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SUMMARY OF PhD THESIS

# Monitoring and valorization of bioactive compounds from elderberries and grape pomace in ripened cheeses

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## SUMMARY OF PhD THESIS

Functional foods are a type of food that not only provides nourishment but also has the ability to enhance health or decrease the risk of disease. These foods are gaining popularity (Beltrán-Barrientos et al., 2016). Furthermore, consumer awareness regarding the impact of food on health is significantly increasing. As a result, numerous brands promoted more nutritious meals, and the production of healthier foods is becoming increasingly prevalent (Palou et al., 2007). Food producers have recently shifted their focus towards dairy products to meet the growing demand. Consequently, there have been several attempts to enhance dairy products, like cheeses, by incorporating functional and antioxidant constituents (Fan and Brzeska, 2016). This is necessary because these meals typically lack bioactive chemicals.

Food commonly incorporates plants, plant mixtures, and even isolated compounds to provide their significant advantages (Carocho et al., 2016). During cheese manufacture, extracts from herbs or medicinal plants enhance the nutritional content of various types of cheese. For instance, Marinho et al. (2015) added rosemary leaves to ripened semi-hard cheese (Marinho et al., 2015). For example, cheese was supplemented with orange carrot powder during the process of milk incubation. The findings revealed that the levels of vitamin A and  $\beta$ -carotene in the cheese samples increased, while the texture and overall acceptance diminished as a result of a deficiency in finely ground carrot powder (Roy, 2018).

*Sambucus nigra* is the scientific name for the black elderberry plant. *Haschberg* and *Sambucus nigra* L. are very adaptable shrubs that are native to the northern hemisphere (Martiş et al., 2021). They are commonly referred to as the standard cultivars of elderberry and European wild black elder, respectively. These shrubs belong to the Adoxaceae family under the order of the Dipsacales. The study of the primary bioactive compounds and antioxidative properties of various cultivated and wild plants of *Sambucus nigra* L. is of significant interest in multiple fields, such as horticulture, the food industry and the pharmaceutical industry.

Researchers recently discovered that elderberry (*Sambucus nigra* L.) is a significant anthocyanin-rich berry. Elderberry and its preparations (extracts, powders, etc.), which contain high levels of anthocyanin, primarily cyanidin glycosides (Marziliano et al., 2021) can be employed as colouring agents in a variety of foods (Najgebauer-Lejko et al., 2021). As a results, there is a growing interest in using elderberries as a functional ingredient and natural additive in food products. Traditional medicine has used elderberries as a medicinal plant for millennia due to the presence of anthocyanins, known phytochemicals that promote human health (Nile et al., 2016). However, it should be mentioned that elderberries contain cyanogenic glycosides, which are somewhat poisonous and cause vomiting, however, this toxicity can be reduced by cooking (Domínguez et al., 2021). Sambunigrin,

prunasin, m-hydroxysubstituted glycosides, zierin, and holocalin are some of the most common cyanogenic glucosides (Kammerer et al., 2007). Nonetheless, the berries have the lowest levels of these poisonous chemicals when compared to the cyanogenic glucosides found in leaves or flowers. The food industry uses elderberries to make pies, jellies, jams, ice creams, yoghurts, and alcoholic beverages (Jeon et al., 2021; Pascariu and Israel-Roming, 2022).

Grape berries (*Vitis vinifera* L.), are utilized in the winemaking sector for making alcoholic beverages through the process of pressing the berries and subsequently fermenting the resulting liquid. The primary by-products produced during the winemaking process consist of a combination of grape seeds and skins, known as grape pomace. Grape pomace typically accounts for around 20 - 25% of the harvested grapes (Theagarajan et al., 2019). The wine business generates a substantial amount of grape pomace, resulting in significant environmental and economic consequences (Beres et al., 2017). The by-products possess high levels of antioxidant polyphenols (anthocyanins) and dietary fiber, which have the potential to positively impact human health. These benefits include antioxidant activity, antibacterial and anti-inflammatory capabilities, as well as possible anticancer and cardiovascular impacts (Fontana et al., 2013; Rațu et al., 2023a, 2023b; Reham et al., 2023;).

Nevertheless, in response to growing consumer demand for functional foods, grape pomace in the form of a powder, has been incorporated into meals that have low levels of polyphenols and dietary fiber. This addition aims to enhance the functional and nutraceutical qualities of these foods. The bioactive components present in wine grape pomace have a wide range of potential uses, such as in functional foods, cosmetics, pharmaceuticals, and supplements (García-Lomillo et al., 2017; Anduaem, 2023). Previous studies have suggested the use of wine grape pomace flours or extracts in various food products such as bread, pasta, yoghurt, cheese, and meat products (da Silva et al., 2017; Marchiani et al., 2016a). Utilizing grape pomace powder as a component in food products serves multiple purposes, including promoting environmentally-friendly production methods, reducing the expenses associated with treating by products, generating additional revenue for grape producers, and attracting consumer attention towards healthier food options. Moreover, incorporating grape by-products into food products could serve as an innovative approach to creating functional foods (that must be safe for nutrition) that contain antioxidants derived from natural sources (Helkar, 2016; Alshaikh et al., 2023).

Organic acids, reducing sugars, amino acids, polypeptides, nucleotides, and other small compounds are the primary constituents of non-volatile aromatic substances. Ripened cheeses are an especially abundant source of these molecular components (Zheng et al., 2021). All of the above elements are by-products of the fermentation and metabolic processes carried out by the microbiota present in the cheese. They have a direct impact on both the taste and the texture of the final product (Mayo et al., 2021; Tahir et al., 2023). The sensory attributes of cheese play a crucial

role in determining the overall eating experience and consumer satisfaction (Drake and Delahunty, 2017). The specific structure and composition of cheese simultaneously activate all human sensory pathways, resulting in a single perception that a consumer experiences during and after cheese. The use of objective sensory science procedures has led to advances in understanding the correlations between these components and cheese's sensory characteristics (Drake and Delahunty, 2017).

According to the main goal of this study, which is to find out how adding elderberries and grape pomace affects the properties of cheese, this study aims to fill in a gap in our knowledge by collecting and analyzing a wide range of samples of cheeses that have been enhanced with different amounts of elderberries and grape pomace. Simultaneously, it intends to systematically evaluate the antioxidant and antibacterial efficacy improvements conferred by these additions throughout the cheese maturing process. This multifaceted approach is very important for figuring out the complex roles that these bioactive components play in improving the quality of cheese. This could lead to new ways of making cheese that improve food safety, make it last longer, and give it better nutrition, all of which are things that modern consumers want in functional foods that are also good for them.

This study aimed to explore the use of grape by-products such as red grape pomace and elderberry, in the form of bioactive powders to obtain three-month old cheese assortments. Powders obtained from red grape pomace (GPP) and elder fruit (EFP) contain antioxidant components such as phenols, anthocyanins and flavonoids, compounds characterized by high bioactivity. This study investigated not only the potential of incorporating different percentages (1%, 2%, and 3%) of GPP and EFP in cheese, to increase its nutritional value, but also the changes that occur during the 2 months of ripening. The results indicated that the incorporation of GPP and EFP led to improvements in the nutritional composition such as phenolics (5.201 - 6.62 mg GAE/g DW), anthocyanins (3.389 - 4.748 mg C3G/g DW), and flavonoids (0.398 - 1.246 mg CE /g DW). The specific carbohydrates in cheese are lactose and galactose. The amount in carbohydrates of ripened cheeses was lower than carbohydrate amounts in fresh cheeses. The organic acids identified were citric acid, tartaric acid, succinic acid and fumaric acid.. Succinic acid represents the largest amount. Regarding the general perception, the cheese sample with 1% pomace and elderberry was the most appreciated. From a microbiological standpoint, the cheese did not undergo any significant undesirable changes. The use of GPP and EFP as bioactive powders in cheese presents a promising strategy to reduce food waste and create innovative, value-added dairy products. The development of such products can not only contribute to sustainable food production, but also provide consumers with more diverse food choices with improved characteristics.

**Key words: By-products, cheese, fatty acid, carbohydrates, organic acids, sensory analysis**