Phenolic compounds from spent hops as inhibitors of glycolytic enzymes.

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Abstract

Diabetes is one of the major health problems in the world, as it is a common disease and has even been considered an epidemic. One of the hallmarks of this disease is high blood glucose (hyperglycemia), which is caused by a defect in the production or action of insulin. One strategy for lowering blood sugar levels in people with type 2 diabetes is to reduce the amount of glucose released from a meal by inhibiting the digestive enzymes that break down carbohydrates, namely α-glucosidase and α-amylase. Due to the occurrence of side effects caused by currently used conventional drugs, it is advisable to search for alternative inhibitors of glycolytic enzymes of natural origin. Plant natural products including polyphenols have been reported to exert such an anti-diabetic activity.

In this work, the CO_2 supercritical extraction of hop cone residue (spent hops), was used as a source of phenolic compounds. The use of a waste product is part of the current trend of reducing the generation of waste by using it as a starting material for the creation of new products. In terms of inhibitory activity towards α-glucosidase and α-amylase, the hops extract and its fractions obtained after separation on the Sephadex LH-20 were examined for their inhibitory activity against α-glucosidase and α-amylase.

The research hypothesis was put forward that the inhibitory properties of phenolic compounds derived from hops depend on the source of glycolytic enzymes and the type of substrates used. In order to verify the above assumption, the anti-amylase activity was tested using three sources of the enzyme: human saliva, human and porcine pancreas in a model with five types of starch. The inhibitory effects of spent hops on α-glucosidase were determined for enzymes derived from *S. cerevisiae* yeast and rat intestines, using five substrates, including two synthetic and three natural substrates. In this work, for the first time, such a variety of enzymes and substrates was used in research on the search on the glycolytic enzyme inhibitors. Additionally, an important aspect of the study was to determine the quantitative and qualitative composition of the extract and its fractions using the UPLC-PDA-Q/TOF-MS method in order to identify the phenolic compounds responsible for the observed biological properties.

The first stage of the research consisted in the separation of phenolic compounds contained in the hops extract on the Sephadex LH-20 with the use of aqueous solutions of methanol with its increasing proportions (from 25 to 80%) and 70% of acetone. Seven fractions were separated based on the spectroscopic data obtained for the eluted samples. The next step involved determining the content of phenolic compounds in the spent hops extract and its fractions using spectrophotometric and chromatographic methods. The obtained results indicate the separation of phenolic compounds present in the spent hops extract, as evidenced by the obtained data regarding the qualitative and quantitative composition of phenolic compounds, as well as the determined values of the mean degree of polymerization of proanthocyanidin. An important aspect of the study was determining the phenolic profile of the extract and fractions, in which 97 phenolic compounds were identified, including

hydroxybenzoic acids (3 compounds), hydroxycinnamic acids (9 compounds), flavonols (28 compounds), stilbenes (1 compound), flavan-3-ol monomers (4 compounds), proanthocyanidins (51 compounds), and other polyphenols (1 compound). It should be emphasized that 11 phenolic compounds were described for the first time as compounds present in hop cones.

The main part of the research focused on determining the anti-diabetic potential of the spent hops extract and its fractions. This potential was evaluated using in vitro methods that measured the inhibitory efficacy against α-glucosidase and α-amylase activities from various origins. α-Glucosidases were investigated using the following substrates: 4-nitrophenyl-β-D-glucopyranoside (*p*NPG), 4-methylumbelliferyl α-D-glucopyranoside (MUG), maltose, sucrose, and dextrins. As for α-amylases, five types of starch were used i. e.: potato, wheat, corn, rice and tapioca starch. The antioxidant potential of the tested samples was also determined, including their ability to scavenge free radical cations ABTS•+ and peroxyl radicals, due to the excessive generation of reactive oxygen species in a state of chronic hyperglycemia. The obtained results indicate that the spent hops extract is a rich source of phenolic compounds, particularly glycosylated flavonols, monomeric flavanols, and proanthocyanidins. A significant variation in the inhibitory properties of the spent hops extract and its fractions was observed depending on the enzyme origin and the substrate used. It has been demonstrated that glycosylated flavonols, monomeric flavanols, and proanthocyanidins are likely active inhibitors of mammalian α-glucosidase. In turn, the inhibitory properties of the spent hops extract against the tested α-amylases are mainly attributed to proanthocyanidins. Furthermore, the spent hops extract and its fractions exhibited high antioxidant potential.

The results presented in this study have demonstrated the anti-diabetic properties of spent hops extract based on the inhibition of glycolytic enzymes responsible for carbohydrate digestion and glucose release. In addition, the significant variation of the results depending on the measurement system used indicates the need for standardization of methods for determining the aforementioned activities, particularly when studying complex plant extracts. Spent hops extracts could be an excellent raw material for enriching functional food or pharmaceutical preparations as dietary supplements for individuals with type 2 diabetes. However, further research confirming the anti-diabetic effects of spent hops and ensuring the safety of its use is necessary.