
PhD THESIS

Research regarding the use of grape pomace in treating inflammatory diseases

(PhD Thesis Summary)

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INTRODUCTION

Grapes are among the most appreciated fruit in the world. (GARCÍA-LOMILLO and GONZÁLEZ-SANJOSÉ, 2017).

In the wine industry, large quantities of residues are produced in short periods of time, which is why a sustainable winemaking process consists in maximizing resources, associated with increasing residue use and reducing gas emissions. (2019)

These residues are rich in bioactive compounds with important properties for human health. In recent years, antioxidant therapy has gained more and more ground, being recommended as a pathogenetic treatment associated to specific treatments for diseases with associated inflammatory processes.

Based on these considerations, the present study has analysed the extracts of grape mark from Fetească Neagră and Pinot noir were analysed from a phytochemical point of view and the antioxidant anti-proliferative in vitro proprieties were analysed and the anti-inflammatory and cardioprotective effects in vivo were tested.

In this context, the central point of this research was the use of the grape marc of Feteasca Neagră grapes and Pinot noir, varieties cultivated in the Parish Mica, Mures County, Romania, for achieving an efficient extract in human alternative medicine. For these reasons, the use of grape mark can at the same time be an important alternative of ecological and economic approach.

THE STRUCTURE OF THE DOCTORAL THESIS

The paper is structured in two parts: the current state of knowledge and my own contribution, summed up in 127 pages and comprising 6 chapters, with 18 tables, 56 figures, an annex and 161 bibliographic references.

THE PURPOSE AND THE OBJECTIVES OF RESEARCH

This research involved five separate but interconnected studies. The first experience was of a bifactorial type and the following were of a multifactorial type.

The purpose of the research was to analyse the phytochemical composition of the substances with antioxidant effect from the fresh and fermented grape marc extracts from the Pinot noir and Fetească Neagră varieties from “Castel Vinum” Trading Company, commune of Mica, Mureş county, harvest 2015, followed by the evaluation of the in vitro antioxidant activity of grape marc, the anti-proliferative effects in vitro, the antioxidant effects in vivo and on the experimental myocardial ischemia in vivo.

The objectives that were pursued to achieve the proposed goals consisted in the following stages:

Phytochemical evaluation of fresh and fermented grape marc extracts from the analysed varieties:

- Determination of total polyphenols
- Determination of total tannins
- Determination of total anthocyanins
- Determination of phenolic acids and flavonols
- Determination of proanthocyanidins and flavan 3-ols monomers
- Determination of stilbenes.

The evaluation of in vitro and in vivo testing of grape marc from Pinot noir and Fetească Neagră varieties consisted in:

- Evaluation of antioxidant activity in vitro of grape marc extracts
- Evaluation of the anti-proliferative effects of grape marc extracts
- Evaluation of the anti-inflammatory effects in vivo of the extracts of grape marc
- Evaluation of cardioprotective effects of grape marc extracts.

PART ONE THE CURRENT STATE OF KNOWLEDGE IN THE APPROACHED SUBJECT

CHAPTER 1 THE SITUATION OF WINE GROWING

It presents information on the history of wine growing, statistics on the culture of vines, wine and grape production, from which it can be concluded that the wine wastes remain in large quantities if not used, but also important aspects regarding the use of by-products wine.

PART TWO THE CURRENT STATE OF KNOWLEDGE IN THE APPROACHED SUBJECT

In the second part of the doctoral thesis the research materials and methods were presented as well as the results of the five studies performed, as well as the conclusions and recommendations that have emerged from this research.

CHAPTER 2 MOTIVATION PURPOSE AND OBJECTIVES OF THE RESEARCH

Chapter two is dedicated to a detailed description of the purpose and objectives pursued in this doctoral thesis. Details have been provided to gain a better understanding of the motivation with which this research began.

CHAPTER 3 RESEARCH MATERIALS AND METHODS

Chapter 3 presents the research materials and methods used to carry out the proposed research. This chapter is structured in three sub-chapters, as follows:

The biological material used: in this subchapter the two varieties of grapes (Fetească Neagră and Pinot noir), used during the experiments are described.

Location of the area of origin of the grape varieties: includes information on the locations where the five studies were conducted;

The research methods include the methodology followed by the five studies presented in the doctoral thesis, respectively: evaluation of phytochemicals, evaluation of antioxidant activity, anti-proliferative in vitro, methods of evaluation of antioxidants in vivo and evaluation of experimental myocardial ischemia of grape marc extracts from the varieties studied.

CHAPTER 4 RESULTS AND DISCUSSIONS

Chapter 4 presents the results obtained from the research, in 5 subchapters, carried out as follows:

The humidity of the grape pomace

Determining the humidity of the grape pomace samples was important regarding the possibility of reporting the results in dry weight.

Analysis of phytochemical compounds. Compounds such as: total polyphenols, total tannins, total anthocyanins, phenolic acids and flavonols, total proanthocyanidins have been determined and flavan-3-olic monomers, anthocyanins and total tannins, phenolic acids and flavonols and total proanthocyanidins, 3-olan monomers and stilbenes from grape pomace samples.

In vitro oxidant activities of grape pomace extracts

Both the extract of the fresh grape pomace and the fermented grape pomace of the Pinot noir variety have had a very good free radical binding capacity (AA), AA having an increase dependent on the concentration of the extract. Both the extract of the grape pomace and that of the fermented grape pomace the Pinot noir and Fetească Neagră varieties have had a very good free radical binding ability (AA). The extracts from fermented grape pomace from Pinot noir and Fetească neagră varieties had a better in vitro antioxidant activity than the extracts from fresh grape marc. The extracts of Pinot noir grape marc had better in vitro antioxidant activity than those of Feteasca neagra. The results show that Pinot noir and Fetească neagră grape pomace extracts have in vitro antioxidant activity correlated with polyphenol content and recommend their evaluation in vivo in experimental models.

Evaluation of anti-proliferative effects in vitro and in vivo antioxidant effects of grape pommace extracts. In the present study PNP, FNP and FNF reduced the proliferation of the analyzed cells. The inhibitory effect of grape pommace extracts may be due to their high content of anthocyanins, which are known for their chemopreventive effects. Combined treatment of plant polyphenols, such as resveratrol, together with chemotherapeutic treatments could inhibit breast cancer cells more effectively (LUO and others., 2017). FNP, FNF and PNP extracts have had significant inhibitory effect on B164A5 cells, which may be recommended as adjuvants in such tumours. Resveratrol has been shown to have the potential to increase sensitivity to apoptotic stimuli (MIKSTACKA., 2018). The higher content of stilbenes from the fresh extracts of Fetească neagră and Pinot noir may explain the more important anti-proliferative effect of these years compared to those of fermented grape marc. Ethanolic extracts of fresh and fermented grape marc from Fetească neagră and Pinot noir inhibited cell proliferation of A549 cancer cell lines - human lung carcinoma cells, MDA-MB-231 - human breast adenocarcinoma cells and B164A5 - murine melanoma cells may recommend them as adjunctive treatments in chemoprevention. Ethanolic extracts of

fresh and fermented grape marc from Fetească neagră and Pinot noir inhibited cell proliferation of normal HaCat keratinocytes.

Antioxidant effects *in vivo*. Ethanolic extracts of fresh and fermented grape marc from Fetească neagră and Pinot noir have reduced the oxidative stress induced by experimental inflammation by lowering ROS and less by increasing CAT .8.

Effects on oxidative stress were concentration dependent for all extracts tested, respectively higher doses have had more important effects. Ethanolic extracts of Fetească neagră grape marc have reduced oxidative stress more than those of Pinot noir.

Evaluation of the effects of Fetească neagră grape marc extract of and Pinot noir on experimental myocardial ischemia

The present study has performed a phytochemical analysis of the extracts from the grape pomace from Pinot noir and Fetească neagră variety and it was shown that the extracts from the fresh grape pomace and from the fermented grape pomace from both varieties are rich sources of polyphenols and had cardioprotective and antioxidant effects *in vivo*. FNP, FNF, PNP and PNF extracts HAVE had cardioprotective effects against isoprenaline-induced lesions. The strongest antioxidant properties *in vivo* were highlighted in fresh grape marc extracts in both varieties. Based on ECG assessments and serum cardiac markers, the most effective cardioprotective extract was FNP

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

Chapter 5 contains the conclusions and recommendations regarding the researches carried out in accordance with the purpose and the objectives intended. Some of the recommendations of the research carried out are:

1. These results encourage the use of fresh grape marc extracts of Fetească Neagră and Pinot noir as adjunctive cardioprotective treatment for myocardial preconditioning.
2. It is particularly recommended to use fresh Fetească neagră grape marc extract in human conditions that involve oxidative stress, inflammation, cell proliferation and impairment of the cardiovascular system.

CHAPTER 6 ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

It presents the originality and innovative contributions of this doctoral thesis. These are presented as follows:

1. The first performance of the phytochemical analysis associated with the demonstration of the antioxidant, antiproliferative and cardioprotective effects of the extracts of Fetească neagră grape marc grown in Romania.
2. For the first time, a comparative analysis of the composition of the polyphenols, of the antioxidant, antiproliferative and cardioprotective effects of the extracts of Festească and Pinot noir grape marc cultivated in Romania has been performed.
3. The comparative analysis of the composition of the polyphenols, of the antioxidant, antiproliferative and cardioprotective effects of the fresh and fermented grape marc

extracts of Fetească neagră and Pinot noir cultivated in Romania, was performed for the first time.

4. The obtained results bring arguments in order to recommend the use of the grape marc of Fetească neagră and Pinot noir grown in Romania as antioxidant, antiproliferative and cardioprotective adjuvants.

SELECTED BIBLIOGRAPHY

1. CUCCIA P., 2015, Ethics+ economy+ environment= sustainability: Gambero Rosso on the front lines with a new concept of sustainability, *Wine Economics and Policy*, 4(1), 69-70.
2. GARCÍA-LOMILLO, J. și M.L. GONZÁLEZ-SANJOSÉ, 2017, Applications of wine pomace in the food industry: approaches and functions, *Comprehensive Reviews in Food Science and Food Safety*, 16(1), 3-22.
3. LUO, J., S. SONG, Z. WEI, Y. HUANG, Y. ZHANG, J. LU, 2017, The comparative study among different fractions of muscadine grape "Noble" pomace extracts regarding anti-oxidative activities, cell cycle arrest and apoptosis in breast cancer, *Food & Nutrition Research*, 61(1), 1412795.
4. MIKSTACKA, R., M. ZIELIŃSKA-PRZYJEMSKA, Z. DUTKIEWICZ, M. CICHOCKI, T. STEFAŃSKI, M. KACZMAREK, W. BAER-DUBOWSKA, 2018, Cytotoxic, tubulin-interfering and proapoptotic activities of 4'-methylthio-trans-stilbene derivatives, analogues of trans-resveratrol, *Cytotechnology*, 1-14