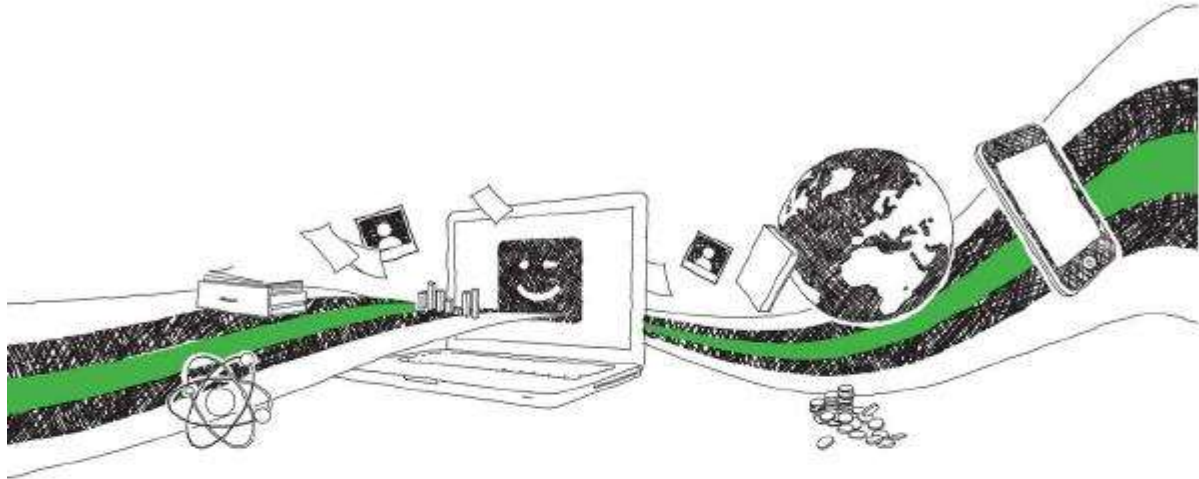


**Violeta Ivanova-Petropulos / Zorana Andonovic
/ Dusko Nedelkovski / Krste Tasev / Klime Beleski**

Application of Grape Pomace as a Natural Food Preservative and Source of Biofuel

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Dusko Nedelkovski, Krste Tasev, Klime Beleski**

APPLICATION OF GRAPE POMACE AS A NATURAL FOOD PRESERVATIVE AND SOURCE OF BIOFUEL



ABSTRACT

Aim of the work

The aim of this work is utilization of the grape pomace piled up as a waste after winemaking and its application in producing biodiesel. Furthermore, a novel yoghurt product was produced, enriched with antioxidants, such as polyphenolics from the grape pomace and berries in order to improve its nutritional value, contributing to food preservation and significantly reducing the risk of diseases. In fact, for the first time we engaged these compounds in preparation of a new yoghurt product, that proved to be last – longer and more beneficial for human health than the traditional one, since the antioxidants play a great role in the prevention of cardiovascular disease, cancer etc. Furthermore, a cost-effective, easily made and environmentally friendly biodiesel was produced, that could represent an alternative to the old fossil petroleum to replace it and resolve the present energy crisis.

Samples and Procedures

Totally, 4 types of Macedonian grape pomace, (from Zupjanka, Prokupec, Kadinal and Vranec varieties) as well as blueberry and aronia, were used. For the extraction of polyphenolics liquid-liquid extraction with ethanol/water/acetic acid, followed by decantation and filtration was used. The phenolic content of the obtained extracts was determined by the Folin-Ciocalteu method (Ivanova et al. 2010); and the total anthocyanins were realized by the Di Stefano et al. (1989) method. Afterwards, three different volumes of the obtained extracts (10, 50 and 100 mL) from each sample were concentrated by rotoevaporation to

dryness. The three different concentrates of each sample were applied on milk together with the lactic bacteria in order to study the influence of polyphenolics during the fermentation; as well as, applied on milk after the fermentation (into the obtained yoghurt). In meantime, the pH value of the newly generated yoghurts was observed by using a pH meter. In the second part the seeds were separated from the grape pomace and served as a new source of oil that was to be transformed into biodiesel. Additionally, blueberry and aronia peels were used for the same purpose. Six organic solutions (ethanol, hexane, benzene, diethyl ether, acetone, acetic acid) were added to the dried seeds and then filtration and distillation followed for obtaining the oil. For dividing the biodiesel transesterification reaction was applied and due to the combustion of the final product it proved to be biodiesel.

Results

The aronia sample was the one containing the highest phenolic content (431 mg/L), while Zupjanka had the lowest content of polyphenols (67.9 mg/L). The grape pomace presenting best results was Prokupec (246 mg/L). All yoghurt samples containing polyphenolics applied before the fermentation, presented higher pH value compared to the control and samples with polyphenolics applied after fermentation. Furthermore, all yoghurt samples containing highest phenolic concentration, showed best results, presenting stable pH value. Zupjanka has shown the best results (pH-4.4 for 1-100) for all concentrations, that is even higher than aronia. After obtaining the yoghurt, sensory analysis was performed, stating that the new product has creamy texture; it tastes good, without unpleasant smell or bitterness. The colour of some samples turned into red-violet, excluding the yoghurt with dry extracts from: Zupjanka and Kardinal

(white grapes). By microbiological analysis the presence of pathogen and other harmful bacteria was proved to be negative, showing that this yoghurt could be a main basis for manufacturing a more beneficial dairy product that could be soon available on the market. Concerning the biodiesel production, the oil content, depending on the solvent used, differed from 0.11 g to 0.36 g. During the extraction acetone and acetic acid showed better results than ethanol, which proved to be a better extractor of antioxidants. Thus, acetic acid was the most proper solvent for obtaining impure biodiesel with a yield of 90.8%. Overall, the sample which showed the highest percentage when mixed with acetic acid was blueberry. On the other hand, from the grape pomace, the type comprising the largest amount of impure biodiesel was aronia with the average yield of 53.46% and then followed: blueberry, Prokupec, Kardinal, Zupjanka and Vranec, respectively.

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1. INTRODUCTION

The geographic location of Republic of Macedonia, the fertile soils and optimal climate conditions are exceptional for breeding vine and specific grape varieties. 35 000 hectares of vineyards with different grape varieties are located at the territory of Macedonia, producing grapes with high quality used for production of high quality wines, red and white. But, the wine industry waste in general is a problem in Macedonia, since it does not have any usage. In the European Union, there is approximately 14.5 million tons of wine industry waste produced from wineries, which is a lot of material that could be recycled and reused for other purposes. In fact, the wine industry waste contains primarily crushed grape skins and seeds rich in beneficial polyphenol compounds that act as antioxidants, antibacterial agents, anticarcinogenic agents, antiviral agents, etc.

1.1 Grape pomace

Grape pomace stands for the solid remains of grapes (skins, stalks and seeds), which are being discarded during winemaking (after grapes are pressed). The largest fraction of winery waste is pomace (Figure 1) which is thrown away ending up in landfills. Fruit processing industries generate tremendous amount of solid wastes which is almost 35-40% dry weight of the total produce used for the manufacturing of juices. During the management of these wastes there is a production of greenhouse gases (GHG) which must be taken into account. Winery leftovers pile up fast and can cause some serious environmental problems if not disposed properly.



Figure 1. Samples of Macedonian grape pomace

1.2 Polyphenols

Polyphenols are secondary metabolites widely distributed in the plant kingdom and the most abundant micronutrients in our diet. Their beneficial effects have been ascribed to their strong antioxidant activity, their ability to scavenge oxygen radicals and other reactive species. These features make phenols a potentially interesting material for the development of functional foods or possible therapy for the prevention of some diseases. As metabolites and antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative illnesses associated to oxidative stress.

The main source of polyphenolic antioxidants is dietary, since they are found in a wide array of foods. For example, most legumes (fruit such as apples, blackberries, blueberries, cantaloupe, cherries, cranberries, grapes (Figure 2), pears, plums, raspberries, and strawberries; and vegetables such as broccoli, cabbage, celery, onion and parsley), are rich in polyphenolic antioxidants. Red

wine, chocolate, green tea, coffee, olive oil, fruit and plant-derived beverages, and many grains and pulses are also good sources.

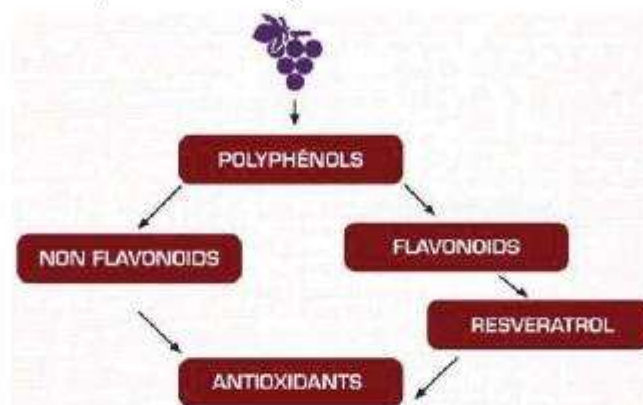


Figure 2. Polyphenols abundance in grapes
(source: <http://www.phenolia.ca/polyphenols>)

Polyphenols are divided into two groups: flavonoids and non-flavonoids (Ivanova et al. 2010; Ivanova et al. 2011). The flavonoids consist of two benzene rings (A and B) linked by an oxygen-containing pyrane ring (Figure 3). This group consists of anthocyanins, flavonols, flavan-3-ols, flavanols. The group of non-flavonoids is composed of phenolic acids (hydroxybenzoic and hydroxycinnamic acids and their derivatives) and stilbenes (resveratrol and resveratrol glucoside). The structure of resveratrol is presented in Fig. 4.



Figure 3. Flavonoid structure

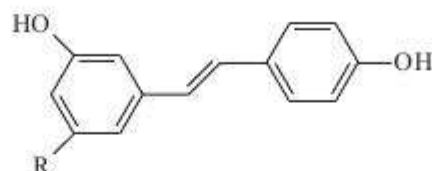


Figure 4. Structure of resveratrol ($R = OH$)

Polyphenols can affect the appearance, taste, mouth-feel, fragrance, and antimicrobial properties of wine. The antioxidant potential of wine is largely attributable to its phenolic composition (Burns et al., 2000).

Since pomace is rich in polyphenols, these material could be used a potential source of antioxidants and their application at different areas (Pecket and Smal, 1980, Montealegre et al. 2006). Awareness is increasing among consumers about the food products that provide health benefits. The increased alertness leads to high acceptance levels and thereby increased demands for functional health products, especially probiotic dairy products. The aim is providing unique and innovative products that do not compromise on quality or performance.

There is a growing interest by the consumers in foods that are recognized as beneficial for human health because they are either low fat, or of greater nutritional value, or contain bioactive materials which are associated with reduced risk of disease. Ideally, these foods should be appealing, taste good, low in price and, most importantly for consumers' acceptability, should contain all-natural ingredients, in a single, easily-accessible product (Petrotos et al. 2012).

1.3 Yoghurt

Yoghurt refers to a product obtained from fermentation of milk by means of cultures of bacteria (*Streptococcus thermophilus* and *Lactobacillus*). For yoghurt production, bacterial cultures are added to the milk to start the fermentation, and to transform the present sugar into lactic acid. The lactic acid then reacts with the proteins in the milk giving the yoghurt hickness and creamy texture. Many yoghurts (Figure 5) provide their benefits by adjusting the microflora (the natural balance of organisms) in the intestines, or by acting directly on body functions, such as digestion or immune function.



Figure 5. Yoghurt

In recent years enormous research has been focused on the functionality of the polyphenols and their application in human health improvement (Mahdavi et al. 2010, Hii et al. 2009). Experimental studies strongly support a role of polyphenols in the prevention of cardiovascular disease, cancer, osteoporosis, diabetes mellitus and neurodegenerative disease (D'Archivio et al. 2007).