

**Studies on the transformations and biological properties
of ellagitannins in plants of the *Rosaceae* family**

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Abstract

Berries of the *Rosaceae* family are a valuable source of ellagitannins, anthocyanins and dietary fiber. The low shelf life of berries means that they are increasingly frozen and processed. Processing involves the creation of waste by-products, i.e. – pomace.

The main goal of the work was to obtain and expand knowledge about the transformations and biological properties of ellagitannins (ETs) isolated from pomace of selected fruits from the *Rosaceae* family, i.e. raspberries, blackberries, strawberries and wild strawberries. An equally important goal was to determine the degree of interaction of ETs with food proteins and the impact of complexation with gelatin on the recovery of these polyphenols in conditions simulating, from a chemical point of view, the digestive system. An innovative element was to determine the impact of the addition of raw raspberry extract on selected properties of pork and its products.

The theoretical introduction presents the importance of berries, the characteristics of ETs and methods of isolating these compounds. Next, the state of knowledge on the chemical transformations and biological properties of ETs and their interactions with selected food ingredients are presented.

The experimental part characterized the materials and presented the methods used in the research work.

In the first stage, unclarified juices and purees were produced from the tested fruits, which were intended to analyze the stability of ETs in storage conditions of various temperatures (-20, 4 and 20°C±2°C) and time (0 - 12 months). The fastest reduction in ETs concentration was recorded during the year, at the highest temperature tested. Moreover, higher stability of ETs was demonstrated in juices than in purees derived from raw materials of the *Rubus* genus. In turn, in the case of products based on raw materials from the *Fragaria* genus, ETs in strawberry products were more stable than in wild strawberry products.

The study showed that pomace from the production of juices and purees is a rich source of ETs, including oligomers. Therefore, crude polyphenol extracts with a high content of ETs (from 1.5 to over 19 g/100 g of dry matter) were obtained from the pomace of the tested fruits. The extracts were further purified on an XAD Amberlite 1600 N adsorption resin and purified using Size Exclusion Chromatography (SEC). The procedure used allowed for the effective separation of ETs from anthocyanins and partially from flavanols. In 100 g of freeze-

dried extracts with the highest degree of purification, ETs, anthocyanins and flavanols were determined in amounts of 12 – 74 g, 0 – 0.5 g and 4 – 18 g, respectively.

In the next stage, the transformations of ETs were determined in freeze-dried raw and purified extracts during one-year storage in various conditions, in 4 variants, i.e. –20, 4, 20 (light), 20 (dark)°C ($\pm 2^\circ\text{C}$). In most of the cases considered, the highest ETs losses were recorded for the highest tested temperature with access to light. Moreover, the degree of purification of the extract was a factor influencing the stability of ETs. In the raw extracts, after 12 months, the initial concentration of ETs decreased by 6 – 29%, while in the purified extracts it was from 1 to 37% for 20°C in the dark and from 12 to 70% in the light. Ellagitannins remained stable during 24-hour exposure to UV-C radiation, regardless of the degree of purification of the extract and the type of fruit.

The next element of the work was the assessment of ETs transformations in aqueous solutions under different pH conditions (3 - 8) and temperature (20 - 80°C $\pm 2^\circ\text{C}$). Purified raspberry and strawberry extracts were selected for the study, being a source of oligomeric ETs and lower molecular weight compounds, respectively. It was shown that high temperature reduced the concentration of ETs, especially at high pH. At lower pH, temperature was less important. In most variants of raspberry extracts, the main transformation product was dimeric sanguin H-10. In turn, the solutions after incubation of strawberry extracts were a complex mixture of lower mass compounds, including bis-HHDP-glucose, gallolyl-HHDP-glucose, agrimonic acid and sanguin H-2 without the gallic acid residue. For all tested extracts, high temperatures combined with high pH resulted in an increase in the share of ellagic acid and its conjugates (EAC) already in the first hours of incubation.

Studies on the stability of ETs from raspberry pomace in solutions with varying degrees of oxygenation have shown that the concentration of these compounds decreases in each analyzed system. After 14 days of incubation of the extracts with oxygen and without aeration, the concentration of ETs and EAC was more than two and four times lower compared to the initial extract. In turn, ETs isolated from strawberry pomace remained stable throughout the aeration period.

In a further step, the interactions of ETs with food proteins were determined in various matrices (buffer solutions with pH 3, 5, 7; water; sodium chloride; sucrose). The protein that most strongly bound ETs, regardless of the matrix, was gelatin (10 - 99% binding). Interactions with other proteins depended on the composition of the matrix. Raspberry ETs combined with

proteins best in a sodium chloride environment (up to 99%) and least strongly in an aqueous environment (<10% for most proteins). In turn, strawberry ETs combined well in water (20 – 59%), and least well in the environment with added sugar (0 – 14%). The dissolved complexes showed lower values of antioxidant potential (DPPH radical inhibition value ranging from 1 to 36%) compared to their hydrolysates (DPPH from 1 to 67%). Additionally, the influence of complexation with gelatin on the stability of ellagitannins in conditions chemically simulating the digestive system was determined. Uncomplexed ETs were stable in most tested conditions, i.e. at pH 1.2 and 7.5 and in the presence of pepsin. The exception was incubation with intestinal enzymes, where the concentration of ETs was reduced by 80 - 90%. In the sediments after interactions with pancreatin, 15 and 60% EAC were determined, respectively, for extracts from raw materials of the *Rubus* and *Fragaria* genera. High recoveries of EAC in sediments after interactions with pancreatin (15 – 60%) may indicate a strong affinity of ETs for enzymes.

The antagonistic properties of ETs towards pathogenic strains contaminating food were determined. Eight extracts with the highest concentration of ETs after purification using the SEC method were used for the study. The extracts showed antibacterial potential at a concentration of 200 mg/mL against bacteria of the genus *Listeria* spp. and 60 mg/mL against bacteria of the genus *Escherichia* spp., *Salmonella* spp. and *Staphylococcus* spp. The minimum inhibitory concentration (MIC) ranged from 0.391 to 12.5 mg/mL, with no difference noted for Gram-negative and Gram-positive bacteria. The extracts also had properties that inhibited the formation of biofilms by *Listeria innocua*.

An innovative element was the application of raw raspberry extract, rich in ETs, to pork processing products. In this work, the problem of the efficiency of extraction of ETs from meat matrix was presented for the first time. In all meat products, unextracted ETs ranged from 20 to 49%. The extraction residue contained ETs, most likely permanently bound to proteins or peptides. Thus, it was proven that the low recovery of ETs was not only due to the thermal degradation of these compounds.

Next, 54 meat products enriched with raw raspberry extract rich in ETs were prepared. The variants were differentiated in terms of the dose of extract and pickling salt as well as thermal treatment. The addition of the extract improved the red color and antioxidant properties in most of the variants considered, regardless of the type of thermal treatment used. The addition of raspberry extract did not affect the juiciness, tenderness and overall

attractiveness of smoked sausages with a reduced dose of pickling salt. However, the complete lack of pickling salts had an impact on reducing the acceptability of sausages. The results of the presented research indicate a high potential for the application of raw extracts rich in ETs obtained from waste by-products.