

Color is an intrinsic attribute of food that affects the selection and perception of food taste. However, many foods are visually altered during storage, so coloring agents are an integral element in food technology. Enhancing the color intensity of foods is often done by adding appropriate substances. In response to the challenges of modern food industry, new colorants, preferably based on natural compounds, are being sought. Unfortunately, despite their lack of toxicity, high coloring power, and their health-promoting properties, many of them have low stability, which limits their potential applications.

Betalains are purple-red (betacyanins) or yellow-orange (betaxanthins) water-soluble pigments found in plants, e.g. red beet (*Beta vulgaris* L.), pitaya fruit (*Hylocereus polyrhizus*), prickly pear (*Opuntia ficus indica*), but also in some fungi, i.e. red toadstool (*Amanita muscaria*). Their consumption can reduce the risk of certain diseases, but also add aesthetic variety to the daily diet.

Recently, products that result from the coupling (conjugation) of natural betalain pigments, isolated from plant sources, with compounds containing the -SH (sulfhydryl) moiety, such as cysteine and glutathione have been synthesized. Such derivatives have been characterized by different colors, which gives them a chance to be used as food additives, but so far, they have not been tested, both chemically and biologically. It is anticipated that such a reaction may contribute to better stability and bioavailability, compared to the starting betalains. A thorough investigation of the products is all the more important because analogous derivatives of other natural compounds reacting similarly with glutathione and cysteine have been found to be produced *in vivo*.

Therefore, this Project focuses on studying the stability and biological properties (antioxidant, cytotoxic, anti-inflammatory) of novel betalain pigment conjugates with sulfhydryl radical scavengers. Starting betalains required to produce the conjugates will come from the root of red beet (*B. vulgaris* L.) and the fruit of Malabar spinach (*Basella alba* L.). These plants contain large amounts of betanin and gomphrenin, respectively, which are representatives of the two main groups of betacyanins. The coupling reaction will be carried out in the presence of an oxidizing agent since conjugation occurs on intermediate forms formed during oxidation of betalain pigments. Comprehensive evaluation of antioxidant properties will be performed using chemical assays, electrochemical techniques, as well as bioassays against three cell lines derived from the human gastrointestinal tract – from the stomach, liver, and colon. Anti-inflammatory activity will be tested by ELISA.

Research on betalain stability will be performed using chemical assays to investigate the effects of degrading factors such as temperature, pH, the presence of metals, chelating agents, oxidants, and antioxidants. The oxidized derivatives will furthermore be produced on a larger scale for full characterization, as the reaction of their formation is a very important part of the degradation processes. In addition, the stability of conjugates in simulated body fluids is derived from three parts of the digestive system (salivary, gastric, and intestinal fluids) and will be also investigated to determine the behavior of the newly formed pigments during digestion.

The results obtained in this Project will expand the state of knowledge on novel compounds and contribute to the multidirectional characterization of alternative coloring agents for food applications. The compounds can be a novel component for functional foods, nutraceuticals, and dietary supplements while giving an interesting color to food. In addition, they could potentially exhibit antioxidant and anti-inflammatory properties, and enrich the daily diet, with valuable compounds that protect against the development of various diseases.