Research, Society and Development, v. 11, n. 14, e55111435888, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i14.35888

Characterization and bioactive compounds of organic Bordeaux grape seed oil and flours (*Vitis labrusca* L.)

Caracterização e compostos bioativos do óleo e farinhas orgânicas de semente de uva Bordeaux (*Vitis labrusca* L.)

Caracterización y compuestos bioactivos del aceite y harinas de semilla de uva orgánicas de Burdeos (*Vitis labrusca* L.)

Received: 09/27/2022 | Revised: 10/12/2022 | Accepted: 10/14/2022 | Published: 10/20/2022

Soraia Silva Pinheiro Caetano
ORCID: https://orcid.org/0000-0001-5766-739X
Federal University of Viçosa, Brazil
E-mail: soraia.pinheiro@outlook.com
Mariana de Moura e Dias
ORCID: https://orcid.org/0000-0003-0822-0721
Federal University of Viçosa, Brazil
E-mail: mmouradias@gmail.com
Augusto César Fonseca Sobreira
ORCID: https://orcid.org/0000-0003-4332-1659
University of São Paulo, Brazil
E-mail: augustosobreira@usp.br
Abigail Brandão Barroso
ORCID: https://orcid.org/0000-0001-8359-4948
Federal University of Viçosa, Brazil
E-mail: abigail.barroso@ufv.br
Helena Maria Pinheiro-Sant'Ana
ORCID: https://orcid.org/0000-0002-2224-5872
Federal University of Viçosa, Brazil
E-mail: helena.santana@ufv.br
Ceres Mattos Della Lucia
ORCID: https://orcid.org/0000-0002-6731-5694
Federal University of Viçosa, Brazil
E-mail: cmdellalucia@ufv.br
Hércia Stampini Duarte Martino
ORCID: https://orcid.org/0000-0002-8565-8439
Federal University of Viçosa, Brazil
E-mail: hercia@ufv.br
Pamella Cristine Anunciação
ORCID: https://orcid.org/0000-0002-6447-5588
Federal University of Viçosa, Brazil
E-mail: nutripamella@gmail.com
Maria do Carmo Gouveia Peluzio
ORCID: https://orcid.org/0000-0003-4665-7043
Federal University of Viçosa, Brazil
E-mail: mcpeluzio@ufv.br

Abstract
The *Bordeaux* grape variety is the most used in the production of red juices. The objective of the work was to evaluate carotenoids, flavonoids, vitamins C, E and B complex, total fatty acids, phytates, tannins, antioxidant capacity, total phenolic compounds and centesimal composition in whole (W) and defatted (D) *Bordeaux* grape seed flours and oil (O). Total fatty acids were evaluated by gas chromatography (flame ionization detector). Analysis of vitamins E and complex B were performed by High Performance Liquid Chromatography (HPLC) (fluorescence detection). Vitamin C, carotenoids and flavonoids were evaluated by HPLC (diode-array detection). The fatty acid composition showed the pattern: C18:2>C18:1>C16:0>C18:0. W and D showed higher concentrations of B complex vitamins than O. W had the highest concentration of lutein and total carotenoids, followed by O and D. O presented increased vitamin E and lower levels of flavonoids and antioxidant capacity. The different grape products affected the occurrence and concentration of bioactive compounds.
Keywords: Complex B vitamins; Vitamin E; Flavonoids; Fatty acids; HPLC.

Resumo

A variedade de uva *Bordeaux* é a mais utilizada na produção de sucos tintos. O objetivo desse trabalho foi avaliar carotenóides, flavonóides, vitaminas C, E e do complexo B, ácidos graxos totais, fitatos, taninos, capacidade antioxidante, compostos fenólicos totais e composição centesimal em uvas *Bordeaux* inteiras (W) e desengorduradas (D), e em farinhas e óleo (O). Os ácidos graxos totais foram avaliados por cromatografia gasosa (detector de ionização de chama). As análises das vitaminas E e do complexo B foram realizadas por Cromatografia Líquida de Alta Eficiência (HPLC) (detecção de fluorescência). Vitamina C, carotenóides e flavonóides foram avaliados por HPLC (detecção de matriz de diodos). A composição de ácidos graxos apresentou o padrão: C18:2>C18:1>C16:0>C18:0. W e D apresentaram maiores concentrações de vitaminas do complexo B do que O. W apresentou a maior concentração de luteína e carotenóides totais, seguido de O e D. O apresentou aumento de vitamina E e menores níveis de flavonóides e capacidade antioxidante. Os diferentes produtos de uva afetaram à ocorrência e concentração de compostos bioativos.

Palavras-chave: Vitaminas do complexo B; Vitamina E; Flavonoides; Ácidos graxos; HPLC.

Resumen

La variedad de uva bordelesa es la más utilizada en la elaboración de zumos tintos. El objetivo de este trabajo fue evaluar carotenoides, flavonoides, vitaminas C, E y complejo B, ácidos grasos totales, fitatos, taninos, capacidad antioxidante, compuestos fenólicos totales y composición proximal en uva bordelesa entera (W) y desgrasada (D), y en harinas y aceite (O). Los ácidos grasos totales se evaluaron por cromatografía de gases (detector de ionización de llama). Los análisis del complejo de vitamina E y B se realizaron mediante cromatografía líquida de alta resolución (HPLC) (detección de fluorescencia). La vitamina C, los carotenoides y los flavonoides se evaluaron por HPLC (detección de matriz de diodo). La composición de ácidos grasos mostró el patrón: C18:2>C18:1>C16:0>C18:0. W y D tenían concentraciones más altas de vitaminas B que O. W tenía la concentración más alta de luteína y carotenoides totales, seguido por O y D. O tenía mayor vitamina E y niveles más bajos de flavonoides y capacidad antioxidante. Los diferentes productos de uva afectaron la ocurrencia y concentración de compuestos bioactivos.

Palabras clave: Vitaminas B; Vitamina E; Flavonoides; Ácidos grasos; HPLC.

1. Introduction

Grapes of all species have attracted a lot of interest worldwide due to its functional and nutritional potential properties. The Bordeaux variety is the most used in the production of red juices (Nicolescu et al., 2022). Only in Brazil, grape cultivation has increased by 15.4% in the last five years and in the wine industry, about 35% of its production is waste (Maicas & Mateo, 2020).

The major compounds of grapes are flavonoids, such as anthocyanins, flavones, and flavonols, phenolics, and E and B complex vitamins (Alara et al., 2021). In this context, in turn 10% of these compounds are present in the grape pulp, 28–35% are present in the peel, and 60–70% are present only in the seeds (Martin et al., 2020).

Some studies related the antioxidant capacity and the total phenolic compounds of grape and its seed oil (Monteiro et al., 2021; Ghafoor et al., 2020), but few related the concentration of bioactive compounds and total dietary fiber in the whole and defatted Bordeaux seed grape flours and oil. Thus, it is necessary to perform studies that allow a more complete characterization of the different nutrients and bioactive compounds in flours and preparations using whole grape seed flours and oils.

In this context, the aim of the present study was to evaluate the composition, occurrence and concentration of total fatty acids, phytates, tannins and bioactive compounds (vitamin C, complex B vitamins, vitamin E, carotenoids, flavonoids, antioxidant capacity and total phenolic compounds) in whole and defatted *Bordeaux* grape seed flours and oil, in addition to assess the potential of their contribution to achieve the nutritional vitamin recommendations for adults.

2. Methodology

The present paper is an observational, quantitative, descriptive and laboratory study, where the proximate composition, as well as the occurrence and concentration of bioactive compounds in flours and oil of grape seeds of the Bordeaux variety were evaluated (Estrela, 2018).

Raw material

Bordeaux grapes were used as raw material to obtain fresh seeds, and whole and defatted flours, as well as grape seed oil. This variety was chosen due to the fact that it is the only one available on the market to purchase its flour (which is defatted) and its oil, both for making different preparations for human consumption.

Seeds, defatted flour and *Bordeaux* grape seed oil were purchased from the company Econatura, located in the city of Garibaldi - Rio Grande do Sul. The cultivation of these grapes was carried out by about 30 families of producers, who each own on average 2 hectares of cultivated area. The cultivation was carried out in accordance with the regulations for national and international organic production (for the European and American market). The harvest took place according to the stage of ripening of the grapes, which occurred between the months of January and February of 2019.

To obtain the whole grape seed flour of the *Bordeaux* variety, the dried seeds were manually separated from the skins and crushed in an analytical mill (Quimis model Q298A21). Immediately after pressing the grapes, the resulting bagasse was sieved to separate the skins and seeds. Then, the skins and seeds were deposited separately in dryers with forced circulation of hot air (average temperature of 65°C), until they reached sufficient moisture concentration to be stored until the moment of use (below 10%). Then, the seeds went through a pressing process, in which the cold extraction of the oil from the grape seeds occurred, without using chemical solvents. Afterwards, the defatted seeds were subjected to the grinding process in a stone mill, until they reached the appropriate texture, and then the defatted flour and oil were packaged. Each repetition of whole flour, defatted flour and oil was represented by different batches during the grape harvest. The oil and flours were covered with aluminum foil and stored in a freezer (-20 ± 1°C) until the conclusion of the analyzes, which were performed within 30 days.

Standards and reagents

The standards of B complex vitamins (thiamine hydrochloride, riboflavin and pyridoxine hydrochloride), carotenoids (lutein and zeaxanthin), flavonoids (3-desoxyanthocyanidins – 3-DXAs: luteolinidin chloride and apigeninidin chloride; flavones: lutein and apigenin and flavanones: naringenin and eriodictyol) and fatty acids (Frame from 4 to 30 C) were purchased from Sigma Aldrich (St. Louis, MO, USA). The standards of vitamin C and vitamin E (ascorbic acid, α, β, γ and δ-tocopherols and tocotrienols) were obtained from Calbiochem (EMD Biosciences, Inc., USA).

Analytical grade reagents (VETEC, São Paulo, Brazil) were used to extract vitamins, carotenoids, flavonoids and total fatty acids. Claradiastase was purchased from Sigma Aldrich (St. Louis, MO, USA). For analysis, HPLC grade reagents (methanol, acetonitrile, formic acid, ethyl acetate, acetone, hexane, isopropanol, glacial acetic acid) were obtained from Tedia (São Paulo, Brazil) and analytical grade (sodium acetate and heptanesulfonic acid) were obtained from Sigma Aldrich (St. Louis, MO, USA).

Analysis of centesimal composition and estimate of total energy value

The determination of humidity, lipids, ash, proteins, and total, soluble and insoluble dietary fiber were carried out in triplicate, according to methodology proposed by AOAC (2012). Carbohydrates were estimated by difference. For calculation of the energy value or total caloric value, the conversion factors of 9 kcal/g for lipids and 4 kcal/g for proteins and carbohydrates were used.

Analysis of total fatty acids

The fatty acids composition was determined by gas chromatography (GC) after methylation by Hartman and Lago methodology (1973). CG was performed using CG-17A Shimadzu/Class model®, with capillary column DB-5 (30 m x 0.25 µm id, 0.25 mm film thickness, J&W Scientific, USA) and a flame ionization detector. The programming of the analysis

presented an initial temperature of 100 °C, being isothermic for 5 minutes, and a posterior heating of 4 °C per minute up to 220 °C, maintaining this temperature for 30 minutes. The temperature of the vaporizer was 200 °C and the temperature of the detector was 240 °C. The carrier gas used was nitrogen at 43.2 cm/s. The split of the sample in the injector was 1/50 and 1 μL of the sample was injected. Fatty acid methyl esters (FAME) were identified by direct comparison of retention time with FAME standard mix (Supelco 37 Component FAME Mix; Sigma-Aldrich®, EUA). Percentage of individual FAME was made in relation to total area of the chromatogram.

Analysis of bioactive compounds

The bioactive compounds were determined in three repetitions. During extraction and analysis, the samples and extracts were protected from sunlight and artificial light using amber glassware, aluminum foil and blackout curtains, and protected from oxygen using hermetically sealed flasks and with nitrogen gas environment.

Extraction and analysis of vitamin C, vitamin E, carotenoids and flavonoids

Vitamin C, in the form of ascorbic acid (AA), was extracted and analyzed according to Campos et al. (2009). For vitamin E, the occurrence and concentration of its eight components (α, β, γ and δ-tocopherols and tocotrienols) were investigated, and their extraction and analysis were carried out simultaneously, according to Pinheiro-Sant'Ana et al. (2011).

The carotenoids (lutein and zeaxanthin) were extracted according to Rodriguez-Amaya et al. (1976) and analyzed according to Panfili et al. (2004). The flavonoids (3-DXAs, flavones and flavanones) were extracted and analyzed simultaneously, using the methodologies proposed by Dykes et al. (2009) and Yang et al. (2012), respectively.

All the compounds were evaluated by High Performance Liquid Chromatography (HPLC). Vitamin C, carotenoids and flavonoids were assessed with diode array detection (DAD) and vitamin E with fluorescence detection.

Extraction and analysis of thiamine, riboflavin e pyridoxine

Total thiamine (thiamine, thiamine monophosphate and thiamine pyrophosphate) and total riboflavin (riboflavin, flavin adenine dinucleotide and flavin mononucleotide) were extracted simultaneously, based on the methods proposed by Ndaw et al. (2000) and Arella et al. (1996). For thiamine, it was derivatized to thiochrome, following the referred methodologies.

The total pyridoxine (pyridoxol, pyridoxal and pyridoxamine) was extracted according to the method proposed by Ndaw et al. (2000), with modifications and analyzed by HPLC with fluorescence detection (Bergaentzlé et al., 1995).

Analysis of antioxidant capacity and total phenolic compounds

The antioxidant capacity was determined using the DPPH$^{\bullet}$ radical method (1,1-diphenyl-2-picrylhydrazyl) (Bloor, 2001) and the Iron Reduction Method (FRAP) (Benzie & Strain, 1996), by spectrophotometry. The total phenolic compounds in the samples were determined using the *Folin Ciocalteau* reagent (Singleton et al., 1999), also by spectrophotometry.

Analysis of phytates and condensed tannins

Phytates were determined by ion exchange chromatography, followed by spectrophotometry (Ellis & Morris, 1986; Latta & Eskin, 1980). The concentration of total condensed tannins was determined by the vanillin/HCl method (Burns et al., 1971), also followed by spectrophotometry.

Identification and quantification of bioactive compounds

All compounds analyzed by HPLC were identified by co-chromatography and comparing the retention time of authentic commercial standards with the components of interest in the samples. In addition, carotenoids, flavonoids and ascorbic acid were identified comparing the absorption spectra of the standards and peaks of interest in the samples, using DAD.

All compounds were quantified using analytical curves constructed from the injection, in duplicate, of six different concentrations of standard solutions. The total concentrations of thiamine, riboflavin, pyridoxine and vitamin C were expressed in mg/100g of flour (dry matter). The total concentrations of carotenoids (lutein + zeaxanthin) and vitamin E (α, β, γ and δ tocopherols and tocotrienols) and their isolated components were expressed in µg/100g (dry matter). The α-tocopherol equivalent was calculated using the equation: (α-tocopherol × 1.0) + (β-tocopherol × 0.5) + (γ-tocopherol × 0.1) + (δ-tocopherol × 0.03) + (α-tocotrienol × 0.3) + (β-tocotrienol × 0.05) (IOM, 2011).

The total concentration of flavonoids (3-DXAs: luteolinidin – LUT; apigeninidin – API; 7-methoxyapigeninidin - 7-MeO-API; and 5-methoxy-luteolinidin - 5-MeO-LUT); and flavones: luteolin and apigenin; and flavanones: eriodictyol and naringenin) and their isolated components was expressed in µg/g of sample (dry matter). The concentrations of total fatty acids were expressed as percentage. DPPH results were expressed in milligrams of trolox per gram of sample (mg trolox/g) and FRAP values expressed in µmol of Fe+2 equivalents per gram of sample. The results of total phenolics were expressed in milligrams of gallic acid equivalents per gram of sample (mg EAG/g).

For phytates, an analytical curve constructed from reading the absorbance of phytic acid solutions with different concentrations was used, in which the phytate concentration was expressed in microgram of phytic acid per gram of sample. For the condensed tannins, the quantification was performed by the construction of an analytical curve from methanolic solutions with different concentrations of catechin, where the results were expressed in milligram of catechin equivalent per gram of sample (mg Ecatequine/g).

Estimate of the contribution potential of flours and oil to achieve the nutritional recommendations

Grape seed flours, being a type of flour, and grape seed oil, were classified as belonging to the cereal and oil and fat groups, where each portion was equivalent to 150 and 73 kcal, respectively. Considering the total energy value of each product, the portion, in grams, of each product was calculated (IOM, 2011).

According to Phillippi (2008), foods can be considered as “source”, “good source” or “excellent source” of nutrients. If the food portions in the present study supplied 5 to 10% of the Dietary Reference Intake (DRI), they were considered as a “source” of the nutrient; if 10 to 20% of the DRI was supplied, they were considered “good source” of the nutrient; and if more than 20% of the DRI was supplied, they were considered an “excellent source” of the nutrient.

Experimental design and statistical analysis

To analyze the nutrients and bioactive compounds in the flours and oil, a completely randomized design was used, and the data were assessed for normality using the Shapiro Wilk test. Then, the data were submitted to One Way ANOVA and, for multiple comparison of means, they were submitted to the Duncan test. Statistical analyzes were performed using the IBM SPSS software, version 19.0, with a significance level (α) of 5%.

3. Results and Discussion

Centesimal composition and total energy value

Both grape seed flours (whole and defatted) presented similar concentrations of carbohydrates, proteins and total fibers, in which 95.51% and 95.82% represented insoluble fibers, and 4.49% and 4.18% represented soluble fibers, respectively (Table 1). The grape seed oil showed the highest concentration of lipids, followed by whole grape seed flour and defatted grape seed flour. Thus, the grape seed oil had increased total energy value, when compared to the whole and defatted flours (about nine and twenty times bigger, respectively).

These results are in accordance with data on grape pomace and grape seed oil produced in Brazil (Sousa et al., 2014; Deng et al., 2011). However, other studies reported different concentrations of proteins, lipids and total fibers, since they were five and three times lower for proteins and lipids, respectively, as well as 15 times higher for total fibers (Garavaglia et al., 2016; Karnopp et al., 2015; Ozvural & Vural, 2011).

Table 1: Proximate and total fatty acids composition (%) in wet matter of seed oil, whole and defatted *Bordeaux* grape seed flours [A, B]

Compound	Oil	Whole flour	Defatted flour
Carbohydrates (%)	nd	8.78 ± 0.59 [a]	8.07 ± 0,97 [a]
Proteins (%)	nd	7.42 ± 0.58 [a]	7.12 ± 1.01 [a]
Lipids (%)	**99.83 ± 0.11 [a]**	**3.92 ± 0.30 [b]**	**0.07 ± 0,01 [c]**
Ash (%)	0.97 ± 0.09 [b]	4.74 ± 0.34 [a]	2.07 ± 0.23 [b]
Dietary Fibers (%)			
Soluble fiber	nd	3.14 ± 0.34 [a]	2.78 ± 0.23 [a]
Insoluble fiber	nd	66.74 ± 0.12 [a]	63.65 ± 0.19 [a]
Total fibers	nd	69.88 ± 2.37 [a]	66.43 ± 1.17 [a]
Total energy value (kcal)			
In 100g of sample	**898.47 [a]**	**100.08 [b]**	**61.39 [b]**
Grams per portion	**8.12**	**149.88**	**244.34**
Fatty Acids (%)			
C 16:0	7.03 ± 0.23 [a]	6.23 ± 0.32 [a]	Nd
C 18:0	3.84 ± 0.19 [a]	2.98 ± 0.35 [a]	Nd
C 18:1	16.68 ± 0.78 [a]	14.88 ± 0.55 [a]	Nd
C 18:2	75.15 ± 1.56 [a]	72.45 ± 1.48 [a]	Nd
C 18:3	nd	Nd	Nd

[A] The results were expressed as the average of 3 repetitions ± standard deviation; [B] Means followed by the same letter on the lines are not statistically different at 5% probability by the Duncan test. Where: % = percentage; g = grams; nd = not detected. One portion of the cereal group was considered to provide 150 calories. One portion of the oil group was considered to provide 73 calories. Source: Authors.

It is worth mentioning that these authors analyzed different grape varieties, which may have contributed to these differences. Besides, several environmental factors, such as regional, climatic and soil differences can affect the composition of the grape seeds, which could cause differences in the concentrations of their compounds (Schultz, 2016). Data on the centesimal composition and total energy value of whole and defatted *Bordeaux* grape seed flours, as well as *Bordeaux* grape seed oils are still incipient, since most studies report the composition of bagasse and grape extract, and not of its seeds, which demonstrates the pioneer of the present study.

Total fatty acids

The major fatty acid was linoleic acid (C18:2), contributing between 73.17% and 75.05% in the different flours and oil (Table 1). Total saturated fatty acids were below 11.00%, while mono- and poly-unsaturated fatty acids totalled up to 89.42% and 90.46% for oil and whole flour, respectively. The fatty acid profile, as well as the concentration of each compound, did not differ between the samples. None of the flours and oil presented concentrations of alpha-linolenic acid (C18:3). The fatty acid composition obtained the following pattern: linoleic acid (C18:2) > oleic acid (C18:1) > palmitic acid (C16:0) > stearic acid (C18:0).

Similar results were shown in the literature, where many authors presented a range from 66.80% to 73.60% of oleic acid in samples of different grapes varieties (Lutterodt et al., 2011; Crews et al., 2006). Other authors showed different results (Bertrand & Ozcan, 2011). It is worth noting that different extraction methods may influence the fatty acid concentrations and profiles of grape seed oils and its flours.

Monounsaturated fatty acids have been identified as hypolipidemic, which act decreasing the low-density lipoproteins (LDL). In addition, they present a hypocholesterolemic and protective effect against the development of atherosclerotic coronary disease (Lira et al., 2005; Spector, 1999; Monteiro, 1998).

In turn, polyunsaturated fatty acids have great biological importance, as they participate in the structure and integrity of membranes and in vital biological processes, such as the synthesis of eicosanoids (prostaglandins and leukotrienes). Thus, these fatty acids contribute to reducing the risk of rheumatic and coronary heart diseases, diabetes and cancer. The presence of these compounds is particularly important in the diet of pregnant and breastfeeding women, as arachidonic acids and DHA are major components of the gray matter of the brain and retina and, therefore, should be available to the fetus (through placenta) and to the newborn (through breast milk) (Lira et al., 2005; Bragagnolo, 1997).

Excessive intake of saturated fatty acids should be avoided in human food, as they increase the blood cholesterol level by reducing the activity of the LDL-cholesterol receptor and reducing the LDL free space in the blood stream (Peñuela-Sierra et al., 2015; Grundy & Denke, 1990). Thus, products that have lower concentrations of these compounds should have their consumption stimulated in human food.

Vitamin C, carotenoids and B complex vitamins

The concentrations of carotenoids and B complex vitamins of Bordeaux grape seed flours and oil were different (Table 2 and Figure 1). For thiamine, riboflavin and pyridoxine, both grape seed flours showed higher concentrations (two, five and three times higher) than the grape seed oil, respectively. The concentrations of ascorbic acid (AA) did not differ between the samples.

For carotenoids (lutein, zeaxanthin and total carotenoids), the major carotenoid was lutein in all samples. The whole seed flour had the highest concentration of lutein and total carotenoids, followed by grape seed oil and defatted grape seed flour ($p<0.05$). However, the defatted seed flour presented the lower concentration of zeaxanthin. These data can be explained by the fact that carotenoids are fat-soluble compounds and AA and complex B vitamins are water-soluble compounds (Egbuonu, 2015).

Shinagawa (2015), when comparing different grape seed oils, found concentrations of total carotenoids three times lower than the present work. These differences can be attributed to the different grape varieties of the studies, as well as different oil extraction methods. In addition, several environmental factors, such as regional, climatic and soil differences can affect the composition of the grape seeds, which could cause differences in the concentrations of their compounds (Schultz, 2016).

Table 2: Occurrence and concentrations of ascorbic acid (mg/100g), carotenoids (µg/100g) and B complex vitamins (µg/100g), in wet matter of seed oil, whole and defatted *Bordeaux* grape seed flours [A, B]

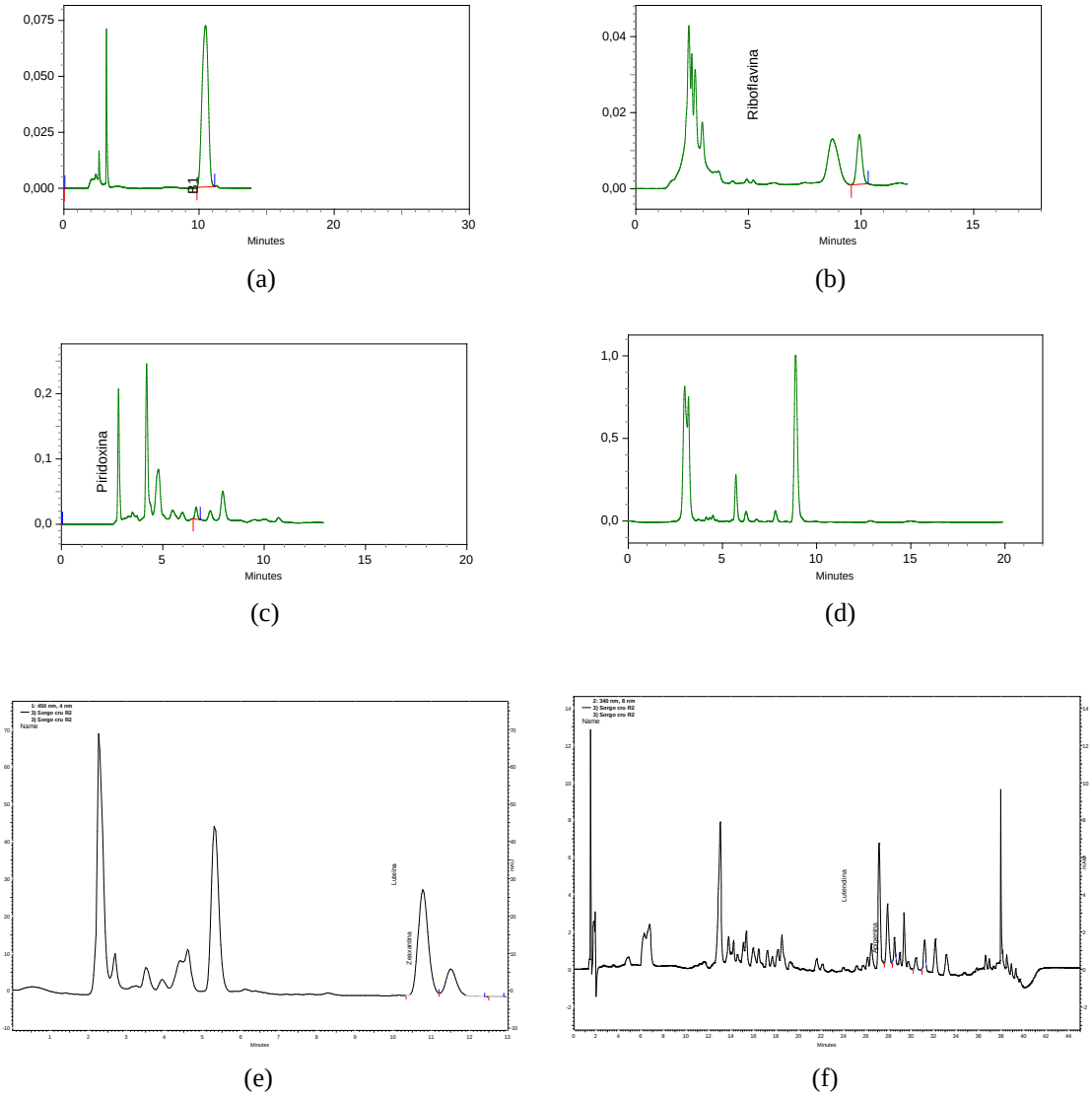
Compound	Oil	Whole flour	Defatted flour
Vitamin C			
Ascorbic acid	10.08 ± 2.34 [a]	13.56 ± 2.78 [a]	12.64 ± 2.71 [a]
Carotenoids			
Lutein	101.12 ± 19.23 [b]	226.70 ± 35.82 [a]	10.71 ± 2.92 [c]
Zeaxanthin	41.13 ± 6.38 [a]	58.15 ± 9.17 [a]	8.22 ± 3.41 [b]
Sum of carotenoids	142.25 ± 17.54 [b]	284.85 ± 34.55 [a]	18.93 ± 7.21 [c]
Complex B vitamins			
Thiamine	17.13 ± 0.97 [b]	41.74 ± 6.36 [a]	32.31 ± 9.79 [a]
Riboflavin	159.73 ± 32.44 [b]	802.51 ± 67.13 [a]	785.02 ± 12.23 [a]
Pyridoxine	51.35 ± 13.41 [b]	296.20 ± 25.38 [a]	189.58 ± 22.12 [ab]

[A] The results were expressed as the average of 3 repetitions ± standard deviation; [B] Means followed by the same letter on the lines are not statistically different at 5% probability by the Duncan test. Source: Authors.

The main benefits to human health associated with lutein, in addition to evidence in reducing the risk of developing age-related macular degeneration, are the beneficial effects on protection against atherosclerosis, cataracts, cancer and other diseases (Stringheta et al., 2009; Krinsky & Johnson, 2005; Alves-Rodrigues & Shao, 2004; Brow et al., 1999). Thiamine is essential to help cells convert carbohydrate into energy and is necessary for the proper functioning of nerve cells and the brain (Rubert et al., 2017; Maihara et al., 2006). Riboflavin, on the other hand, is an essential nutrient that maintains the functions of metabolism under normal conditions, acting as a cofactor in enzymatic reactions, especially in the electron transport system (Rubert et al., 2017; Delgadillo & Ayala, 2009).

Vitamin C is of great importance due to its antioxidant activity: it protects the body against chronic diseases such as cataracts and cardiovascular diseases, prevents anemia, stimulates the immune system, in addition to be present in the development and regeneration of muscles, skin, bones and teeth (Vieira, 2020; Munyaka et al., 2010; Strutzel et al., 2007). Data on the occurrence and concentration of AA, lutein, zeaxanthin and B complex vitamins in grape oil, whole and defatted grape seed flours are still incipient, which demonstrate the pioneer of the present study.

Figure 1: Typical chromatograms obtained by High Performance Liquid Chromatography in the analysis of flours and oil. (a) Thiamine; (b) Riboflavin; (c) Pyridoxine; (d) Vitamin E; (e) Carotenoids; (f) Flavones. Chromatographic conditions described in Methodology.



Source: Authors.

Vitamin E

The concentrations of total vitamin E and α-tocopherol equivalent, as well as their profile, differed according to the types of flours and oil (Table 3 and Figure 1). For all the samples, the major isomer was α-tocopherol, which represented 91.85%, 93.59% and 97.25% of the total carotenoids for grape seed oil, whole grape seed flour and defatted grape seed flour, respectively. Some isomers, like β-tocopherol, β-tocotrienol, γ-tocotrienol and δ-tocotrienol were not detected. The grape seed oil had the highest concentrations of α-tocopherol, total vitamin E and α-tocopherol equivalents, followed by whole grape seed flour and defatted grape seed flour ($p<0.05$).

These values were different from those reported by Gauer et al. (2018) and Shinagawa et al. (2017), who obtained different concentrations and profiles of vitamin E. It is noteworthy that these authors analyzed seeds and oils from different

grape varieties, which may have contributed to these differences. In addition, several environmental factors, such as regional, climatic and soil differences can affect the composition of the grapes, which could cause differences in the concentrations of their compounds (Schultz, 2016).

The most recognized role for vitamin E in human health is as a lipid soluble antioxidant required for the preservation of cell membranes, where it reacts quickly with peroxyl radicals to preserve polyunsaturated fatty acids, preventing oxidative stress (Clarke et al., 2008; Is & Woodside, 2001).

Table 3: Occurrence and concentration of vitamin E (mg/100g), in wet matter of seed oil, whole and defatted *Bordeaux* grape seed flours [A, B]

Compound	Oil	Whole flour	Defatted flour
α-tocopherol	199.20 ± 45.54 [a]	98.92 ± 25.65 [b]	3.89 ± 0.21 [c]
α-tocotrienol	1.89 ± 0.98 [a]	0.54 ± 0.12 [a]	0.11 ± 0.09 [a]
β-tocopherol	nd	nd	nd
γ-tocopherol	12.34 ± 2.61 [a]	4.55 ± 1.33 [a]	nd
β-tocotrienol	nd	nd	nd
γ-tocotrienol	nd	nd	nd
δ-tocopherol	3.44 ± 0.91 [a]	1.69 ± 0.79 [a]	nd
δ-tocotrienol	nd	nd	nd
Total vitamin E	216.87 ± 83.21 [a]	105.70 ± 14.92 [b]	4.00 ± 0.89 [c]
α-T equiv.	201.10 ± 34.89 [a]	99.59 ± 12.54 [b]	3.92 ± 0.57 [c]

[A] The results were expressed as the average of 3 repetitions ± standard deviation; [B] Means followed by the same letter on the lines are not statistically different at 5% probability by the Duncan test. Where: α-T equiv. = α-tocopherol equivalents; nd = not detected. Source: Authors.

Flavonoids

None of the samples evaluated in the present study showed 3-DXAs and flavanones (Table 4 and Figure 1). The concentrations of total flavones, luteolin and apigenin differed according to the flour and oil. Both grape seed flours presented higher concentrations for luteolin, apigenin and total flavones, and the grape seed oil showed the lowest concentration of these compounds.

Table 4: Occurrence and concentration of flavones (mg/100g), in wet matter of seed oil, whole and defatted *Bordeaux* grape seed flours [A, B]

Compound	Oil	Whole flour	Defatted flour
Flavones			
Luteolin	112.34 ± 32.54 [b]	317.45 ± 34.76 [a]	285.13 ± 43.11 [a]
Apigenin	17.33 ± 8.12 [b]	39.66 ± 19.44 [a]	27.18 ± 6.32 [a]
Total flavones	129.67 ± 23.34 [b]	357.11 ± 65.39 [a]	312.31 ± 54.85 [a]

[A] The results were expressed as the average of 3 repetitions ± standard deviation; [B] Means followed by the same letter on the lines are not statistically different at 5% probability by the Duncan test. Source: Authors.

The occurrence and concentration of flavonoids were different from those reported by some authors, who found higher values for flavanones. However, none of these studies presented concentrations of 3-DXAs and flavones, results that are in agreement with the present study (Arola-Arnal et al., 2013; Ross et al., 2011; Scola et al., 2010). It is worth mentioning that these authors analyzed seeds and extracts of different varieties of grapes, which may have contributed to these differences. In

addition, several environmental factors, such as regional, climatic and soil differences can affect the composition of the grape seeds and its by-products, which could cause differences in the concentrations of their compounds (Schultz, 2016).

Flavonoids have multiplex biological effects, such as high antioxidant capacity, anti-inflammatory and antitumor properties, power to reduce capillary fragility and permeability, inhibition of collagen destruction to platelet aggregation. Thus, flavonoid intake is associated with longevity and the reduction in the incidence of cardiovascular diseases (Pereira et al., 2008; Araújo, 2008; Filho et al. 2001).

Antioxidant capacity, total phenolics, phytates and condensed tannins

The concentrations of antioxidant capacity by DPPH and FRAP methods, total phenolics, phytates and condensed tannins differed according to the product (flour or oil) and had the same pattern. Both grape seed flours showed the highest concentrations of these compounds, followed by the grape seed oil (Table 5). The antioxidant capacity of the oil was, at least, three times lower than the flours and the concentration of phenolic compounds in the oil was two times lower than the flours. Both flours had increased concentrations of phytates and condensed tannins (two and four times higher, respectively), when compared to the grape seed oil.

Table 5: Occurrence and concentration of antioxidant capacity by DPPH (mg trolox/g) and FRAP (µmol EFe^{+2}/g), total phenolic compounds (mg EAG/g), phytates (mg AF/g) and condensed tannins (mg Ecat/g), in wet matter of seed oil, whole and defatted *Bordeaux* grape seed flours [A, B]

Compound	Oil	Whole flour	Defatted flour
Total phenolics	0.09 ± 0.01 [b]	0.22 ± 0.01 [a]	0.18 ± 0.01 [a]
Phytates	1.34 ± 0.15 [b]	4.87 ± 0.32 [a]	5.19 ± 0.24 [a]
Tannins	0.08 ± 0.01 [b]	0.95 ± 0.02 [a]	0.99 ± 0.05 [a]
Antioxidant capacity			
DPPH	27.65 ± 5.25 [b]	89.44 ± 12.32 [a]	72.32 ± 15.01 [a]
FRAP	0.05 ± 0.01 [b]	0.39 ± 0.09 [a]	0.27 ± 0.04 [a]

[A] The results were expressed as the average of 3 repetitions ± standard deviation; [B] Means followed by the same letter on the lines are not statistically different at 5% probability by the Duncan test. Source: Authors.

These results were different from those reported by some authors, who found lower concentrations of antioxidant capacity (by both methods) and total phenolics for grape seed flours and grape seed oils (Wen et al., 2016; Peighambardoust, 2014; Scola et al., 2010). As for phytates and tannins, the data in the present study were higher than those reported by Muhammad et al. (2018) and Addo et al. (2018).

Phytates bind to iron and calcium in the human body, which could impair their absorption and cause deficiencies. Tannins are scattered in different parts of the plants and are present in a greater quantity in fruit and cereal seeds. These compounds have several medicinal properties and also the ability to form complexes with proteins, in addition to inhibiting the action of endogenous proteins, such as digestive enzymes. Its toxic amount to the human organism is not known yet (Addo et al., 2018; Shils et al., 2006).

Percentage of contribution to the supply of daily vitamin recommendations

Following the DRIs for male individuals and the age group between 19 and 30 years old, whole and defatted Bordeaux grape seed flour presented as an excellent source of riboflavin, pyridoxine and vitamins C and E, as well as source of thiamine (Table 6). *Bordeaux* grape seed oil was classified as an excellent source of vitamin E.

Table 6: Percentage of contribution of seed oil, whole and defatted *Bordeaux* grape seed flours to achieve the daily nutritional recommendations of vitamins, for adult men from 19 to 30 years old

Compound	Oil (%)		Whole flour (%)		Defatted flour (%)	
	100 g	Per portion (8.12 g)	100 g	Per portion (149.88 g)	100 g	Per portion (244.34 g)
Thiamine	1.43	0.12	3.48	5.21	2.69	6.58
Riboflavin	12.29	1.00	61.73	92.52	60.39	147.55
Pyridoxine	3.95	0.32	22.78	34.15	14.58	35.63
Vitamin C	11.20	0.91	15.07	22.58	14.04	34.32
Vitamin E	1,445.80	117.40	704.67	1,056.15	26.67	65.16

Calculated based on the information that one portion of oil is equivalent to 73 kcal and whole and defatted flour are equivalent to 150 kcal. Calculated based on the Recommended Dietary Allowance of vitamins for adults aged 19 to 30 years (U. S INSTITUTE OF MEDICINE, 2011). Where: % = percentage; g = grams. Source: Authors.

4. Conclusion

The different grape products (flour and oil) affected the occurrence and concentration of bioactive compounds. Considering their nutritional value and potential benefits to human health, the intake of whole and defatted *Bordeaux* grape seed flour, as well as *Bordeaux* grape seed oil, should be encouraged in human feeding. In addition, the present study contributed data to the food composition tables and to the use of the grape by-product in the food industry after the production of juices, wines and other products.

This paper will serve as a basis for future studies that evaluate the effects of long-term consumption of *Bordeaux* grape seed flours and oil on important parameters of human health (markers of lipid metabolism, glucose, iron and zinc; markers of oxidative stress and inflammatory response; food intake; anthropometry and body composition), gut microbiota, diabetes, hormones, gene expression, among others. In addition, the present paper will serve as a reference for studies that elaborate preparations containing these flours and oil, in order to introduce them in human food.

Acknowledgments

The authors thank the company Econatura for donating the samples, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for the partnership and the granting of doctoral and scientific initiation grants.

References

Addo, P. W. et al. (2018). Antinutrient contents of watermelon seeds. *MOJ Food Processing and Technology*, 6 (2), 237-239.

Alara, O. R. et al. (2021). Extraction of phenolic compounds: a review. *Current Research in Food Science*, 2, 200-214.

Alves-Rodrigues, A. & Sha, A. (2004). The science behind lutein. *Toxicology Letters*, 150, 57-83.

AOAC. Association of Official Analytical Chemists. (2012). *Official methods of analysis of the AOAC*, Washington, D.C.

Araújo, J. M. (2008). *Química de Alimentos: Teoria e Prática*. Editora UFV.

Arella, F. et al. (1996). Liquid chromatographic determination of vitamins B1 and B2 in foods. A collaborative study. *Food Chemistry*, 56(1), 81-86.

Arola-Arnal, A. et al. (2013). Distribution of grape seed flavanols and their metabolites in pregnant rats and their fetuses. *Molecular Nutrition & Food Research*, 57(10), 1741-1752.

Benzie, I. & Strain J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76.

Bergaentzlé, M. et al. (1995). Determination of vitamin B_6 in foods by HPLC—a collaborative study. *Food Chemistry*, 52(1), 81-86.

Bertrand, M. & Ozcan, M. M (2011). Oil yields, fatty acids compositions and tocopherol contents of grape seed oils from Turkey. *Carpathian Journal of Food Science and Technology*, 3, 63-71.

Bloor, S. J. (2001). Overview of methods for analysis and identification of flavonoids, in: Lester, P. (Ed.), *Methods in Enzymology*. Academic Press.

Bragagnolo, N. (1997). *Fatores que influenciam o nível de colesterol, lipídeos totais e composição de ácidos graxos em camarão e carne*. 123f. Tese (doutorado) - Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Campinas, SP.

Brow, L. et al. (1999). A prospective study of carotenoid intake and risk of cataract extraction in US men. *The American Journal of Clinical Nutrition*, 70, 517-524.

Campos, F. M. et al. (2009). Optimization of methodology to analyze ascorbic and dehydroascorbic acid in vegetables. *Química Nova*, 32, 87-91.

Clarke, M. W. et al. (2008). Vitamin E in human health and disease. *Critical Reviews in Clinical Laboratory Sciences*, 45(5), 417-450.

Crew, C. et al. (2006). Quantitation of the main constituents of some authentic grape seed oils of different origins. *Journal of Agricultural and Food Chemistry*, 54, 6261-6264.

Delgadillo, J & Ayala, G. (2009). Efectos de la deficiencia de riboflavina sobre el desarrollo del tejido dentoalveolar, en ratas. *Anales de la Facultad de Medicina*. UNMSM. Facultad de Medicina, 19-27.

Deng, Q. et al. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International*, 44(9), 2712-2720.

Dykes, L. et al. (2009). Flavonoid composition of red sorghum genotypes. *Food Chemistry*, 116, 313-317.

Egbuonu A. C. C. (2015). Comparative assessment of some mineral, amino acid and vitamin compositions of watermelon (*Citrullus lanatus*) rind and seed. *Asian Journal of Biochemistry*, 10(5), 230-236.

Ellis, R. & Morris, E. R. (1986). Appropriate resin selection for rapid phytate analysis by ion-exchange chromatography. *Cereal Chemistry*, 63(1), 58-59.

Estrela, C. (2018). *Metodologia Científica: Ciência, Ensino, Pesquisa*. Editora Artes Médicas.

Filho W, D. et al. (2001). Flavonóides antioxidantes de plantas medicinais e alimentos: importância e perspectivas terapêuticas. In: *Plantas medicinais sob a ótica da química medicinal moderna*. Chapecó: Universidade do Oeste de Santa Catarina, 317-334.

Garavaglia, J. et al. (2016). Grape seed oil compounds: Biological and chemical actions for health. *Nutrition and Metabolic Insights*, 9, S32910.

Gauer, P. O. et al. (2018). Evaluation of oil and flour for human nutrition obtained from conventional and organic grape seed Bordô from a winery in the South of Brazil. *Grasas y Aceites*, 69(1), e237-e237.

Ghafoor, K. et al. (2020). Influence of grape variety on bioactive compounds, antioxidant activity, and phenolic compounds of some grape seeds grown in Turkey. *Journal of Food Processing and Preservation*, 44(12), e14980.

Grundy, S.M. & Denke, M.A. (1990). Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research*, 31, 1149.

Hartman, L. & Lago, R.C.A. (1973). Rapid preparation of fatty acid methyl ester from lipids. *London Laboratory Practice*, 22, 475-476.

Institute of Medicine. (2011). *Dietary reference intakes: a risk assessment model for establishing upper intake levels for nutrients*. Washington (DC): National Academy Press.

Is, Y. & Woodside, J. V. (2001) Antioxidant in health and disease. *Journal of Clinical Pathology*, 54(3), 176-186.

Karnopp, A. R. et al. (2015). Effects of whole-wheat flour and bordeaux grape pomace (*Vitis labrusca* L.) on the sensory, physicochemical and functional properties of cookies. *Food Science and Technology*, 35, 750-756.

Krinsky, N. I. & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, 26, 459-516.

Latta, M. & Eskin, M. A. (1980). Simple and rapid colorimetric method for phytate determination. *Journal of Agricultural and Food Chemistry*, 28(6), 1313-1315.

Lira, G. M. et al. (2005). Composição centesimal, valor calórico, teor de colesterol e perfil de ácidos graxos da carne de búfalo (*Bubalis bubalis*) da cidade de São Luiz do Quitunde-AL. *Revista do Instituto Adolfo Lutz* (Impresso), 64(1), 31-38.

Lutterodt, H. et al. (2011). Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chemistry*, 128(2), 391-399.

Maicas, S. & Mateo, J. J. (2020). Sustainability of Wine Production. *Sustainability*, 12(2), 1-10.

Maihara, V. A. et al. (2006). Avaliação nutricional de dietas de trabalhadores em relação a proteínas, lipídeos, carboidratos, fibras alimentares e vitaminas. *Food Science and Technology*, 26, 672-677.

Martin, M. E. et al. (2020). Grape (*Vitis vinifera* L.) seed oil: a functional food from the winemaking industry. *Foods*, 9(10), 1360.

Monteiro, G. C. et al. (2021). Bioactive compounds and antioxidant capacity of grape pomace flours. *LWT*, 135, 110053.

Monteiro, E. M. (1998). *Influência do cruzamento ile de France x corriedale (F1) nos parâmetros de qualidade da carne de cordeiro*. Tese (doutorado). Universidade de São Paulo, São Paulo.

Muhammad, S. (2019). Physico-chemical, fatty acids and amino acids composition of the seeds of african grapes (*Lannea microcarpa*) fruits. *Journal of Applied Sciences*, 19(2), 82-87.

Munyaka, A. W. et al. (2010). Thermal stability of L-ascorbic acid and ascorbic acid osidase in broccoli (*Brassica oleracea* var. *italica*). *Journal of Food Science*, 75(4), 336-340.

Ndaw, S. et al. (2000). Extraction procedures for the liquid chromatographic determination of thiamin, riboflavin and vitamin B6 in foodstuffs. *Food Chemistry*, 71(1), 129-138.

Nicolescu, C. M. et al. (2022). Romanian organic and conventional red grapes vineyards as potential sources of high value-added products, in a circular economy approach. *Grapes and Wine*, 107.

Ozvural, E. B. & Vural, H. (2011). Grape seed flour is a viable ingredient to improve the nutritional profile and reduce lipid oxidation of frankfurters. *Meat Science*, 88(1), 179-183.

Panfili, G. at al. (2004). Improved normal-phase high-performance liquid chromatography procedure for the determination of carotenoids in cereals. *Journal of Agricultural and Food Chemistry*, 52(21), 6373-6377.

Peighambardoust, S. H. et al. (2014). Physicochemical, nutritional, shelf life and sensory properties of Iranian Sangak bread fortified with grape seed powder. *Journal of Food Processing and Technology*, 5(10).

Peñuela-Sierra, L. M. et al. (2015). Ácidos graxos poliinsaturados e ácido linoléico conjugado na carne suína. Benefícios para a saúde humana: Revisão. *PUBVET*, 9, 287-347.

Pereira, R. J. & Das Graças Cardoso, M. (2012). Metabólitos secundários vegetais e benefícios antioxidantes. *Journal of Biotechnology and Biodiversity*, 3(4).

Phillippy, B. Q. et al. (2003). Ion chromatography of phytate in roots and tubers. *Journal of Agricultural and Food Chemistry*, 51(2), 350-353.

Pinheiro-Sant'Ana, H. M. et al. (2011). Method for simultaneous analysis of eight vitamin E isomers in various foods by high performance liquid chromatography and fluorescence detection. *Journal of Chromatography*, 1218(47), 8496-8502.

Rodriguez-Amaya, D. B et al. (1976). Carotenoid pigment changes in ripening *Momordica charantia* fruits. *Annals of Botany*, 40(3), 615-624.

Ross, C. F. et al. (2011). Influence of heating on the polyphenolic content and antioxidant activity of grape seed flour. *Journal of Food Science*, 76(6), C884-C890.

Rubert, A. et al. (2017). Vitaminas do complexo B: uma breve revisão. *Revista Jovens Pesquisadores*, 7(1), 30-45.

Schultz, H. R (2016). Global climate change, sustainability, and some challenges for grape and wine production. *Journal of Wine Economics*, 11(1), 181-200.

Scola, G. et al. (2010). Flavan-3-ol compounds from wine wastes with in vitro and in vivo antioxidant activity. *Nutrients*, 2(10), 1048-1059.

Shils, M. E. et al. (2006). *Modern nutrition in health and disease*. Lippincott Williams & Wilkins. Philadelphia.

Shinagawa, F. B. et al. (2015). Grape seed oil: a potential functional food? *Food Science and Technology*, 35, 399-406.

Singleton, V. L. et al. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.

Sousa, E. C. et al. (2014). Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. *Food Science and Technology*, 34, 135-142.

Spector, A. A. (1999). Essentiality of fatty acids. *Lipids*, 34, S1–S3.

Stringheta, P. C. et al. (2009). Luteína: propriedades antioxidantes e benefícios à saúde. *Alimentos e Nutrição Araraquara*, 17(2), 229-238.

Strutzel, E. et al. (2007). Análise dos fatores de risco para o envelhecimento da pele: aspectos gerais e nutricionais. *Revista Brasileira de Nutrição Clínica*, 22(2), 139-145.

Vieira, A. C. R. (2020). *Atividade antioxidante da vitamina C: aplicações na indústria farmacêutica e de alimentos e formas de evitar a oxidação mantendo sua estabilidade*. 49 f. Trabalho de Conclusão de Curso (Graduação) – Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia.

Wen, X. et al. (2016) Characterisation of seed oils from different grape cultivars grown in China. *Journal of Food Science and Technology*, 53(7), 3129-3136.

Yang, L. et al. (2012). Sorghum phenolics demonstrate estrogenic action and induce apoptosis in nonmalignant colonocytes. *Nutrition and Cancer*, 64(3), 419-427.