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Hurdles and potentials in value-added use of peanut and grape by-products as sources of phenolic compounds

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Thesis presented to obtain the degree of Doctor in Science.
Area: Food Science and Technology

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Bachelor of Food Science

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This work is decidated to family, friends, and mentors,

whose support was everything I needed to make my dreams come true.

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CONTENTS

RESUMO

Desafios e potencialidades na agregação de valor a subprodutos da agroindústria do amendoim e da uva como fonte de compostos fenólicos

Estudos recentes têm demonstrado que subprodutos da indústria processadora de amendoim e uva podem ser mais ricos em compostos bioativos em comparação às suas matérias-primas. No entanto, alguns desafios tecnológicos precisam ser enfrentados antes da sua aplicação como fonte de compostos nutracêuticos ou na prevenção da oxidação lipídica em sistemas alimentares. Este estudo discute os recentes avanços na aplicação de subprodutos da indústria processadora de amendoim e uva como fontes de compostos fenólicos. Especial ênfase foi dada a sua caracterização por cromatografia líquida acoplada à espectrometria de massas, aos potenciais benefícios à saúde e à segurança microbiológica. As principais conclusões estão apresentadas nos capítulos 2, 3 e 4. O primeiro capítulo trata de compostos bioativos de subprodutos da indústria de suco de uva e da produção vinícola. A fração da qual foram extraídos os compostos fenólicos ligados à parede celular foi predominante. Em geral, esta fração também foi a mais eficaz na inibição da oxidação do LDL - colesterol *in vitro* quando comparada à fração que continha os fenólicos livres e os esterificados. Os compostos fenólicos de todas as frações inibiram o dano oxidativo ao DNA induzido por radicais peroxila. O terceiro capítulo fala sobre os efeitos da irradiação gama sobre a carga microbiana, a composição fenólica e as propriedades antioxidantes da película de amendoim. A irradiação gama (5,0 kGy) diminuiu a contagem microbiana do produto. Os compostos fenólicos totais, o teor de proantocianidinas e a capacidade dos extratos em neutralizar radicais como o ABTS, DPPH e espécies reativas de oxigênio como o peróxido de hidrogênio e radicais hidroxila, assim como o poder redutor da amostra, aumentaram devido à irradiação gama em ambas as frações (contendo fenólicos livres e ligados à parede celular). A bioatividade dos compostos fenólicos livres contra a oxidação do LDL-colesterol *in vitro* e contra os danos oxidativos ao DNA aumentou com a irradiação gama. Os compostos fenólicos foram positivamente ou tentativamente identificados, distribuindo-se entre: fenólicos livres > esterificados > ligados. Houve aumento na concentração de dímeros de procianidina A em todas as frações, enquanto a concentração de dímeros de procianidina B diminuiu. Essas alterações podem ser explicadas pela conversão molecular, despolimerização e formação de ligações cruzadas. No quarto e último capítulo, enzimas selecionadas foram aplicadas à matéria-prima inicial (experimento I) ou nos resíduos contendo apenas compostos fenólicos insolúveis (experimento II). Pronase e Viscozyme aumentaram a extração de compostos fenólicos insolúveis (ligados à parede celular). Viscozyme liberou maiores quantidades de ácido gálico, catequina e dímero de prodelfinidina A em comparação ao tratamento com Pronase. Além disso, os ácidos *p*-cumárico e ácido caféico, bem como o dímero de procianidina B, foram extraídos com Viscozyme, mas não com Pronase. A solubilidade desempenha um papel importante na biodisponibilidade de compostos fenólicos. Desta forma, o terceiro estudo oferece uma alternativa para a exploração de compostos fenólicos de subprodutos da indústria vinícola como ingredientes alimentares com propriedades funcionais ou suplementos alimentares.

Palavras-chave: Segurança microbiológica; Irradiação gama; Ácidos fenólicos; Flavonóides; Proantocianidinas; Bioatividade; Extração enzimática

ABSTRACT

Challenges and potentials in value-added use of peanut and grape by-products as sources of phenolic compounds

Recent studies have demonstrated that peanut and grape processing by-products may be richer sources of bioactive compounds as compared to their original raw material and feedstock; however, before their application as a source of nutraceuticals or in the prevention of lipid oxidation in food systems, certain technological challenges have to be addressed. This study discusses recent advances in the application of plant food processing by-products as sources of phenolic compounds with special emphasis on the profiling and screening of phenolics using high-performance liquid chromatography-mass spectrometry, their potential health benefits, and microbiologial safety. The major findings are summarized in chapters 2, 3, and 4. The first chapter deals with phenolics from grape by-products. In general, insoluble-bound phenolics were more effective in inhibiting copper-induced human LDL-cholesterol oxidation *in vitro* than free and esterified phenolics. Phenolic extracts from all fractions inhibited peroxyl radical-induced DNA strand breakage. The third chapter brings about the effects of gamma-irradiation on the microbial growth, phenolic composition, and antioxidant properties of peanut skin. Gamma-irradiation at 5.0 kGy decreased the microbiological count of the product. Total phenolic and proanthocyanidin contents, ABTS radical cation, DPPH radical, hydrogen peroxide, and hydroxyl radical scavenging capacities as well as the reducing power of the sample were increased upon gamma-irradiation in both the free and insoluble-bound phenolic fractions. The bioactivity of the free phenolics against *in vitro* human LDL-cholesterol oxidation and copper induced DNA strand breakage was improved upon gamma-irradiation. Phenolic compounds were positively or tentatively identified and their distribution was in the decreasing order of free > esterified > insoluble-bound forms. Procyanidin dimer A was increased in all phenolic fractions, whereas procyanidin dimer B decreased. Gamma-irradiation induced changes may be explained by molecular conversion, depolymerization, and cross-linking. In the fourth chapter, the ability of selected enzymes in improving the extraction of insoluble-bound phenolics from the starting material (experiment I) or the residues containing insoluble-bound phenolics (experiment II) were evaluated. Pronase and Viscozyme improved the extraction of insoluble-bound phenolics. Viscozyme released higher amounts of gallic acid, catechin, and prodelphinidin dimer A compared to Pronase treatment. Furthermore, p-coumaric and caffeic acids, as well as procyanidin dimer B, were extracted with Viscozyme but not with Pronase treatment. Solubility plays an important role in the bioavailability of phenolic compounds, hence this study may assist in better exploitation of phenolics from winemaking by-products as functional food ingredients or supplements.

Keywords: Microbiological safety; Gamma-irradiation; Phenolic acids; Flavonoids; Proanthocyanidins; Bioactivity; Enzyme extraction

2 LOW MOLECULAR WEIGHT PHENOLICS OF GRAPE JUICE AND WINEMAKING BY-PRODUCTS: ANTIOXIDANT ACTIVITIES AND INHIBITION OF OXIDATION OF HUMAN LOW-DENSITY LIPOPROTEIN CHOLESTEROL AND DNA STRAND BREAKAGE

Adapted with permission from DE CAMARGO, A. C.; REGITANO-D'ARCE, M. A. B.; BIASOTO, A. C. T.; SHAHIDI, F. Low molecular weight phenolics of grape juice and winemaking byproducts: antioxidant activities and inhibition of oxidation of human low-density lipoprotein cholesterol and DNA strand breakage. **Journal of Agricultural and Food Chemistry,** Easton, v. 62, p. 12159–12171, 2014.

Abstract

Bioactive compounds belonging to phenolic acids, flavonoids, and proanthocyanidins of grape juice and winemaking by-products were identified and quantified by HPLC-DAD-ESI-MS^n. The concentration of phenolic compounds in different grape cultivars was in the order Tempranillo > Cora > Syrah > Isabel. The insoluble-bound fraction was most prominent, contributing 63 and 79% to the total for Isabel and Tempranillo, respectively. Juice-processing by-products had a higher content of free than esterified phenolics, but the opposite was noted for winemaking by-products. Insoluble-bound phenolics were up to 15 and 10 times more effective as antioxidants than those of free and esterified fractions, respectively, as evaluated by the DPPH, ABTS, and H_2O_2 scavenging activities and reducing power determinations. In general, insoluble-bound phenolics (100 ppm) were more effective in inhibiting copper-induced human LDL-cholesterol oxidation than free and esterified phenolics, exhibiting equal or higher efficacy than catechin. Phenolic extracts from all fractions inhibited peroxyl radical-induced DNA strand breakage. These findings shed further light for future studies and industrial application of grape by-products, which may focus not only on the soluble phenolics but also on the insoluble-bound fraction.

Keywords: Processing byproduct; Phenolic acids; Flavonoids; Proanthocyanidin; LDL-cholesterol; DNA

2.1 Introduction

Fruits, vegetables, nuts, and cereals have been in the spotlight due to extensive literature support demonstrating their health benefits. Other than providing carbohydrate, protein, lipid, minerals, and vitamins, a balanced diet also provides a wide range of bioactive compounds. Polyphenols are recognized for rendering several health benefits such as potential anticancer, antimicrobial, and antioxidative effects (CHANDRASEKARA; SHAHIDI, 2011a; CHENG et al., 2012). However, commercial products have not always been considered as a viable source of bioactives. In this regard, several studies have demonstrated the high content of polyphenols in different commercial food products such as chocolate, tomato sauce, grape juice, and wine.

The processing of foods and beverages generates a large amount of by-products. The juice industry is one of the major suppliers of by-products as a consequence of fruit seasonality as well as different climates and soil adaptation of the feedstock. Many tons of grapes are produced each year, and a large part of their final consumption is through grape juice and wine. Consequently, a considerable amount of by-products (e.g., grape skin and seeds) is generated, creating an environmental burden.

Several by-products have been studied as a source of polyphenols, and the information available demonstrates their potential for being exploited (LEE et al., 2006; SHAHIDI; ALASALVAR; LIYANA-PATHIRANA, 2007; DE CAMARGO et al., 2012a). Grape by-products have also been shown to serve as a good source of dietary fiber (LLOBERA; CANELLAS, 2007) and for extending the shelf life of high-lipid foods due to their antioxidant activity (SHIRAHIGUE et al., 2010). The potential health benefits of such products/by-products stems from their high polyphenol content, which has been evidenced by both *in vitro* and *in vivo* studies (JARA-PALACIOS et al., 2013; EVANS; WILSON; GUTHRIE, 2014).

Polyphenols are water-soluble and found in the free, esterified, and insoluble-bound forms, the latter fraction being linked to the cell walls of source materials. Numerous studies have evaluated the phenolic profile, antioxidant properties, and potential biological activities of grapes and their by-products (SANDHU; GU, 2010; CHENG et al., 2012; GONZÁLEZ-CENTENO et al., 2012); however, there are clear gaps in the existing knowledge about the contribution of the free, esterified, and insoluble-bound fractions to the total phenolic/polyphenolic contents that affect the antioxidant properties of grape by-products. Moreover, grape by-products are generated by both juice- and winemaking operations.

The grape variety, its maturation stage, and crop production area are some of the crucial factors influencing the different phenolic profiles found in grape juice, wine, and their by-products. Grape juice and winemaking have different processes, among them the fermentation process, which leads to alcohol generation, as the main one. For red wines, this stage is conducted in two steps called primary and secondary fermentations. The primary fermentation may take 1-2 weeks in the presence of grape skins, which are responsible for color development of red wines. Furthermore, the primary fermentation is carried out by yeast cells, and their ability to absorb phenolic compounds may result in a higher or lower phenolic content in the wine and in its byproduct. The secondary step is conducted by bacterial fermentation, in the absence of grape skins, which are removed in the process. Thus, grape skins have no influence in the secondary fermentation.

Reactive oxygen species (ROS) are detrimental to health as they take part in oxidative processes both *in vivo* and *in vitro* (WETTASINGHE; SHAHIDI, 2000). ROS are involved in cell damage, cancer development, inflammation, and heart disease. Furthermore, ROS play an important role in aging and the development of Parkinson's disease. ROS are constantly generated via mitochondrial metabolism, which can worsen with unhealthy habits such as smoking. High-lipid foods are also affected by ROS generation, which can be influenced by heating, UV light, and gamma-irradiation, leading to sensory changes, decreasing the shelf life of products and becoming an economic burden (DE CAMARGO et al., 2012b). Hydrogen peroxide generates hydroxyl radicals in the presence of ferrous ion or via UV light dissociation. Although generation of the highly reactive short-lived hydroxyl radicals is of much concern as it induces DNA damage and lipid and protein oxidation, the presence of H_2O_2 itself causes enzyme inactivation and cell damage. The scavenging activity of phenolics against H_2O_2 is attributed to electron donation, but neutralization of H_2O_2 to H_2O is also contemplated (WETTASINGHE; SHAHIDI, 2000). Moreover, ferric ion catalyzes the oxidation of proteins and lipids, thus being detrimental to food and biological systems.

The present study focused on the unraveling of the phenolic profile of grape by-products, and the contribution of each fraction to potential bioactivity as well as differences between by-products generated by the juice- and winemaking industries. Total phenolic content (TPC), proanthocyanidin content (PC), scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and H_2O_2, and reducing power (RP) were evaluated. The detailed phenolic profile was investigated using HPLC-DAD-ESI-MS^n, and the potential health benefits of the extracts were evaluated against copper-induced LDL-cholesterol oxidation and peroxyl radical induced DNA strand breakage.

2.2 Materials and Methods

Grape juice (BRS Cora and Isabel) by-products were donated by Embrapa Semiárido (Petrolina, Pernambuco state, Brazil). Syrah and Tempranillo (winemaking by-products) were donated by Ouro Verde Farm, Grupo Miolo (Casa Nova, Bahia state, Brazil) and Santa Maria winery (Lagoa Grande, Pernambuco state, Brazil), respectively. Original grapes used in the grape juice- and winemaking processes had 20 and 22 ºBrix, respectively. Brazilian grape juice is mainly produced using the Isabel cultivar, due to its large production. BRS Cora, hereafter named Cora, is a Brazilian variety developed by Embrapa through crossing between ‘Muscat Belly A’ and ‘H.65.9.14’ varieties. Cora and Isabel grapes were grown in Petrolina, Pernambuco state, Brazil. Syrah grape was grown in Casa Nova, Bahia state, Brazil, and Tempranillo was grown in Lagoa Grande, Pernambuco state, Brazil. The microbiological evaluation (*Salmonella* spp., yeasts and molds, coliform bacteria, and coagulase-positive *Staphylococcus*) demonstrated that grape by-products were safe to use as a functional ingredient and/or supplement (data not shown).

Folin-Ciocalteu's reagent, vanillin, DPPH, ABTS, mono- and dibasic potassium phosphates, hydrogen peroxide, potassium ferricyanide, ferric chloride, copper sulfate, human LDL-cholesterol, ethylenediaminetetraacetic acid trisodium salt (Na_3EDTA), tris acetate, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), agarose, bromophenol blue, xylene cyanol, glycerol, trolox, caffeic, gallic, protocatechuic, and *p*-coumaric acids, (+)-catechin, and (−)-epicatechin were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Sodium carbonate, sodium hydroxide, sodium chloride, potassium persulfate, trichloroacetic acid, diethyl ether, ethyl acetate, hexane, acetone, methanol, acetonitrile, formic acid, hydrochloric acid, sodium hydroxide, and pBR 322 from *Escherichia coli* were purchased from Fisher Scientific Ltd. (Ottawa, ON, Canada). SYBR safe gel stain was purchased from Probes (Invitrogen, Eugene, OR, USA).

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3 GAMMA-IRRADIATION INDUCED CHANGES IN MICROBIOLOGICAL STATUS, PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF PEANUT SKIN

Reprinted with permission from DE CAMARGO, A. C.; REGITANO-D'ARCE, M. A. B.; GALLO, C. R.; Shahidi, F. Gamma-irradiation induced changes in microbiological status, phenolic profile and antioxidant activity of peanut skin. **Journal of Functional Foods**, Amsterdam, v. 12, p. 129-143, 2015.

Abstract

The effects of gamma-irradiation on the microbial growth, phenolic composition, and antioxidant properties of peanut skin were evaluated. Gamma-irradiation at 5.0 kGy decreased the microbiological count of the product. Total phenolic and proanthocyanidin contents, ABTS radical cation, DPPH radical, H_2O_2, and hydroxyl radical scavenging capacities as well as the reducing power of the sample were increased upon gamma-irradiation in both the free and insoluble-bound phenolic fractions. However, a decrease in the esterified phenolics was noticed. The bioactivity of the free phenolics against *in vitro* human LDL-cholesterol oxidation and copper induced DNA strand breakage was improved upon gamma-irradiation. Phenolic acids, flavonoids, and proanthocyanidins were positively or tentatively identified by HPLC-DAD-ESI-MS^n and their distribution was in the decreasing order of free > esterified > insoluble-bound forms. Procyanidin dimer A was increased in all phenolic fractions, whereas procyanidin dimer B decreased. Gamma-irradiation induced changes may be explained by molecular conversion, depolymerization, and cross-linking.

Keywords: Microbiology; LDL-cholesterol oxidation; DNA strand breakage inhibition; HPLC-DAD-ESI-MS^n; Polyphenol; Proanthocyanidin

3.1 Introduction

The role of food phenolics and polyphenolics in the prevention of cardiovascular disease and certain types of cancer is well recognized. Polyphenols have also been reported as having positive *in vivo* effect in reducing obesity and visceral fat, as potential anti-inflammatory compounds (TERRA et al., 2007), and in the management of pre-diabetic and/or diabetic conditions (ROOPCHAND et al., 2013). The antioxidant properties of phenolic compounds have been extensively reported. Studies on vegetable oils, fruits, cereals, spices, teas, and nuts, among other foods and beverages, have highlighted the potential health benefits of polyphenols. By-products such as the skin of Brazil nut (JOHN; SHAHIDI, 2010), hazelnut (ALASALVAR et al., 2009), almond (WIJERATNE; ABOU-ZAID; SHAHIDI, 2006) and peanuts (SARNOSKI et al., 2012) also serve as a rich source of antioxidants.

Peanut skin has almost 20-fold higher total phenolics than whole peanuts and more than 100-fold free radical scavenging capacity (DE CAMARGO et al., 2012a, 2012c), which explains the interest of the peanut industry in exploring the potential applications of this low-cost feedstock. However, there is a concern about the microbiological status of peanut and its by-products due to possible presence of mycotoxinogenic fungi. Worldwide regulations for aflatoxins limit their level in food to less than 20 μg/kg (20 ppb). In addition, it is difficult and sometimes impossible to attain such low values due to environmental conditions in most places where peanuts are produced and stored, making this an additional economic burden (DORNER, 2008).

Gamma-irradiation is an ionizing radiation with high energy that removes one electron from water, creating highly reactive species including free radicals. The interaction of such species with the DNA of microorganisms brings about their death (KILCAST, 1995). Insects are known to be vectors of mycotoxin-producing fungi (NESCI; MONTEMARANI; ETCHEVERRY, 2011). Additionally, low doses of gamma-irradiation (0.2–0.8 kGy) are also efficient for killing and sterilizing insects (FARKAS, 2006). The effectiveness of gamma-irradiation in inhibiting mycotoxinogenic fungi has already been reported (DE CAMARGO et al., 2012c). Nevertheless, antioxidants or by-products intended for use as functional food ingredients need to satisfy microbiological standards for a broad spectrum of microorganisms, such as coagulase-positive *Staphylococcus, Escherichia coli,* and *Salmonella*. In addition, gamma-irradiation is detrimental to antioxidants such as tocopherol (DE CAMARGO et al., 2012b) and ascorbic acid. Thus, investigating the effects of gamma-irradiation on antioxidant compounds and their activity is necessary. Opposite to tocopherols, monophenols that are mainly found in the lipid fraction of peanuts, other phenolics and polyphenolics are concentrated in the water-soluble fraction, and phenolic acids generally exist in the free, esterified, and insoluble-bound forms, the latter being linked to the cell wall components. Soluble phenolic extracts are often defined as the crude phenolic extracts in the literature, accounting for both the free and esterified forms. Phenolics from the crude extract may be found in the glucosides as well as in the aglycone forms.

Although the effect of gamma-irradiation on the total phenolic content and antioxidant capacity of crude phenolics of peanut skin extract has already been studied (DE CAMARGO et al., 2012a), there is no information available about its effect on different fractions of phenolic extracts. Moreover, there is a lack of data on handling microbiological contamination and effects of gamma-irradiation on the individual phenolic compounds of peanut skin. Thus, the objective of the present study was to investigate the application of gamma-irradiation to decrease the microbiological count of peanut skin and its effect on the content of phenolic compounds and antioxidant properties in the free, esterified and insoluble-bound phenolic fractions.

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4 ENZYME-ASSISTED EXTRACTION OF PHENOLICS FROM WINEMAKING BY-PRODUCTS: ANTIOXIDANT POTENTIAL AND INHIBITION OF ALPHA-GLUCOSIDASE AND LIPASE ACTIVITIES

Reprinted with permission from DE CAMARGO, A. C.; REGITANO-D'ARCE, M. A. B.; BIASOTO, A. C. T.; SHAHIDI, F. Enzyme-assisted extraction of phenolics from winemaking by-products: antioxidant potential and inhibition of alpha-glucosidase and lipase activities, **Food Chemistry**, Easton, 2016a. In press.

Abstract

Phenolics in food and agricultural and processing by-products exist in the soluble and insoluble-bound forms. The ability of selected enzymes in improving the extraction of insoluble-bound phenolics from the starting material (experiment I) or the residues containing insoluble-bound phenolics (experiment II) were evaluated. Pronase and Viscozyme improved the extraction of insoluble-bound phenolics as evaluated by total phenolic content, antioxidant potential as determined by ABTS and DPPH assays, and hydroxyl radical scavenging capacity, reducing power as well as evaluation of inhibition of alpha-glucosidase and lipase activities. Viscozyme released higher amounts of gallic acid, catechin, and prodelphinidin dimer A compared to Pronase treatment. Furthermore, *p*-coumaric and caffeic acids, as well as procyanidin dimer B, were extracted with Viscozyme but not with Pronase treatment. Solubility plays an important role in the bioavailability of phenolic compounds, hence this study may assist in better exploitation of phenolics from winemaking by-products as functional food ingredients and/or supplements.

Keywords: HPLC-DAD-ESI-MS^n; Phenolic acids; Flavonoids; Proanthocyanidin; Diabetes; Obesity

4.1 Introduction

Grapes and their derived beverages are important sources of food phenolics (DA SILVA et al., 2015; TAO et al., 2016). However, winemaking generates a large amount of by-products (e.g. skins and seeds). These by-products serve as rich sources of phenolics belonging to several classes of compounds such as phenolic acids, flavonoids, including anthocyanins, as well as proanthocyanidins (CHENG et al., 2012; DE CAMARGO et al., 2014a).

Phenolic and/or polyphenolic compounds have attracted much attention due to their wide range of potential health benefits, as substantiated by both *in vitro* and *in vivo* studies (DE CAMARGO et al., 2014a; VICENTE; ISHIMOTO; TORRES, 2014). The role of food phenolics in preventing degenerative, vascular and heart disease and as anti-inflammatory and antimicrobial agents have also been reported (ALASALVAR; BOLLING, 2015; SHAHIDI; AMBIGAIPALAN, 2015). Additionally, phenolic compounds may play an important role in amiliorating certain types of cancer, including colorectal cancer (SHAHIDI;

AMBIGAIPALAN, 2015). The chemical structures of these molecules are as important as their detection and concentration, which may reflect in a different correlation between a particular molecule and its activity.

Phenolic compounds are present in the soluble (free and esterified) and insoluble-bound forms, the proportion of each one depends not only on the starting material but also on their cultivar and an eventual processing to which they are subjected. For example, the esterified phenolics from lentils were generally in higher amount, but some cultivars also showed higher content in the fraction containing insoluble-bound phenolics (ALSHIKH; DE CAMARGO; SHAHIDI, 2015), whereas berry seed meals had higher content of insoluble-bound phenolics (AYOUB; DE CAMARGO; SHAHIDI, 2016). Peanut skin submitted to gamma-irradiation had increased free and insoluble-bound phenolic contents upon processing (DE CAMARGO et al., 2015). As antioxidants, phenolic compounds may counteract oxidative reactions in food subjected to treatments such as gamma-irradiation and pasteurization as well as during long-term storage, which may affect its shelf-life and sensory characteristics (DE CAMARGO et al., 2012a; DA SILVA et al., 2014). Furthermore, biologically relevant molecules such as lipids, proteins, lipoproteins and DNA may also be protected from oxidatively reactive compounds.

In a previous study at this department (DE CAMARGO et al., 2014a), it has been demonstrated that, regardless of the process (juice or winemaking), insoluble-bound phenolics were major fractions in grape processing by-products. The same study also provided evidence about the dominant benefits of insoluble-bound phenolics of grape by-products in inhibiting copper-induced human LDL-cholesterol oxidation and peroxyl radical-induced DNA strand breakage. These results demonstrated the potential of the insoluble-bound phenolics from winemaking by-products as their major source of bioactive compounds.

Enzyme-assisted extraction has been regarded as an alternative method for improved extraction of food phenolics (MONTELLA et al., 2013; PAPILLO et al., 2014), especially the insoluble-bound phenolics, which are linked to carbohydrates and proteins of cell wall matrices. However, to the best of the authors' knowledge, there is no literature providing the effect of enzyme-assisted extraction on the ratio of soluble to insoluble-bound phenolics from winemaking by-products although this has been reported for germinating lentils by Yeo and Shahidi (2015). Thus, in the present study, winemaking by-products (cv. Tempranillo) were treated with Pronase and Viscozyme to improve the solubility of phenolics present in the sample. The effects were studied based on the change in the distribution pattern of soluble/insoluble-bound phenolics as well as their chemical profile, antioxidant properties (antiradical activity) and reducing power. The resultant products were also evaluated for their effect in deactivating alpha-glucosidase and lipase, which have a key role in the prevention and management of diabetes and obesity, respectively.

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5 GENERAL CONCLUSIONS

The initial microbiological status of peanut skin from two cultivars indicated that these by-products of the peanut industry should be subjected to a decontamination process. Gamma-irradiation diminished the microbiologial count of peanut skins; therefore, providing a safer product. Furthermore, this was the first study demonstrating how gamma-irradiation affected the phenolic profile of peanut skin, which included the fractions containing soluble (free and esterified) and insoluble-bound phenolics using HPLC-DAD-ESI-MS^n. It was possible to suggest that molecular conversion, depolymerization, and cross-linking were induced by the process. The fraction containing free phenolics were the major ones in two different cultivars of peanut skin. Gamma-irradiation increased the content of free phenolics, antioxidant activity, reducing power, as well as the inhibition capacity of *in vitro* human LDL-cholesterol oxidation and inhibition of DNA strand breakage of this fraction. Therefore offering a product with a greater potential to serve as a functional ingredient or food supplement. By-products of grape juice and winemaking industries were also evaluated for their microbiological status, which demonstrated it would not be necessary to use any decontamination method for such samples. The phenolic profile and bioactivities of grape by-products were also evaluated for their different fractions for the first time. Grape juice and winemaking by-products showed different phenolic distribution in the soluble fraction and it was possible to suggest a cluster for grape juice versus winemaking by-products. The first cluster (grape juice by-products) had higher free phenolics whereas the second one (winemaking by-products) showed higher esterified phenolic contents. Furthermore, all samples had the insoluble-bound fraction as the richest source of phenolics. The same trend was observed for their antioxidant activities, reducing power, inhibition of *in vitro* human LDL-cholesterol oxidation and inhibition of inhibition of DNA damage. Because of the major contribution of insoluble-bound phenolics from grape by-products two enzymes were tested in order to improve their extraction so that they could potentially become readily bioavailable. Pronase and Viscozime released insoluble-bound phenolics, but the latter was more efficient. Furthermore, different molecules such as procyanidin dimers B were extracted only with Viscozyme. The effect of enzyme-assisted extraction in the antiradical activity and inhibition of alpha-glucosidase and lipase demonstrated that this technology offers promise for further industrial exploitation. Therefore, the microbiological, chemical, and technological knowledge generated in this doctoral thesis makes a solid contribution for different steps to be considered for the full exploitation of these plant food by-products as sources of phenolic compounds for potential application as antioxidants, and potentially for prevention of cardiovascular diseases, cancer, diabetes, and obesity.