filtro obtidos dos 10 voluntários, para cada grupo. Após extração das proteínas, as mesmas serão submetidas à cromatografia líquida de fase reversa interligada a um espectrômetro de massas (nLC-ESI-MS/MS). Quantificação proteômica livre de marcadores será feita utilizando o software Protein Lynx Global Service (PLGS).

O projeto possui mérito científico e encontra-se bem delineado. O projeto deve ser cadastrado na plataforma Brasil.

Prof. Dr. Fabrício Mezzomo Collares

Coordenador da Comissão de Pesquisa ODONTOLOGIA UFRGS

Porto Alegre RS Rua Ramiro Barcelos, 2492 CEP 90035-003 Fones 0xx51- 33 08 51 67

ANEXO B – Parecer do Comitê de Ética em Pesquisa (CEP-UFRGS)

UFRGS - PRÓ-REITORIA DE PESQUISA DA UNIVERSIDADE FEDERAL DO RIO GRANDE DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Diferenças na composição da película adquirida e saliva em provadores de vinho profissionais com e sem erosão dentária: estudo proteômico.

Pesquisador: Jonas de Almeida Rodrigues

Área Temática:

Versão: 3

CAAE: 58440116.7.0000.5347

Instituição Proponente: Universidade Federal do Rio Grande do Sul

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.024.551

Apresentação do Projeto:

Trata-se de um projeto de pesquisa da Faculdade de Odontologia relacionado ao processo de erosão dentária e características da saliva que podem servir como proteção frente a esse processo.

Objetivo da Pesquisa:

O objetivo deste trabalho será comparar o perfil protéico em películas adquiridas do esmalte (PAE) formadas in vivo e saliva, em provadores de vinho profissionais com lesões de erosão dentária, provadores de vinho sem lesões de erosão dentária e pacientes controle (não provadores de vinho e que não tenham erosão dentária).

Avaliação dos Riscos e Benefícios:

Riscos e benefícios foram devidamente considerados.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um estudo composto por 3 braços paralelos, no qual 30 voluntários serão divididos em 3 grupos, a saber: a) provadores de vinho com erosão dentária; b) provadores de vinho sem erosão dentária; c)pacientes controle (não provadores de vinho e BEWE. Uma hora antes da coleta de saliva, os voluntários (n=10 por grupo) escovarão os dentes com pasta de dentes não abrasiva e então não ingerirão nenhum

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UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3308-3738 **Fax:** (51)3308-4085 **E-mail:** etica@propesq.ufrgs.br

alimento ou bebida. Quinze minutos antes da coleta, farão um bochecho com água deionizada.
Depois de 120 min e após a formação PAE, os dentes serão isolados com rolos de algodão a película será removida com um papel de filtro umedecido em ácido cítrico a 3%. Será feito um "pool' com os papeis de filtro obtidos dos 10 voluntários, para cada grupo. Após extração das proteínas, as mesmas serão submetidas à cromatografia líquida de fase reversa interligada a um espectrômetro de massas (nLC-ESIMS/MS).
Quantificação proteômica livre de marcadores será feita utilizando o software ProteinLynx Global Service (PLGS).

vai colaborar com a realização dos ensaios foram apresentados pelos pesquisadores e encontram-se em condições de aprovação.
- O tamanho amostral de 10 participantes por grupo foi escolhido com base na variável de resposta a ser utilizada (comparação do perfil proteico de películas adquiridas). Os pesquisadores argumentam que este n tem se mostrado adequado para comparação do perfil proteico de películas adquiridas formadas sob diferentes situações em estudos encontrados na literatura [Delecrode et al., 2015a; Delecrode et al., 2015b; Lee et al., 2013].
- Os autores atenderam à solicitação do CEP de incluir um TCLE para os participantes do grupo controle.
- Os pesquisadores atenderam à solicitação de incluir uma carta de anuência do enólogo responsável pela Vinícula Aurora, demonstrando concordância da instituição com a realização do estudo.

Conclusões ou Pendências e Lista de Inadequações:
O projeto está em condições de aprovação.

Considerações Finais a critério do CEP:
Aprovado.

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UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3308-3738 **Fax:** (51)3308-4085 **E-mail:** etica@propesq.ufrgs.br

Continuação do Parecer: 2.024.551

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_762603.pdf	20/03/2017 17:26:40		Aceito
Projeto Detalhado / Brochura Investigador	PROJETO.pdf	20/03/2017 17:25:51	Daiana Back Gouvea	Aceito
Outros	20170317093921668.pdf	20/03/2017 17:24:14	Daiana Back Gouvea	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tclev2.pdf	23/11/2016 17:39:35	Nicole Marchioro dos Santos	Aceito
Outros	COMPESQ.pdf	05/08/2016 18:00:10	Daiana Back Gouvea	Aceito
Folha de Rosto	Jonas_de_Almeida_Rodrigues.pdf	05/08/2016 17:58:59	Daiana Back Gouvea	Aceito
Declaração de Instituição e Infraestrutura	Laboratorio.pdf	04/08/2016 19:49:37	Daiana Back Gouvea	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 20 de Abril de 2017

Assinado por:
MARIA DA GRAÇA CORSO DA MOTTA
(Coordenador)

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Bairro: Farroupilha **CEP:** 90.040-060
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Telefone: (51)3308-3738 **Fax:** (51)3308-4085 **E-mail:** etica@propesq.ufrgs.br

APÊNDICE A – Termo de Consentimento Livre e Esclarecido

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA

PROJETO DE PESQUISA:
"DIFERENÇAS NA COMPOSIÇÃO DA PELÍCULA ADQUIRIDA E SALIVA EM PROVADORES DE VINHO PROFISSIONAIS COM E SEM EROSÃO DENTÁRIA: ESTUDO PROTEÔMICO"

Termo de Consentimento Livre e Esclarecido

Elaborado com base na Resolução 466 do Conselho Nacional de Saúde, publicada no DOU Nº112, 2012.

Caro participante,

Estamos realizando uma pesquisa para avaliar as características da saliva que podem proteger os dentes da erosão dentária. A erosão dentária é um desgaste dos dentes que ocorre devido a ingestão de bebidas ácidas e/ou condições sistêmicas como refluxo gastroesofágico e bulimia, não sendo causada por bactérias.

Nosso grupo de pesquisa convida provadores de vinho profissionais a participar deste estudo, uma vez que o vinho é uma bebida ácida com potencial de provocar erosão dentária.

Esta autorização deverá ser dada com o conhecimento do senhor (a) sobre todos os procedimentos a serem executados e seus objetivos, no uso de sua liberdade e sem sofrer qualquer tipo de pressão. **Sua participação é voluntária.**

Durante essa pesquisa serão realizados exames clínicos sobre a saúde oral, bem como a aplicação de um questionário sobre saúde geral, hábitos alimentares e de higiene oral. Além disso será realizado coleta de saliva e de película adquirida (saliva aderida ao dente). Nessa etapa todas as medidas de biossegurança, como uso de equipamento de proteção individual e uso de instrumental odontológico estéril, serão adotadas pelos pesquisadores.

Os benefícios relacionados à participação nesse estudo envolvem o diagnóstico de eventuais problemas de saúde oral, bem como orientações sobre erosão dentária e medidas preventivas.

Os riscos ao participar dessa pesquisa são baixos, envolvendo apenas a possibilidade de sentir leve desconforto durante o exame clínico e as coletas de saliva e película adquirida.

Toda e qualquer dúvida no decorrer do estudo poderá ser esclarecida pelos envolvidos nesta pesquisa através do telefone **(51)33085027.** Os pesquisadores Prof. Dr. Jonas de Almeida Rodrigues e as cirurgiãs-dentistas Daiana Back Gouvêa e Nicole Marchioro dos Santos estarão sempre à disposição para esclarecimentos. Possíveis problemas ou dúvidas podem ser reportados diretamente ao Comitê de Ética Central da UFRGS pelo telefone (51) 33083738.

Eu, _____ declaro que fui informado dos objetivos e procedimentos que serão realizados nessa pesquisa, bem como sei dos meus direitos e dos deveres dos pesquisadores. Declaro, ainda, que recebi uma cópia deste termo. Estou ciente de que posso a qualquer momento retirar a presente autorização por minha livre vontade e sem qualquer prejuízo, bastando para isso comunicar por escrito o responsável pelo estudo.

Este documento foi elaborado em **duas vias** e é assinado pelo participante e pelo pesquisador.

DATA: __/__/__ ASSINATURA:_____

ENDEREÇO:_____

TELEFONE: (___)_____

DATA: ___/___/___ ASSINATURA: _____

Jonas de Almeida Rodrigues – Pesquisador Responsável