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

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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 to 5 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 valued. 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 valued (BLASZCZYK ET AL., 2019).

Resveratrol (C14H12O3), 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; GÜLCİN, 2010; HUYUT ET AL., 2018). It is considered as having an antioxidant capacity of 4 up to 5 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; SCHIFFLIN, 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 CHANDRAWAI, 2017). Resveratrol is a compound considered phytoalexin (Pannu and Bhatnagar, 2019). Like for other similar compounds included in this class, also for resveratrol, adaptive mechanisms 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 research. 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 qualitative and quantitative 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.
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 (SNOUSI 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; DEMIROVAN 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 (GÖLMOHAMADI ET AL., 2013), and low quantity of solvents. The
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**BIBLIOGRAPHY**


