



Research Article

Gábor Lehoczki, Kármén Szabó, Lili Kandra, Gyöngyi Gyémánt*

Inhibition studies on α -amylase using isothermal titration calorimetry

<https://doi.org/10.1515/amylase-2018-0002>

Received March 1, 2018; accepted May 3, 2018

Abstract: The control of postprandial blood glucose level via the inhibition of α -amylase is a relevant strategy for the treatment of type 2 diabetes. Several antidiabetic plants are known but there is no information about their α -amylase inhibitory activity. This in vitro study tries to reveal the answer. Hot water extracts of 58 medicinal plants and spices were examined. Activity measurements of human salivary α -amylase (HS α A) on 0.14 m/v % starch substrate was carried out by isothermal titration calorimetry in the presence or absence of plant extracts. Water soluble antioxidant capacity of each extract was measured with photo-chemiluminescence method. Results have confirmed the inhibitory activity of several plant extracts against HS α A. The green tea, cinnamon and allspice, furthermore leaves of blackberry, raspberry and strawberry deserve particular mention ($IC_{50} \leq 1.2$ mg/mL). A few extracts had significant water-soluble antioxidant capacity compared to ascorbic acid and a weak correlation was recognised between the obtained IC_{50} and antioxidant capacity values. Inhibition of amylases located in digestive system can be reached via daily intake of most active extracts. These plants could be inserted effectively into a diabetic diet as food supplements.

Keywords: isothermal titration calorimetry; α -amylase; plant extracts, enzyme inhibition; food supplement

Abbreviations: HP α A, human pancreatic α -amylase; HPLC, high performance liquid chromatography; HS α A, human salivary α -amylase; IC_{50} , half maximal inhibitory concentration; ITC, isothermal titration calorimetry; MALDI TOF, matrix assisted laser desorption ionisation time of flight.

*Corresponding author: Gyöngyi Gyémánt, Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4002 Debrecen, Hungary; E-mail: gyemant@science.unideb.hu

Gábor Lehoczki, Kármén Szabó, Lili Kandra, Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4002 Debrecen, Hungary

1 Introduction

α -Amylases (α -1,4-D-glucan glucanohydrolases; EC 3.2.1.1) are classical calcium-containing enzymes, which constitute a family of endo-amylases catalysing the cleavage of α -1,4-D-glycosidic bonds in glycogen, starch and related carbohydrates with retention of α -anomeric configuration in the products [1]. They play a dominant role in carbohydrate metabolism in microorganisms, plants and higher organisms. From 1991, when the sequence-based classification of all glycoside hydrolases, transferases and isomerases was established [2], the α -amylase family has been known as the family 13 of glycoside hydrolyses. The α -amylase family enzymes carry strictly conserved three essential catalytic residues: Asp206 – catalytic nucleophile, Glu230 – proton donor, Asp 297 – involved in the substrate binding (Taka-amylase A numbering) [3].

In humans, α -amylase is one of the major secretory products in pancreas and salivary glands playing a role in digestion of starch and glycogen. Subsite structure of human salivary α -amylase (HS α A), containing four glycone and three aglycone binding sites, was determined earlier in our laboratory [4]. Primary structures of HS α A and human pancreatic α -amylase (HP α A) are highly homologous and this sequence identity results in structural and functional similarity [5].

Due to the rapid spread of diabetes and obesity, significant attention has been paid to the new α -amylase and α -glycosidase inhibitors during the last decades. One group of oral anti-diabetic agents is represented by the enzyme inhibitors, which slow down the absorption of glucose and reduce postprandial glucose level by inhibiting digestive enzymes [6]. Since the enzymes to be inhibited are present in the digestive system, the intake of inhibitors with food may be one of the possible ways of therapy. According to a review by Sales et al. [7], approximately 800 plant species have been shown to have a smaller or greater antidiabetic effect and the number is still rising. For most plants, the mechanism responsible for antidiabetic activity has not been studied

so far. Most of the analysed plant extracts were obtained by solvent extraction, mainly alcoholic (methanol [8], ethanol [9]), chloroform [10] or hexane [11] extraction. By these methods, the apolar and slightly polar components can be extracted, such as their typical and frequently studied representatives, the different plant colorants and secondary metabolites. The extraction of polar constituents is usually carried out using buffer [12] or aqueous alcohol [13] as solvents. In these cases, the extracts contain mainly various glycosides, organic acids and their derivatives or proteinaceous substances [14].

α -Amylase inhibition by natural compounds, such as tannins [15-17] or anthocyanins [18], has been studied in our group earlier using a short chromogenic substrate for activity measurement. In comparison with the results obtained on short and natural (starch) substrate, we experienced a magnitude difference between the calculated inhibition constant values. The methods used for α -amylase activity measurements on starch are often labour intensive. Some of them have insufficient sensitivity and require boiling or heating steps for colour development causing practical difficulties of usage in high-throughput screening assays. Therefore, there is a demand for a more effective method to measure inhibitory activity of plant extracts on amylase enzyme using starch substrate, and isothermal titration calorimetry (ITC) can be the method of choice.

As a result of the continuous development of instrumental analytics, such classical methods as calorimetry started to spread again. Although, the main application of microcalorimetry is the investigation of molecular interactions, it is also used to determine kinetic parameters in enzyme catalysed reactions [19] or examine the metabolic activity of living cells [20]. Since every physical or chemical change is followed by heat change, ITC can be considered as a universal detector [21]. Interactions between tea polyphenols and porcine pancreatic α -amylase have been determined through thermodynamic analysis using ITC method in binding experiments [22]. We have published recently an ITC-based method [23], which can be used economically for both enzyme kinetic and inhibition studies. In our experiments, a well-known hydrolysis reaction, HS α A catalysed digestion, was used as a model system.

In the present study we applied the same method for the inhibition measurements of different plant extracts. We selected plants from the most commonly used spices and herbs recommended for the treatment of diabetes in folk medicine and some other widely known herbs native to Central Europe. We hypothesized that some antidiabetic plants exert their blood sugar lowering effect through

inhibition of α -amylase enzymes of gastrointestinal tract, namely HS α A and HP α A. Plant extracts were used in a concentration range of 0-15 mg/mL and their half maximal inhibitory concentration (IC_{50}) values and maximum inhibitory activities were determined.

2 Materials and methods

Substrates, enzymes and buffer components were purchased from Sigma Aldrich (St. Louis, MO, USA) and Serva (Serva GmbH, Heidelberg, Germany). Since the commercial HS α A (Type IXA, lyophilized powder, 1,000-3,000 units/mg protein) was stabilized with ammonium-chloride, it was purified using Amicon 30K (Millipore, MA, USA) centrifugal filter unit. The activity of the buffer solved enzyme was adjusted to the requirement of the ITC measurement.

2.1 Water extraction of plants

The dried raw plant materials were purchased from Herbária (Budapest, Hungary), which manufactures every product according to Hungarian Pharmacopoeia (Ph. Hg. VIII). The hard parts of plants (bark, root) (5 g) were cooked for 30 minutes in 100 mL water, then cooled to room temperature. The infusion method (common method of making tea) was used for extracting leaves and flowering tops scalded them in boiling water and cooled to room temperature in covered beakers. The infusions were centrifuged at 10,000 rpm for 10 minutes, then the residual solid materials were removed using regenerated cellulose filter with pore size of 0.2 μ m (Phenomenex). The obtained clear infusions were freeze dried and stored at -20 °C. Having measured the residual solid materials, the water-soluble dry-matter content was calculated related to the weight of starting substance. Data are summarised in Table S1 as yields.

2.2 Inhibition measurements

For the inhibition studies, MicroCal ITC₂₀₀ microcalorimeter (Northampton, MA, USA) equipped with sample cell (204 μ L) and injector (40 μ L) was used. We published the applied method earlier [23]. Briefly, the sample cell contained both starch (1.4 mg/mL) and inhibitor solutions diluted with 50 mM MES buffer, pH=6.2. Each measurement started with an injection of 2 μ L of HS α A (1.1 μ M), then the occurring heat change was defined. The obtained raw data were evaluated with the Origin software (Origin7 SR4, OriginLab Corp., USA) supplemented with

ITC upgrade. The IC_{50} values were calculated using the logarithmic dose-response curves, considering that 100% activity is the reaction rate obtained with an uninhibited sample. Each measurement was carried out at 37 °C.

2.3 Water soluble antioxidant capacity

The water soluble antioxidant capacity was measured with ACW method [24] using PHOTOCHEM® equipment (Analytik Jena AG, Jena, Germany) and the official ACW kits. Ascorbic acid calibration was used in the concentration range of 0.2-3.0 nM. Each stock sample solution was made from 2 mg lyophilised plant extract dissolved in 1 mL distilled water, then diluted further until the obtained antioxidant capacity value got into the calibrated range. The obtained curves were evaluated with the manufacturer's software (Analytik Jena AG, Jena, Germany).

2.4 Mass spectrometry

Mass spectra of plant samples were obtained in positive-ion mode using a Biflex III matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometer (Bruker, Billerica, Massachusetts, USA) equipped with delayed-ion extraction. Desorption/ionisation of the sample molecules was affected with a 337 nm nitrogen laser. Spectra from multiple (at least 100) laser shots were summarised using 19 kV accelerating and 20 kV reflectron voltage. External calibration was applied using the $[M+Na]^+$ peaks of cyclodextrins with degree of polymerization 6-8, m/z : 995.31, 1157.36, 1319.41 Da, respectively. The samples were dissolved in water and measured in 2,5-dihydroxy benzoic acid matrix. Ten μ L sample and 10 μ L matrix solution were mixed, then 0.5 μ L was applied to the sample target and allowed to dry at room temperature. The calculated values used for comparison were derived from IUPAC exact isotopic weights using XMASS 5.0 software from Bruker.

3 Results and discussion

In our previous paper it has been already proven that ITC is a suitable method for enzyme inhibition assay providing good results for a well-defined system [23]. On the basis of this experience we have investigated aqueous plant extracts with such physical features or effect (e.g., colour, turbidity caused by protein aggregation) that make not easy to apply the conventional activity measurement methods.

Our aim was to model the dietary intake of plant components and their effects on enzymatic hydrolysis of starch. For this purpose, the pre-boiled starch of 0.14% (the K_M value of starch-HS α A reaction) was used as substrate and hot water extracts of plant materials (infusion) as inhibitors. Some of the tested plant extracts, including onion, mustard seed, nutmeg, dandelion, nettle, mallow, white bean, rhubarb, coneflower and galega, did not show any inhibitor activity.

In four cases (onion, mustard seed, nutmeg and white bean pod), a higher reaction rate was observed compared to the control value, which was presumably caused by the significant amount of cleavable glycosidic bonds of the components in the aqueous extract. The presence of maltooligomers was verified in the cases of onion, coneflower and dandelion by MALDI TOF mass spectrometry (Fig. S1-S3). Starch content of mustard [25], white bean pod [26], nutmeg [27] and onion [28] were published earlier. Extracts of parsley, coriander, anise, spinach, lovage, chervil and garlic reduced the reaction rate but 50% inhibition was not achieved in the concentration range tested. The plants, for which IC_{50} values were successfully calculated, were divided into two groups: spices and medicinal herbs.

IC_{50} values of spices are shown in Figure 1 (see also Table S1). Cinnamon and allspice proved to have the lowest IC_{50} values of 0.6 and 0.8 mg/mL, respectively. These two spices completely inhibited HS α A enzyme in higher concentration. The maximal inhibition was above 90% using clove and oregano, but IC_{50} values were higher.

The rate of starch hydrolysis was decreased by 25 of the 33 examined medicinal herbs; IC_{50} values are illustrated in Figure 2 and summarised again in Table S1.

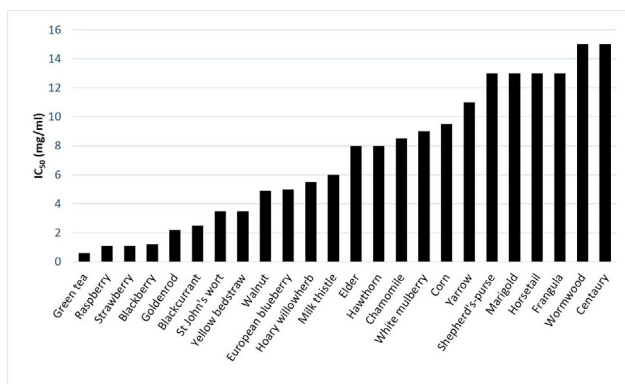


Figure 1. IC_{50} values of spices having inhibitory effect on HS α A-catalysed starch hydrolysis reaction.

Green tea showed the highest inhibitory activity resulted in IC_{50} value of 0.4 mg/mL. Leaf extracts made from

different kinds of berries were able to reduce the enzyme activity by more than 90% resulted in IC_{50} values of 1.1-1.2 mg/mL. According to the data blackcurrant and golden rod also exhibit a significant HSαA inhibitory potency but the reduction of enzyme activity to 50% needs higher concentrations.

Some of the herbs tested in this project have been proven to be α-amylase inhibitors. Cinnamon extracts inhibited PPαA [29] and HSαA [30] during *in vitro* experiments. They were effective in rat starch tolerance test and also in human clinical trials as antidiabetic agent [31]. Similarly, inhibition of α-amylase by green tea extract has been published recently [22, 32-33]. Based on these results cinnamon was selected as a positive control for spices and green tea as a positive control for medicinal herbs. Indeed, these samples showed the best inhibition in our experiments; IC_{50} values were similarly low (Table 1).

IC_{50} of allspice extract is close to cinnamon's value, furthermore clove and oregano have also unequivocal inhibitory potency. In case of medicinal herbs, leaves of different berry species have similar IC_{50} values (1.1 mg/mL). Two-fold concentration of goldenrod or blackcurrant extract was demanded to 50% inhibition.

We were able to identify some compounds using MALDI TOF mass spectrometry analysis of water extracts. Results are summarised in Table S2 and the MALDI TOF spectra as shown in Figures S4-S11. The identified compounds are flavonoids (quercetin, miricetin, kampferol), tannins (elagic acid, gallic acid, procyanidins, ericifolin, eugeniin, chebulagic acid) and triterpenoids (ursolic or oleanolic acid), which were suggested recently as “the most promising carbohydrate-hydrolysing enzymes inhibitors” [34]. These compounds were identified earlier in cinnamon [35], clove [37], allspice [37] and oregano [38], and very recently in leaves of bayberry [39]. Some representatives of these groups had been found as amylase inhibitors in our experiments (tannins [16,17] and anthocyanins [18]) or published by

others [40] as α-glucosidase inhibitors. Medicinally active constituents usually occur in plants in groups of closely related compounds, and various constituents of each group may potentiate each other's effect. This synergistic effect of plant compounds is considered by herbalist to be a great advantage in treatments with medicinal plants. We explained the better inhibition effect with the larger number and higher concentration of inhibitor compounds present in plant extract.

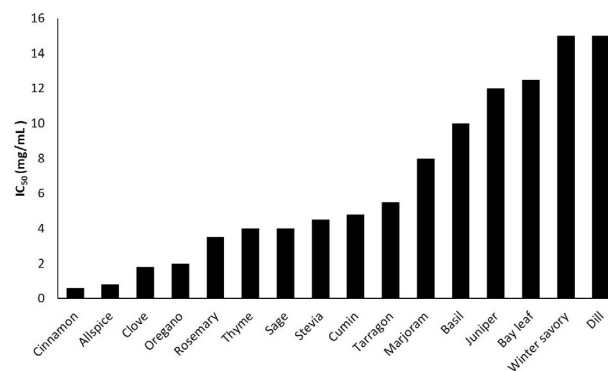


Figure 2. IC_{50} values of medicinal plant extracts measured on HSαA-catalysed starch hydrolysis.

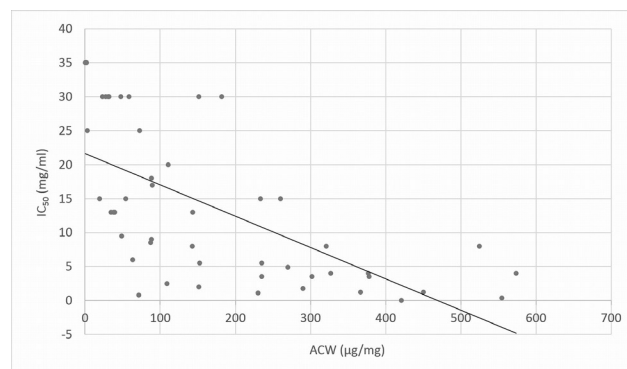


Figure 3. Correlation between the water soluble antioxidant capacity and IC_{50} values of plant extracts.

Table 1. Inhibition data for plant extracts causing full inhibition of HSαA.

Plant	Part used	IC_{50} (mg/mL)	Max. inhibition (%)
Green tea – <i>Camelia sinensis</i>	Leaf	0.4	100
Cinnamon – <i>Cinnamomum verum</i>	Bark	0.6	97
Allspice – <i>Pimenta dioica</i>	Fruit	0.8	100
Raspberry – <i>Rubus idaeus</i>	Leaf	1.1	90
Strawberry – <i>Fragaria vesca</i>	Leaf	1,1	100
Blackberry – <i>Rubus fruticosus</i>	Leaf	1.2	98
Clove – <i>Syzygium aromaticum</i>	Flower bud	1.8	90
Oregano – <i>Origanum vulgare</i>	Whole plant	2	95
Goldenrod – <i>Solidago gigantean</i>	Flowering tops	2.2	85
Blackcurrant – <i>Ribes nigrum</i>	Leaf	2.5	97

3.1 Antioxidant capacity of studied plants

Increased oxidative stress has been postulated in the diabetic state. Supplementation with plant-derived antioxidants may have an additional beneficial chemoprotective role in diabetes. For this reason, amylase inhibition and antioxidant activities were measured for some Iranian herbal plants [41], but no correlation was found between the two series of data. In contrast, porcine pancreatic α -amylase inhibition by ethanol extract of oregano was associated with antioxidant activity of extract [38].

Water soluble antioxidant capacity of plant extracts was determined and expressed as ascorbic acid (Fig. S12). The values were lower for all of the spices and medicinal herbs than that of reference compound. In most cases, spices exhibited a lower antioxidant effect than the medicinal herbs, and we observed that the better inhibition and the higher antioxidant effect were typical for certain plants. Linear regression methods were used to analyse the relationship between IC_{50} and water soluble antioxidant capacity values (Fig. 3). A weak negative correlation ($R^2 = 0.411$) was obtained, which suggests that plants with higher antioxidant capacity are better amylase inhibitors (with lower IC_{50}), probably due to the phenolic compounds having both effects.

4 Conclusion

Here we report several medicinal herbs and spices as inhibitors of starch hydrolysing enzyme HS α A. Amylase inhibitory effect of cinnamon, green tea, oregano and clove were published earlier, but these are the first results for similar efficiency of allspice and leaves of different berries. Knowing the water soluble dry matter content of the investigated plants we concluded that the effective concentration of active ingredients can be achieved if we consume infusions made from the plants with the lowest IC_{50} . Therefore, these plant infusions could be appropriate complements of diabetes therapy.

Acknowledgement: The research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-2016-00008. The authors are grateful for the financial support of TÁMOP-4.2.1.B-09/1/KONV and OTKA CK77515.

Conflict of interest: The authors declare no conflict of interests.

References

- Horvathova V., Janecek S., Sturdik E., Amyolytic enzymes: molecular aspects of their properties, *Gen. Physiol. Biophys.*, 2001, 20, 7–32.
- Henrissat B., A classification of glycosyl hydrolases based on amino acid sequence similarities, *Biochem. J.*, 1991, 280, 309–316.
- Janecek S., Svensson B., MacGregor E.A., α -Amylase: an enzyme specificity found in various families of glycoside hydrolases., *Cell. Mol. Life Sci.*, 2014, 71, 1149–1170.
- Kandra L., Gyémánt G., Remenyik J., Ragunath C., Ramasubbu N., Subsite mapping of human salivary α -amylase and the mutant Y151M, *FEBS Lett.*, 2003, 544, 194–198.
- Ramasubbu N., Paloth V., Luo Y., Brayer G.D., Levine M.J., Structure of human salivary α -amylase at 1.6 Å resolution: implications for its role in the oral cavity, *Acta Crystallogr. Section D*, 1996, 52, 435–446.
- Kahn S.E., Cooper M.E., Del Prato S., Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future, *Lancet*, 2014, 383, 1068–1083.
- Sales P.M., Souza P.M., Simeoni L.A., Magalhaes P.O., Silveira D., α -Amylase inhibitors: a review of raw material and isolated compounds from plant source, *J. Pharm. Pharm. Sci.*, 2012, 15, 141–183.
- Kobayashi K., Baba E., Fushiya S., Takano F., Batkhuu J., Dash T., Yoshizaki F., Screening of Mongolian plants for influence on amylase activity in mouse plasma and gastrointestinal tube, *Biol. Pharm. Bull.*, 2003, 26, 1045–1048.
- Prashanth D., Padmaja R., Samiulla D.S., Effect of certain plant extracts on α -amylase activity, *Fitoterapia*, 2001, 72, 179–181.
- Bhat M., Zinjarde S.S., Bhargava S.Y., Kumar A.R., Joshi B.N., Antidiabetic Indian plants: a good source of potent amylase inhibitors, *Evid. Based Complement. Alternat. Med.*, 2011, 2011, 810207.
- Ali H., Houghton P.J., Soumyanath A., α -Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*, *J. Ethnopharmacol.*, 2006, 107, 449–455.
- Funke I., Melzig M.F., Traditionally used plants in diabetes therapy: phytotherapeutics as inhibitors of α -amylase activity, *Rev. Bras. Farmacogn.*, 2006, 16, 1–5.
- Sheliya M.A., Begum R., Pillai K.K., Aeri V., Mir S.R., Ali A., Sharma M., *In vitro* α -glucosidase and α -amylase inhibition by aqueous, hydroalcoholic, and alcoholic extract of *Euphorbia hirta* L., *Drug Dev. Ther.*, 2016, 7, 26–30.
- Azmir J., Zaidul I.S.M., Rahman M.M., Sharif K.M., Mohamed A., Sahena F., Omar A.K.M., Techniques for extraction of bioactive compounds from plant materials: a review, *J. Food Eng.*, 2013, 117, 426–436.
- Kandra L., Gyémánt G., Zajác Á., Batta G., Inhibitory effects of tannin on human salivary α -amylase, *Biochem. Biophys. Res. Commun.*, 2004, 319, 1265–1271.
- Zajác Á., Gyémánt G., Vittori N., Kandra, L., Aleppo tannin: structural analysis and salivary amylase inhibition, *Carbohydr. Res.*, 2007, 342, 717–723.
- Gyémánt G., Zajác Á., Bécsi B., Ragunath C., Ramasubbu N., Erdődi F., Batta G., Kandra L., Evidence for pentagalloyl glucose

- binding to human salivary α -amylase through aromatic amino acid residues, *Biochim. Biophys. Acta*, 2009, 1794, 291–296.
- [18] Homoki J.R., Nemes A., Fazekas E., Gyémánt G., Balogh P., Gál F., Al-Asri J., Mortier J., Wolber G., Babinszky L., Remenyik J. Anthocyanin composition, antioxidant efficiency, and α -amylase inhibitor activity of different Hungarian sour cherry varieties (*Prunus cerasus* L.), *Food Chem.*, 2016, 194, 222–229.
- [19] Freyer M.W., Lewis E.A., Isothermal titration calorimetry: experimental design, data analysis, and probing macromolecule/ligand binding and kinetic interactions, *Methods Cell Biol.*, 2008, 84, 79–113.
- [20] Braissant O., Wirz D., Gopfert B., Daniels A.U., Use of isothermal microcalorimetry to monitor microbial activities, *FEMS Microbiol. Lett.*, 2010, 303, 1–8.
- [21] Bianconi M.L., Calorimetry of enzyme-catalysed reactions, *Biophys. Chem.*, 2007, 126, 59–64.
- [22] Sun L., Gidley M.J., Warren F.J., The mechanism of interactions between tea polyphenols and porcine pancreatic α -amylase: analysis by inhibition kinetics, fluorescence quenching, differential scanning calorimetry and isothermal titration calorimetry, *Mol. Nutr. Food Res.*, 2017, 61, 1700324.
- [23] Lehoczki G., Szabó K., Takács I., Kandra L., Gyémánt G., Simple ITC method for activity and inhibition studies on human salivary α -amylase, *J. Enzyme Inhib. Med. Chem.*, 2016, 31, 1648–1653.
- [24] Popov I., Volker H., Lewin G., Photochemiluminescent detection of antiradical activity. V. Application in combination with the hydrogen peroxide-initiated chemiluminescence of blood plasma proteins to evaluate antioxidant homeostasis in humans, *Redox Rep.*, 2001, 6, 43–48.
- [25] Singh R.P., Singh P.N., Impact of bee pollination on seed yield, carbohydrate composition and lipid composition of mustard seed, *J. Agric. Res.*, 1992, 31, 128–133.
- [26] Mateos-Aparicio I., Redondo-Cuenca A., Villanueva-Suárez M.J., Zapata-Revilla M.A., Tenorio-Sanz M.D., Pea pod, broad bean pod and okara, potential sources of functional compounds, *LWT – Food Sci. Technol.*, 2010, 43, 1467–1470.
- [27] Gopalakrishnan M., Chemical composition of nutmeg and mace, *J. Spices Arom. Crops*, 1992, 1, 49–54.
- [28] Davis F., Terry L.A., Chope G.A., Faul C.F., Effect of extraction procedure on measured sugar concentrations in onion (*Allium cepa* L.) bulbs, *J. Agric. Food Chem.*, 2007, 55, 4299–4306.
- [29] Adisakwattana S., Lerdsuwankij O., Poputtachai U., Minipun A., Suparpprom C., Inhibitory activity of cinnamon bark species and their combination effect with acarbose against intestinal α -glucosidase and pancreatic α -amylase, *Plant Foods Human Nutr.*, 2011, 66, 143–148.
- [30] Takács I., Takács Á., Pósa A., Gyémánt G., HPLC method for measurement of human salivary α -amylase inhibition by aqueous plant extracts, *Acta Biol. Hungarica*, 2017, 68, 127–136.
- [31] Beejmohun V., Peytavi-Izard M., Mignon C., Muscente-Paque D., Deplanque X., Ripoll C., Chapal N., Acute effect of Ceylon cinnamon extract on postprandial glycemia: α -amylase inhibition, starch tolerance test in rats, and randomized crossover clinical trial in healthy volunteers, *BMC Complement. Altern. Med.*, 2014, 14, 351.
- [32] Gao J., Xu P., Wang Y., Wang Y., Hochstetter D., Combined effects of green tea extracts, green tea polyphenols or epigallocatechin gallate with acarbose on inhibition against α -amylase and α -glucosidase *in vitro*, *Molecules*, 2013, 18, 11614–11623.
- [33] Miao M., Jiang B., Jiang H., Zhang T., Li X., Interaction mechanism between green tea extract and human α -amylase for reducing starch digestion, *Food Chem.*, 2015, 186, 20–25.
- [34] Loizzo M.R., Bonesi M., Nabavi S.M., Sobarzo-Sánchez E., Rastrelli L., Tundis R., Hypoglycaemic effects of plants food constituents via inhibition of carbohydrate hydrolysing enzymes: from chemistry to future applications, pp. 136–161. In: Andrade P.B., Valentão P., Pereira D.M. (Eds.), *Natural Products Targeting Clinically Relevant Enzymes*, Chapter 6, Wiley, 2017.
- [35] Mateos-Martín M.L., Fuguet E., Quero C., Pérez-Jiménez J., Torres J.L., New identification of proanthocyanidins in cinnamon (*Cinnamomum zeylanicum* L.) using MALDI-TOF/TOF mass spectrometry, *Anal. Bioanal. Chem.*, 2012, 402, 1327–1336.
- [36] Bao L.M., Eerdunbayaer, Nozaki A., Takahashi E., Okamoto K., Ito H., Hatano T., Hydrolysable tannins isolated from *Syzygium aromaticum*: structure of a new C-glucosidic ellagitannin and spectral features of tannins with a tergalloyl group, *Heterocycles*, 2012, 85, 365–381.
- [37] Shamaladevi N., Lyn D.A., Shaaban K.A., Zhang L., Villate S., Rohr J., Lokeshwar B.L., Ericifolin: a novel antitumor compound from allspice that silences androgen receptor in prostate cancer, *Carcinogenesis*, 2013, 34, 1822–1832.
- [38] McCue P., Vattem D., Shetty K., Inhibitory effect of clonal oregano extracts against porcine pancreatic amylase *in vitro*, *Asia Pac. J. Clin. Nutr.*, 2004, 13, 401–408.
- [39] Fu Y., Qiao L., Cao Y., Zhou X., Liu Y., Ye X., Structural elucidation and antioxidant activities of proanthocyanidins from Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) leaves, *PLoS One*, 2014, 9, e96162.
- [40] Yin Z., Zhang W., Feng F., Zhang Y., Kang W., α -Glucosidase inhibitors isolated from medicinal plants, *Food Sci. Human Wellness*, 2014, 3, 136–174.
- [41] Dehghan H., Sarrafi Y., Salehi P., Antioxidant and antidiabetic activities of 11 herbal plants from Hyrcania region, Iran, *J. Food Drug Anal.*, 2016, 24, 179–188.

Supplemental Material: The online version of this article

(DOI: 10.1515/amylase-2018-0002) offers supplementary material.