A Face Multidisciplinar das Ciências Agrárias 4

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(Organizadores)

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APRESENTAÇÃO

Nos primórdios do desenvolvimento da agricultura, os recursos naturais disponíveis propiciaram o surgimento das atividades agropecuárias, e desta forma, a necessidade de atuação dos profissionais de ciências agrárias tornou-se consolidada. Durante séculos, novos conhecimentos foram adquiridos, fundamentados teoricamente sobre as práticas agrícolas, conduzindo ao aperfeiçoamento do processo produtivo de acordo com a evolução da sociedade.

Diante do atual cenário, a obra “A Face Multidisciplinar das Ciências Agrárias” em seus volumes 3 e 4 engloba respectivamente 24 e 27 capítulos capazes de possibilitar ao leitor a experiência de ampliar o conhecimento sobre a economia e sociologia no campo, conservação pós-colheita, tecnologia de alimentos, produção vegetal, qualidade de produtos agropecuários, metodologias de ensino e extensão nas escolas, epidemiologia e cadeia produtiva da produção animal.

Em virtude da pluralidade existente desta grande área, os trabalhos apresentados abordam temas de expressiva importância as questões sociais e econômicas do Brasil. E, portanto, evidenciamos profunda gratidão pelo empenho dos autores, que em conjunto, contribuíram para o desenvolvimento e formação deste e-book.

Espera-se, agregar ao leitor, conhecimentos sobre a multidisciplinaridade das ciências agrárias, de modo a atender as crescentes demandas por alimentos primários e transformados, preservando o meio ambiente para às gerações futuras.

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SUMÁRIO

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CAPÍTULO 27

ULTRASOUND EXTRACTION AND FATTY ACID PROFILE OF GRAPE SEED OIL

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ABSTRACT: This study aimed to extract grape seed oil by ultrasound extraction using the experimental design methodology and determine the fatty acid profile of the oils of Cabernet Sauvignon and Cabernet Franc grape varieties. In the ultrasound extraction, the study variables were temperature and solvent: sample ratio, and oil yield was the response variable. The working region chosen for ultrasound extraction was under the temperature conditions of around 40 °C and solvent: sample ratio of around 10:1. The oil yield of the Cabernet Sauvignon and Cabernet Franc seeds was 15.2% and 13%, respectively, while the ultrasound extraction efficiency was higher than 92%. The oils of the Cabernet Sauvignon and Cabernet Franc varieties had iodine index values (134.7 cg I_2/g and 136 cg I_2/g) and refraction (1.475 and 1.477) and free acidity (0.852 and 0.760% oleic acid), respectively. The sum of unsaturated fatty acids of the oils obtained ranged between 81-84%. The ultrasound method can be used as an alternative to the reuse of winery residues from the grape seed oil extraction. The oil obtained had a good nutritional quality due to its high content of unsaturated fatty acids, thus allowing its use in pharmaceutical and food industries.

KEYWORDS: linoleic fatty acid, oleic fatty acid, *Vitis vinifera*

EXTRAÇÃO VIA ULTRASSOM E PERFIL DE ÁCIDOS GRAXOS DE ÓLEO SEMENTE DE UVA

RESUMO: Este estudo teve como objetivo

extrair o óleo da semente de uva via extração por ultrassom utilizando como metodologia um planejamento experimental e determinar o perfil de ácidos graxos dos óleos obtidos a partir das sementes das variedades Cabernet Sauvignon e Cabernet Franc. Na extração por ultrassom, as variáveis de estudo foram temperatura e razão solvente: amostra e como variável resposta o rendimento de óleo obtido. A região de trabalho escolhida para extração por ultrassom foi sob condições de temperatura de 40 ° C e a razão solvente: amostra de 10: 1. O rendimento de óleo das sementes de Cabernet Sauvignon e Cabernet Franc foi de 15,2% e 13%, respectivamente, enquanto a eficiência de extração por ultrassom foram superiores a 92%. Os óleos das variedades Cabernet Sauvignon e Cabernet Franc apresentaram valores de índice de iodo (134,7 cg I2 / ge 136 cg I2 / g) e refração (1,475 e 1,477) e acidez livre (0,852 e 0,760% de ácido oleico), respectivamente. O conte de ácidos graxos insaturados dos óleos obtidos variou entre 81-84%. O método de ultrassom pode ser usado como uma alternativa para a reutilização dos resíduos vinícolas na extração de óleo a partir de sementes de uva. O óleo obtido apresentou boa qualidade nutricional devido ao seu alto teor de ácidos graxos insaturados, permitindo seu uso nas indústrias farmacêuticas e alimentícias.
PALAVRAS-CHAVE: ácido graxo linoleico, ácido graxo oleico, *Vitis vinifera*

1 I INTRODUCTION

The world production of grapes and wines in 2012 was 67 million tons (FAO, 2013) and 252 million hectoliters (DUBA; FIORI, 2015), respectively. In Brazil, in 2011, grape production presented an increase of 49.55% compared to 2000. The country occupies the 11th place in the world rankings of the area planted with vines, reaching a production of 1.542.070 tons of grapes on about 88.000 hectares (FAO, 2013).

The marc consists of stems (25%), seeds (25%) and shell (50%). In recent decades research has shown numerous possibilities of valuation of these by-products through recovery of oil, phenolic compounds and fiber (DUBA; FIORI, 2015). Grape seeds have 10 to 20% of oil in their composition depending on the variety (HANGANU et al., 2012).

The nutritional quality of this oil is due to its high content of unsaturated fatty acids (about 90%), particularly oleic (C18:1) and linoleic (C18:2), essential for human metabolism (HANGANU et al., 2012; DUBA; FIORI, 2015). According to Shinagawa et al. (2015) the consumption of grape oil is still sluggish in Brazil, but in view of the great increase in wine consumption and consequently the generation of waste, grape oil becomes a value-added food.

Traditional methods for the extraction of oil are pressing (DELI et al., 2011) and the hot solvent extraction (ADAM et al., 2012). The extraction by pressing, obtains a high quality oil. However, this extraction has the disadvantage of oil retention in the press-cake that causes low extraction yield (DELI et al., 2011; ADAM et al., 2012). In the method via hot solvent, the oil is extracted with apolar solvents at temperatures near the boiling point of the solvent, which may be responsible for the formation of free fatty acids. In this extraction method, hexane is typically used during 20 hours of process (GÓMES et al., 1996; AGOSTINI et al., 2012).

Ultrasound technology is based on sound waves generated from a certain frequency, which cannot be heard by humans (SARI; EKINCI, 2017). The ultrasound by extraction method is an alternative to the usual methods of oil extraction and has been applied in the extraction of organic compounds present in particulate materials (ADAM et al., 2012; SETYANINGSIH et al., 2015).

The extraction by ultrasound is associated with cavitation phenomenon. During the propagation of ultrasonic waves in a liquid, cavitation microbubbles are formed due to local pressure variations; the collapse (implosion) of these microbubbles can generate pressures and relatively high temperatures in micro liquid system, facilitating the destruction of the solid surface that is in contact and consequently providing a more effective contact between the solid matrix and the solvent (ADAM et al., 2012; SETYANINGSIH et al., 2015; TAO et al., 2014). This extraction method has been applied with promising results, presenting the advantages of simplicity of equipment, initial cost savings, and in the extraction of organic compounds, increased performance and decreased processing time (TAO et al., 2014).

Ultrasound extraction method has attracted interest since it has positive points (time and yield) compared to the classical method of pressing and solvent extraction (ADAM et al., 2012; SETYANINGSIH et al., 2015). Accordingly, the objective of this work was to study the extraction by ultrasound process of grape seed oil, using the methodology of experimental design, and determine the profiles of fatty acids of grape seed oils of Cabernet Sauvignon and Cabernet Franc varieties.

2 I MATERIAL AND METHODS

2.1 Sample Preparation

The samples used were grape seeds of Cabernet Sauvignon and Cabernet Franc varieties from marcs provided by a winery from the region of Bagé/RS. The seeds were separated by screening, washed and subjected to a heat treatment for enzymatic inactivation in a circulating air oven (Tecnal TE 394/2, Sao Paulo, Brazil) at 80 °C for 10 min (ROCKENBACH et al., 2010) and stored in amber vials at a temperature below 30 °C. To perform the ultrasound extraction experiments, the seeds were ground in an analytical mill (IKA A11 Basic Mill, São Paulo, Brazil).

2.2 Ultrasound Extraction

The ultrasound extraction was performed with 10 g sample of crushed grape seeds of Cabernet Sauvignon variety placed in direct contact with an organic solvent (hexane) in a polished mouth Erlenmeyer flask which was connected to a condenser. This apparatus was placed in an ultrasonic bath (Unique USC-2800A, São Paulo, Brazil; frequency of 40 kHz and 150 W power) at a determined temperature for 30 minutes. In the end of the extraction, separation of the solid and liquid fractions was carried out by vacuum filtration and evaporation of the solvent on a rotary evaporator (Quimis Q344B2, Sao Paulo, Brazil) obtaining the oil. The amount of solvent: sample ratio and the extraction temperature were studied by a experimental design.

2.3 Statistical Methodology

The ultrasound extraction experiments were sorted according to Experimental Planning Matrix Full Factorial 2^2 with central point, totaling 5 assays performed in duplicate. The study factors used in the ultrasound extraction were temperature (20, 40 e 60°C) of extraction and the ratio of the solvent: sample masses (1:2; 1:10 and 1:18); the response variable of the experiments was the yield of the extraction process. The values of levels of study factors used were determined from preliminary tests and references (ADAM et al., 2012). The results of the experimental design matrix shown in Table 1 were evaluated statistically by analysis of variance (ANOVA) at the 5% level of significance using statistical software, to obtain the variables that influenced the considered response, as well as the best working region for the ultrasound extraction, which was determined by the response square.

The fatty acid profiles of grape seeds oils of Cabernet Sauvignon and Cabernet Franc varieties were obtained in duplicate and the results presented by mean ± average deviation. The profiles of both oils were compared by Tukey's mean difference test (BOX et al., 1978), using a statistical software. The values were considered significant at a significance level of 5% ($p < 0.05$).

2.4 Analytical Methodology

The lipid content in grape seeds of the Cabernet Sauvignon and Cabernet Franc varieties was determined by Soxhlet, according to the official method of the Adolfo Lutz Institute (2008). The oil samples obtained by ultrasound method (best condition obtained from the results of the experimental design) were analyzed for free acidity (FFA), Iodine Value (IV) and Refractive Index (RI), according to the Adolfo Lutz Institute official methodology (2008).

The identification and quantification of fatty acids was performed by chromatographic analysis of oils from grape seeds of the Cabernet Sauvignon and Cabernet Franc varieties. For injection in the equipment, the methyl esters were prepared according to the methodology described by Metcalfe & Schimitz (1966). The fatty acid methyl esters were identified by gas chromatography (GC) in a chromatograph (Varian CX-3400, Palo Alto, California, USA) equipped with DB-17 capillary column J & W Scientific (Phenylmethylpolysiloxane 50%). The analysis of fatty acid methyl esters was performed in duplicate by injecting 1.0 μl sample (SPLIT ratio 1:50) in the capillary column (30 m length x 0.25 mm internal diameter with a film of 0.25 mm thick). The chromatograph conditions were: 250 °C injector temperature, temperature of the flame ionization detector 300 °C, carrier gas was helium with a flow of 1.00 ml min^{-1}, linear velocity of 24 $cm.s^{-1}$ and initial temperature of the column 100 °C, kept at this temperature for 1 min; thereafter, increasing the temperature 6°$C.min^{-1}$ to 160 °C and 230 °C. The fatty acid methyl esters were identified by direct comparison of retention times with standard (SUPELCO TM 37, Bellefonte, Palo Alto, USA) and quantified by area normalization.

3 I RESULTS AND DISCUSSION

The whole samples had mean diameter of Sauter and porosity of 2.911 ± 0.106 mm and 0.407 ± 0.019. After the milling process the porosity of the bed was increased by about 23%. The increase in the porosity facilitates the extraction of oil, since there is an increase of the surface area of contact with the solvent, facilitating the transfer of mass in the process.

3.1 Experimental Planning

Table 2 shows the matrix and the results of the 2^2 factorial design with center point for the study of the extraction process of oil from grape seeds by ultrasound.

Experiments	Temperature	Solvent:Sample (*cod)	η (%)
1	20	1:2	8.39 ± 0.51
2	60	1:2	9.11 ± 0.78
3	20	1:18	14.08 ± 0.45
4	60	1:18	15.22 ± 0.25
5	40	1:10	14.29 ± 0.42

Table 2: matrix and results of the factorial design 2^2 with center point for the extraction of grape seed oil of the Cabernet Sauvignon variety by ultrasound.

Mean value’standard deviation (*n*=2); * cod = variable level in coded form

Analyzing the results of **Table 2**, it is seen that there was a variation in the process yield from 8% to 15%, which is equivalent to an increase of 80%. Thus, the increased amount of solvent promotes the extraction of oil. This result can be explained by the increase of the process driving force (difference in the oil concentration between the solid matrix and the solvent) in the presence of a greater quantity of solvent, which causes an increase in the mass transfer rates of the matrix (ground grape seed) to the liquid phase (solvent). **Figure 2** shows the Pareto chart of the standardized effects of study variables on the yield of the process.

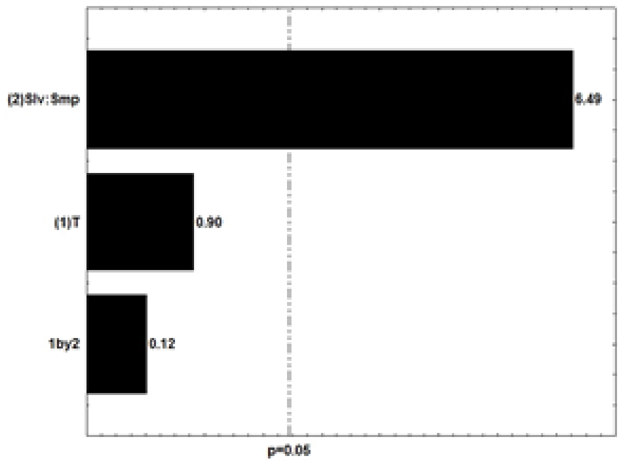

Figure 2: Pareto Chart of the effects of temperature (T) and mass ratio solvent:sample (Slv:Smp) on the response yield of the ultrasound oil extraction process.

Through Pareto chart (**Figure 2**) one can see the effects of the variables that influence the efficiency of oil extraction; it can be seen in this graph that only the solvent: sample ratio was statistically significant ($p < 0.05$) positively affecting the response variable, i.e. the increase in the extraction yield. The variable temperature and its interaction with the variable solvent: sample showed no significant effect ($p > 0.05$).

In yours studies Zhang et al. (2009) found too a positive effect of the variable solvent: sample ratio on almond (Prunus amygdalus) oil ultrasound extraction efficiency (frequency 40 kHz power 150 W), by increasing the efficiency from 56.1% to 72.3%, varying the solvent: sample ratio from 5:1 to 20:1, respectively. The same behavior was verified by Goula (2013) on the extraction of pomegranate oil by ultrasound, obtaining a larger amount of oil using greater solvent: sample ratios. In the present work it was also found that there was an increase in extraction efficiency from 54% to 98% by varying the solvent: sample ratio from 2:1 to 18:1.

Thus, increasing the solvent: sample ratio (2:1 to 18:1), the oil yield was statistically higher. Higher solvent concentrations in addition to increasing the extraction driving force, promote greater contact with the sample inside its pores, reducing the mass transfer resistance. Extraction temperatures (20 ºC to 60 ºC) did not affect significantly ($p > 0.05$) in the yield of the extraction process in the studied zone. This behavior is positive in the extraction, since high temperatures increase the rate of chemical reactions favoring the hydrolysis of triacylglycerol with formation of free fatty acids and also the lipid oxidation reactions.

Figure 3 shows the response surface of the experimental design, where you can check the best work area to be used for extraction of oil via ultrasound. The effects presented by the study variables are reaffirmed through these response surface were its possible to see that the yield of extraction ranged from 8.89% to 15.73% when the solvent: sample ratio increased from 2:1 to 18:1.

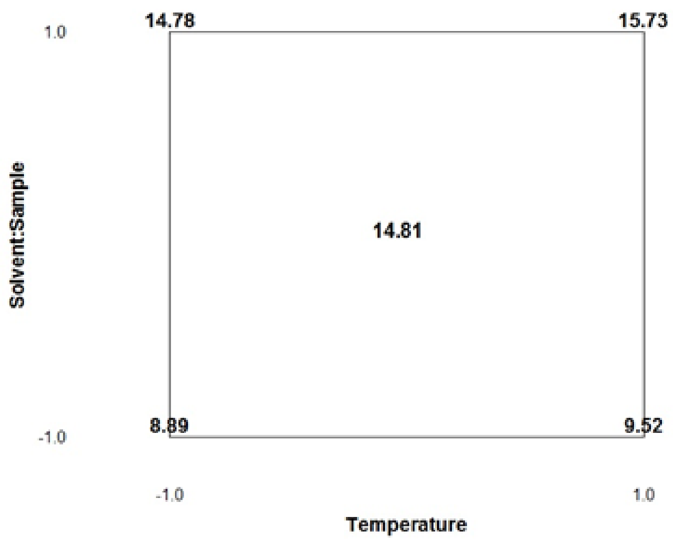

Figure 3: Square response for the oil extraction yield obtained by ultrasound

Through the response surface analysis (**Figure 3**) it is seen that the highest oil content was obtained for the extraction conditions with the highest solvent: sample ratio (18:1), and the center point (10:1). Between levels 10:1 and 18:1, there was a little variability in the efficiency of the oil extraction. This fact may have occurred, possibly due to saturation of the solid matrix by the solvent in the ratio 18:1 at the process time of 30 minutes, not increasing the mass transfer coefficients.

Considering that the best results are based on the higher sample-solvent ratio and that the central point showed a similar behavior, and in terms of lower energy and solvent costs, the central point was choice as the best condition for the process of extraction of grape oil.

3.2 Extraction by ultrasound and characterization of oils from the Cabernet Franc and Sauvignon varieties

The oils present in grape seeds from the Cabernet Franc and Cabernet

Sauvignon varieties were obtained in the best ultrasound extraction condition by experimental design (*see* 2.3), in order to compare the performance and process efficiency, as well as the physicochemical characteristics and profiles of fatty acids of these oils. The Cabernet Sauvignon and Cabernet Franc presented an efficiency of 96.75 and 92.91 % respectly.

The seeds of Cabernet Sauvignon and Cabernet Franc grapes variety showed 15.73 ± 1.02% oil content and 12.70 ± 0.75%, respectively. These values were within the range mentioned in literature (10 to 20%) (HANGANU et al., 2012). Baydar et al. (2007) extracted by soxhlet the seed oil from four varieties of grapes and obtained oil contents ranging from 12 to 16%. Baydar & Akkurt (2001) investigated the content and quality of grape seed oil of 18 cultivars, obtaining oil quantities from 11.6 to 19.6%. In these studies it can be seen that the variation in oil content depends on the grape variety. Furthermore, it is suggested that there might be a relationship between the oil content in the seed and fruit ripening time (BAYDAR & AKKURT, 2001).

Porto et al. (2013) extracted grape seed oil (Raboso Piave variety) by ultrasound and Soxhlet method, using hexane as the solvent. They obtained an oil content of 14.1% and 14.7% for extraction via ultrasound and via solvent, respectively. One can see that ultrasound extraction efficiency was 96%, similar to that obtained in this work for the Cabernet Sauvignon variety. However, it is worth mentioning that the small differences observed within the same species may be related to the soil, since this directly affects the chemical composition, as a greater proportion of proteins, which interact with oil.

From the results presented and considering that the ultrasound extraction method is performed with less time and lower temperature than the method via solvent (70 °C and 20 hours), according to Gómes et al., (1996) and has higher efficiency than the pressing method, the choice of the ultrasound method becomes more interesting and advantageous. Thus, it is possible to avoid losses in oil quality due to relatively high temperatures and process times; Furthermore, it is possible to increase the extraction efficiency, since oils extracted from the studied varieties showed efficiencies above 92%. The oils from grape seeds of the Cabernet Sauvignon and Cabernet Franc varieties presented iodine index (134 and 136 cg I_2*g^{-1}) and refractive index according as the values stipulated by ANVISA (National Health Surveillance Agency) for commercial grape seed oils (BRAZIL, 1999) however, the acidity values were above the maximum allowed by law.

The acidity is related to the nature and quality of raw material used, the quality and purity of the lipid, as well as the processing and the storage conditions of the lipid; the free acidity comes from the partial hydrolysis of the triglycerides, a reaction that is catalyzed in the presence of light and heat. Thus, the acidity of the oil present in the samples used in this work above the maximum allowed, may have occurred due to the raw material being derived from the fermentation of wine processes. Another reason may be related to poor discharge conditions of the samples. Comparing the iodine value of grape seed oil with other oil sources, grape seed oil has a higher degree of unsaturation than rice (99 - 108 cg I_2*g^{-1}) and canola oils (110 - 126 cg I_2*g^{-1} cg I_2*g^{-1}), and values in the range of soybean (120 - 143 cg I_2*g^{-1}) and sunflower oils (110 - 143 cg I_2*g^{-1}) (BRAZIL, 1999).

3.3 Fatty Acid Profiles of oils

The fatty acid profiles of grape seed oils of the Cabernet Sauvignon and Cabernet Franc varieties, are presented in **Table 5**.

Fatty acid	Cabernet Sauvignon*	Cabernet Franc*
C16:0 (palmitic)	6.80 ± 0.01^a	6.15 ± 0.01^b
C18:0 (stearic)	4.50 ± 0.01^a	3.55 ± 0.02^b
C18:1 (oleic)	13.10 ± 0.01^a	14.75 ± 0.01^b
C18:2 (linoleic)	70.13 ± 0.02^a	69.36 ± 0.01^b
Σni	5.47 ± 0.01^a	6.19 ± 0.02^b
ΣSFA	16.30 ± 0.02^a	9.70 ± 0.01^b
ΣMFA	13.10 ± 0.01^a	14.75 ± 0.01^b
ΣDFA	70.13 ± 0.02^a	69.36 ± 0.01^c
ΣUFA	83.23 ± 0.02^a	84.11 ± 0.02^b
ΣUFA/ ΣSFA	5.35^a	4.70^b

Table 5: Fatty acid profiles and lipids class of grape seed oil of the Cabernet Sauvignon and Cabernet Franc varieties.

* mean value ± standard error (n = 3 triplicate) Σni = unidentified sum.

* mean value ± standard error (n = 3 triplicate) ΣMFA = sum of monounsaturated fatty acids, ΣDFA = sum of di-unsaturated fatty acids. ΣUFA = sum of unsaturated fatty acids.

In **Table 5**, it is seen that Cabernet Sauvignon and Cabernet Franc oils presented in greater quantity and significantly different the palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) fatty acids. These percentages of fatty acids are similar to those cited by the National Health Surveillance Agency (BRAZIL, 1999) for palmitic (5.5 to 11%), oleic (12 to 28%) and linoleic (58 to 78%) acids. It is worth mentioning that the differences in the fatty acid profiles may be related to genetic factors (FERNANDES et al., 2013), since the samples are of different varieties and these were harvested at the same time and grown under similar environmental conditions.

Researching the yield and composition of grape seed oils by supercritical extraction means for Cabernet Sauvignon and Cabernet Franc, Beveridge et al. (2005) obtained similar results of stearic (4.92%) and oleic (12.71%) fatty acids for

Cabernet Sauvignon. The content of polyunsaturated fatty acids (72.57%) was higher than that found in the present study. For the Cabernet Franc variety, the authors found stearic fatty acid (5.22%) in greater quantities, oleic fatty acid (13.02%) was smaller and linoleic fatty acid (70.28%) was similar with that in this work.

Regarding the class of lipids, it is apparent that the sum of unsaturated fatty acids ranged from 83.23 to 84.11%. The degree of unsaturation is relatively high, though, lower than that presented by Passos et al. (2010), which was approximately 90%, and greater than that cited by Rockenbach et al. (2010). The latter authors investigated the fatty acid composition of grape seed oil and obtained values ranging from 70.4% to 77.1%. The oils extracted in this study have a high proportion of unsaturated fatty acids, presenting high relevance, since they are recommended for human consumption. As for the ΣUFA/ΣSFA ratio, this was 5.35 for Cabernet Sauvignon and 4.70 for Cabernet Franc varieties. Diets in which the ΣUFA/ΣSFA ratio is greater than 0.45 (Department of health and social security –DHSS, 1984) are considered nutritionally healthy.

4 I CONCLUSION

In the study of ultrasound extraction, it was verified that the temperature does not affect the yield of the process; on the other hand, the solvent:sample ratio had significant influence in the response ($p < 0.05$), where larger amounts of solvent gave the highest yield. The values of oil yield ranged from 8.9% to 15.7%. Considering less solvent used and decrease in the process temperature, the central point was choice as the best condition for the process of extraction of grape oil.

The Cabernet Sauvignon and Cabernet Franc varieties showed an oil yield of 15.2% and 13%, respectively. The efficiency of the ultrasound extraction method was superior to 92% for both varieties. Thus, the ultrasound extraction is advantageous due to lower temperature and shorter extraction time in relation to the solvent method and also in relation to pressing, due to a higher extraction efficiency.

The sum of unsaturated fatty acids of the varieties studied ranged from 83 to 84%. Thus, based on the results of the present study, the reuse of wineries waste for extraction of grape seed oil is a good alternative to adding value to the waste, since the oils obtained have a high content of unsaturated fatty acids, especially linoleic acid, an essential fatty acid beneficial to health.

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