REVIEW OF METHODOLOGIES DESTINED TO QUANTIFY THE RESVERATROL NUTRIENT IN FOREST FRUITS

Daniela BURDUHOS, Antonia ODAGIU, Ioan OROIAN
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca
Corresponding author: antonia.odagiu@usamvcluj.ro

Abstract. *The resveratrol (3, 5, 4' – trihydroxystilbene) is a phytoalexin, meaning that it is produced by different plants in response to some conditions, as environmentally unfriendly factors, wounds, fungal infections, ultraviolet rays, etc. Resveratrol has a strong antioxidant capacity. It is considered as having an antioxidant capacity of 4 up to5 times stronger than beta-carotene, 20 times stronger than vitamin C, and even 50 times stronger than vitamin E. Actually, resveratrol is a well-known nutritional principle considered valuable for reducing risk of cardiovascular diseases, but it is also presumed to be involved in extension of the life span, by activation of longevity gene. Even though several fruit species (grapes, berries and peanuts), are resveratrol sources, grapes skins and seeds are mostly studied, in this aim. Few research is dedicated to the study of resveratrol content in berries, even though high amounts of this principle may be found in these species, which could be higher valuated. In order to perform an accurate quantification of resveratrol occurrence in these sources, selecting the appropriate methodology is a critical step. This study is a short review of the available methodologies destined to quantify the resveratrol from berries. The methods included in the category of non-conventional aim those that use a series of high performance techniques, based on physics methods, as: ultrasounds, microwaves, high pressure, and pulsed electric field. A large diversity of methodologies is available for obtaining resveratrol from different matrices, including berries. They may be classified in conventional and unconventional methods. The solvent extraction is practiced at large extent, but it has a series of disadvantages, like long duration and pollutant action on the environment, due to the solvents involved. All these methods are environmentally friendly (because small amounts or no solvents are used), short duration, and have high accuracy, but high investments are needed in equipment, which are very expensive. .This study aims to review the available methodologies destined to quantify the resveratrol from berries, in order to select the most appropriate.*

Keywords*: antioxidant capacity, berries, health*

INTRODUCTION

Even though several fruit species (grapes, berries and peanuts), are resveratrol sources, grapes skins and seeds are mostly studied, in this aim (RISUELO AND LA MESA, 2019; WANG ET AL., 2005a). Few research is dedicated to the study of resveratrol content in berries (e.g. cranberry, blueberry, mulberry, raspberry, etc.), even though high amounts of this principle may be found in these species, which could be higher valuated (BŁASZCZYK ET AL., 2019).

Resveratrol ($C_{14}H_{12}O_3$), with the scientific name 3,5,4' – trihydroxystilbene, is a nonflavonoid polyphenol. There are three main reasons, which are the basis of the interest that this liposoluble compound is interesting for researchers, namely the benefits on human health, the role in decreasing of cardiovascular diseases, and involvement in plant defence mechanisms (Bravo, 1996; Frankel and Meyer, 1998; Stivala et al., 2001).

Resveratrol has a strong antioxidant capacity (DE LA LASTRA AND VILLEGAS, 2007; GÜLÇIN, 2010; HUYUT ET AL., 2018). It is considered as having an antioxidant capacity of 4 up to5 times stronger than beta-carotene, 20 times stronger than vitamin C, and even 50 times stronger than vitamin E. It is considered that it may explain the so called "French Paradox", namely that the French, though they eat fatty foods, smoke and consume much wine, are

healthier and slimmer, compared to people in other countries. The deeper motivation is that French people consume lot of red wine, rich in resveratrol (Simini, 2000). Research emphasizes that resveratrol is very well absorbed by the human body, but its bioavailability is reduced due to its rapid metabolism and elimination. After consuming a dose of resveratrol, only very small quantities reach the plasma, and the concentration is maximum at up to 60 minutes after consumption. The recommended dose for disease prevention is not yet known (AHMAD, 2003; WANG ET AL., 2005B; RHO ET AL., 2006; CATALGOL ET AL., 2012).

Actually, resveratrol is a well-known nutritional principle considered valuable for reducing risk of cardiovascular diseases, but it is also presumed to be involved in extension of the life span, by activation of longevity gene, SirT1, respectively (LAGOUGE ET AL., 2006; VALENZANO ET AL., 2006; SCHIFFRIN, 2009; ZORDOKY ET AL., 2009; LEKLI ET AL., 2010).

The phytoalexins are substances, usually with low molecular weight, produced by plants in response to abiotic and biotic stress conditions, as environmentally unfriendly factors, wounds, fungal infections, ultraviolet rays, etc. (JEANDET ET AL., 2014; SINGH AND CHANDRAWAt, 2017). Resveratrol is a compund considered phytoalexin (Pannu and Bhatnagar, 2019). Like for other similar compounds included in this class, also for resveratrol, adaptative mechanims and transduction pathways were developed in order to realize their role of coping the adverse factors. These mechanisms involve transcription factors with implications in phytoalexins' biosynthesis (DELAUNOIS ET AL., 2009; JEANDET ET AL., 2019).

In order to perform an accurate quantification of resveratrol occurrence in less used sources as berries, selecting the appropriate methodology is a critical step. This study aims to review the available methodologies destined to quantify the resveratrol from berries, in order to select the most appropriate.

MATERIAL AND METHODS

The present study was performed using specific methodology destined to bibliographical reserch. Several databases were consulted, and articles important for research were analyzed. A synthesis was elaborated, taking into account both, general traits of resveratrol and methodologies of resveratrol quanlitative and quantiative determinations. Starting from the general flow of raw material processing up to classical and modern extraction and separation methodologies are reviewed.

RESULTS AND DISCUSSIONS

Presently, there are available a series of strategies destined for obtaining resveratrol from different matrices, including berries. They include a large diversity of methodologies, and may be classified in two large categories, conventional and unconventional (Fig. 1).

The conventional methodologies used for polyphenolic compounds (class of substances that includes resveratrol) extraction belong to the classical methods well-known from old times. Traditionally used are maceration and solvent extraction, respectively.

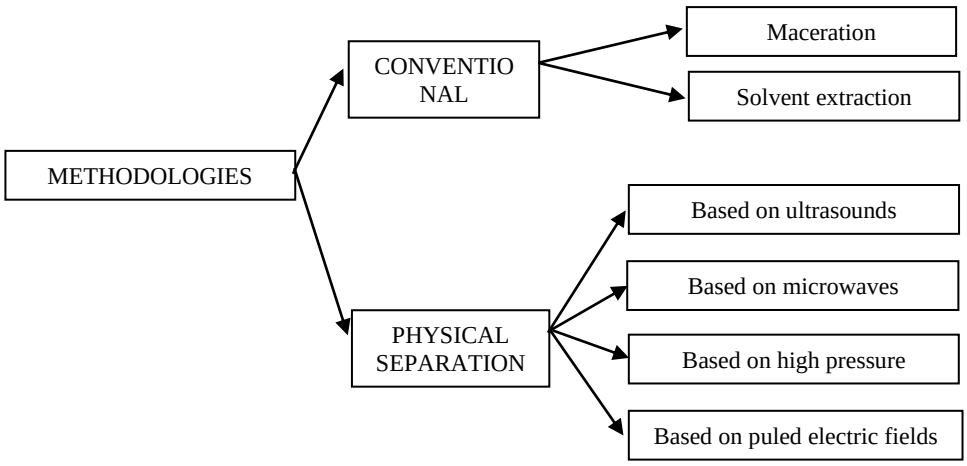

Figure 1. Extraction methods with potental to be used for obtaining resveratrol from berries matrices

Maceration involves the extraction of interest substances, resveratrol in this case, from the berries raw material into a specific solvent or solvent mixtures, at room temperature, over several hours (SNOUSSI ET AL., 2012). Solvent extraction also lead to resveratrol obtaining using a specific solvent or solvent mixtures (e.g. methanol, acetone, water) in different ratios, at room temperature, or higher temperatures. Both maceration and extraction are methodologies that need a supplementary step, in order to deliver the target compound. This step is represented by the flirtation operation. In laboratories, this last step may be eliminated by using the Soxhlet methodology, which consists in several repeated extraction at the boiling point of the extraction solvent (or mixture of solvents). The interest product, resveratrol in this case, is collected in a laboratory dish, which is part of the Soxhlet installation (Wang et al., 2006). The following solvents are preferred for extraction, and/or maceration of monocomponents of a polyphenolic compounds mixture: ethanol, methanol, acetone, water, hexane, ethyl acetate. Studies show that higher effectiveness is obtained when mixtures of solvents are used. Recommended is the mixture ethanol – water (40 – 70 : 60 – 30, v/v), not only due to the extraction yield, but also because ethanol is considered as safe, and it is usually obtained from renewable sources (BENZIE ET AL., 1996; BOROWSKA ET AL., 2009; DEMIRDOVEN ET AL., 2015; IVANOIVIC ET AL., 2014; RODRIGUES ET AL., 2015; SHORTLE ET AL., 2014; STOICA ET AL., 2013; WANG ET AL., 2016). For the particular case of cranberry (*Vaccinium oxycoccus* (Hill) A. Grey), according TO BOROSKA ET AL. (2009), an efficient solvent for extraction is methanol acidified with HCl, 0.1%, while for blueberry (*Vaccinium myrtillus* L.) GÜDER ET AL. (2015) mention Soxhlet methodology as most suitable, mulberry (*Morus* L.), and (DRAGASIC ET AL., 203) mention the suitability of the extraction mixture methanol : water : acetic acid for raspberry (*Rubus idaeus* L.). Up to date, the unconventional methodologies, candidate to be used for resveratrol extraction from berries, include a series of high performance techniques, based on physics methods, using: ultrasounds, microwaves, high pressure, and pulsed electric field. All these methods are environmentally friendly.

The methodology of extraction based on **ultrasounds** uses low frequencies around 20 kHz, at room temperature (GOLMOHAMADI ET AL., 2013), and low quantity of solvents. The

extraction mechanism is based on disruption of cell walls produced by cavitation, which is induced by ultrasound application (ADJÉ ET AL., 2010).This method is simple, has high efficiency, and equipment is not expensive. The use of this methodology eliminates expenses with solvents and heating (BARBA ET AL., 2016; IVANOVIC ET AL., 2014). According to GOLMOHAMADI ET AL. (2013), appropriate conditions for ultrasound assisted extraction from raspberry (*Rubus idaeus* L.) does not involve the use of any solvent, just frequency of 35 kHz, while according to (ȘTEFĂNUȚ ET AL., 2011), appropriate extraction conditions from blueberry (*Vaccinium myrtillus* L.) and mulberry (*Morus* L.) matrices are the use of methanol acidified with HCl, 0.1%, at frequency of 59 kHz, and 25 °C.

When extraction is performed using **microwaves**, the methodology is also characterized by the absence of solvents, or the use of very low solvent quantities. When solvents are used, according to (MENDES ET AL., 2016), the best solid/solvent ratio frames within the limit 30 - 34% (v/v). The microwaves are used for berries' cell damages, in order to create appropriate conditions for releasing the target compound (GALVÁN ET AL., 2012). The use of this methodology has advantages, because it is rapid, and needs no large size equipment. Low CO emissions are reported, when method is applied (LUCCHESI ET AL., 2004). In cranberry (*Vaccinium oxycoccus* (Hill) A. Grey), and blueberry (*Vaccinium myrtillus* L.), appropriate microwave assisted extraction needs the use of the mixture ethanol (70%) : water (1 : 2, v/v), at 300 W.

The use of **high pressure** for resveratrol extraction from berries, involves an unconventional method, or a combination of methodologies, function of variant used, Supercritical CO_2 Extraction, or Pressurized Liquid Extraction. Thus, the Pressurized Liquid Extraction functions at high temperatures, and because this contributes to enhance extraction kinetics, results that the methodology is much rapid, compared to the classical one, solvent extraction, respectively (PAES ET AL., 2014). The other method, Supercritical CO_2 Extraction, respectively, uses not only high pressures (over 220 bar), but also, high temperatures, and these parameters confer the methodology the supercritical trait. In order to perform the extraction, when this methodology is used, it is needed a supplementary polar solvent (about 5%), so called "entrainer", because it is needed to increase target extract solubility, and CO_2 is well-known as a non-polar molecule. The method application allows a rapid mass transfer, and as result a shorter extraction time. According to LAROZE ET AL. (2010), in order to perform the Supercritical CO_2 Extraction from the species *Vaccinium oxycoccus* (Hill) A. Grey), blueberry (*Vaccinium myrtillus* L.) and raspberry (*Rubus idaeus* L.), appropriate work parameters are pressure of 80 – 300 bar at 60°C at CO_2 flow of 2.5 L/h.

The principle of the extraction method that use Pulsed Electric Fields (PEF), is based on electroporation technique, which involves the damage of the walls of the plant cells using electric field, with pulse amplitude within the interval 100V/m – 80 kV/m. Thus, the electric field cause the formation of pores in the cell walls, and target compound located inside the cell is extracted through the pores in the extraction liquid (Barba et al., 2016). The process involves repetitive extraction cycles, with very short cycle duration (l second). BOBINAITÉ ET AL. (2014) propose for extractions from blueberry (*Vaccinium myrtillus* L.) a puls amplitude of 3 kV/cm.

CONCLUSIONS

This study is a short review of the available methodologies destined to quantify the resveratrol from berries. Resveratrol is a phytoalexin, with strong antioxidant capacity, considered valuable for reducing risk of cardiovascular diseases, but it is also presumed to be involved in extension of the life span, by activation of longevity gene. Even though several fruit species (grapes, berries and peanuts), are resveratrol sources, grapes skins and seeds are

mostly studied, in this aim. In order to perform an accurate quantification of resveratrol occurrence in these sources, selecting the appropriate methodology is a critical step.

A large diversity of methodologies is available for obtaining resveratrol from different matrices, including berries. They may be classified in conventional and unconventional methods. The solvent extraction is practiced at large extent, but it has a series of disadvantages, like long duration and pollutant action on the environment, due to the solvents involved. The methods included in the category of non-conventional aim those that use a series of high performance techniques, based on physics methods, as: ultrasounds, microwaves, high pressure, and pulsed electric field. All these methods are environmentally friendly (because small amounts or no solvents are used), short duration, and have high accuracy, but high investments are needed in equipment, which are very expensive.

BIBLIOGRAPHY

AHMAD, K.A., CLEMENT, M.V., PERVAIZ, S., 2003 – Pro-oxidant activity of low doses of resveratrol inhibits hydrogen peroxide-induced apoptosis. Ann. N. Y. Acad. Sci. 1010, pp. 365–373.

BRAVO, A. E., 1996 – Resveratrol in wine: contribution to potential cardiovascular protective activity, Alimentaria, pp.71-72.

BETUL, C., SAIME, B.,YAVUZ, T., NESRIN, K.O., 2012 – Resveratrol: French Paradox Revisited, Front Pharmacol. 3, pp. 141.

BŁASZCZYK, A., S. SADY, M. SIELICKA, 2019 - The stilbene profile in edible berries Phytochem Review, 18, pp. 37–67

DELAUNOIS, B, CORDELIER, S., CONREUX, A., CLÉMENT, C., JEANDET, P., 2009 – Molecular engineering of resveratrol in plants, Plant Biotechnology Journal, 7, pp. 2-12.

FRANKEL E.N., MEYER, A.S., 1998 – Antioxidants in grapes and grape juices and their potential health effects, Pharmac. Biol, pp.14-20.

GÜLÇIN, I., 2010, Antioxidant properties of resveratrol: a structure activity insight. Innov Food Sci Emerg 11, pp. 210–218.

JEANDET, P., CLÉMENT, C., CORDELIER, S., 2019 – Regulation of resveratrol biosynthesis in grapevine: new approaches for disease resistance? Journal of Experimental Botany, 70(2), pp. 375–378.

JEANDET, P., HÉBRARD, C., DEVILLE, M.A., CORDELIER, S., DOREY S., AZIZ, A., CROUZET, J., 2014 – Deciphering the Role of Phytoalexins in Plant-Microorganism Interactions and Human Health, Molecules 19, pp. 18033-18056

LAGOUGE, M., ARGMANN, C., GERHART-HINES, Z., MEZIANE, H., LERIN C., DAUSSIN F., 2006 – Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell, 127(6), pp. 1109–22.

de la LASTRA, C.A., VILLEGAS, I., 2007 – Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. Biochemical Society Trans; 35(Pt5), pp.1156–60.

LEIKI, I., RAY, D., DAS, D.K., 2010 – Longevity nutrients resveratrol, wines and grapes, Genes Nutr. Mar; 5(1), pp. 55–60.

PANNU, N., BHATNAGAR, A., 2019 – Resveratrol: from enhanced biosynthesis and bioavailability to multitargeting chronic diseases, Biomedicine & Pharmacotherapy, 109, pp. 2237-2251.

RHO K.A., KIM M.K., 2006 – Effects of different grape formulations on antioxidative capacity, lipid peroxidation and oxidative DNA damage in aged rats. J Nutr Sci Vitaminol (Tokyo), 52, pp. 33–46

RISULEO G., LA MESA C., 2019 – Resveratrol: Biological Activities and Potential Use in Health and Disease. In: Gupta R., Srivastava A., Lall R. (eds) Nutraceuticals in Veterinary Medicine. Springer, Cham

SCHIFFRIN, E.L., 2010 – Antioxidants in hypertension and cardiovascular disease. Mol. Interv., 10, pp. 354–362

SIMINI B. , 2000 – from French paradox to Cretan miracle. Lancet., 355, pp. 48.

SINGH, R., CHANDRAWAT, K.S., 2017 – Role of Phytoalexins in Plant Disease Resistance, International Journal of Current Microbiology and Applied Sciences, 6(1), pp. 125-129

STIVALA, L.A.M., SAVIO, F., CARAFOLI, P., PERUCCA, L., BIANCHI, L., MAGA, G., FORTI, U.M., MAGNONI, A., ULBINI, E., PROSPERI, V., VANNINI, V., 2001 – Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol,J. biol. chem., p.p. 22586-22594.

VALENZANO, D.R., TERZIBASI, E., GENADE, T., CATTANEO, A., DOMENICI, L., CELLERINO, A., 2006 – Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr. Biol. 16, pp. 296–300

ZORDOKY, B.N., ROBERTSON, I.M., DYCK J.R., 2014 –Preclinical and clinical evidence for the role of resveratrol in the treatment of cardiovascular diseases. Biochim Biophys Acta., 852, pp.1155–1177.

ZÜBEYIR, H., SEKEROĞLU, M.R., BALAHOROGLU, R., HUYUT M.T., 2018 – Characteristics of resveratrol and serotonin on antioxidant capacity and susceptibility to oxidation of red blood cells in stored human blood in a time-dependent manner, Journal of International Medical Research, 46(1), pp. 272–283

WANG, K.H., LAI, Y.H., CHANG, J.C., KO, T.F., SHYU, S.L., CHIOU, R.Y., 2005a – Germination of peanut kernels to enhance resveratrol biosynthesis and prepare sprouts as a functional vegetable. J. Agric. Food Chem., 53, pp. 242–246.

WANG, Z., ZOU, J., CAO, K., HSIEH, T. C., HUANG, Y., WU, J.M., 2005b – Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. Int. J. Mol. Med., 16, pp. 533–540