Impact of vinification procedure on fruit wine inhibitory activity against α-glucosidase

Uroš Čakar, Nađa Grozdanić, Boris Pejin, Vesna Vasić, Mira Čakar, Aleksandar Petrović, Brižita Djordjević

www.elsevier.com/locate/sdj

PII: S2212-4292(18)30151-2
DOI: https://doi.org/10.1016/j.fbio.2018.06.009
Reference: FBIO311

To appear in: *Food Bioscience*

Received date: 13 February 2018
Revised date: 28 June 2018
Accepted date: 28 June 2018

Cite this article as: Uroš Čakar, Nađa Grozdanić, Boris Pejin, Vesna Vasić, Mira Čakar, Aleksandar Petrović and Brižita Djordjević, Impact of vinification procedure on fruit wine inhibitory activity against α-glucosidase, *Food Bioscience,* https://doi.org/10.1016/j.fbio.2018.06.009

Impact of vinification procedure on fruit wine inhibitory activity against α-glucosidase

Uroš Čakar[a]*, Nađa Grozdanić[b], Boris Pejin[c*], Vesna Vasić[d], Mira Čakar[a], Aleksandar Petrović[e] & Brižita Djordjević[a]

[a] *University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia*

[b] *Institute for Oncology and Radiology of Serbia, Belgrade, Serbia*

[c] *University of Belgrade, Institute for Multidisciplinary Research – IMSI, Department of Life Sciences, Belgrade, Serbia*

[d] *University of Belgrade, Vinča Institute of Nuclear Sciences, Belgrade, Serbia*

[e] *University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia*

E-mails: brspjn@gmail.com

borispejin@imsi.rs

E-mails: uroslion@gmail.com

urosc@pharmacy.bg.ac.rs

*Address correspondence to these authors at the University of Belgrade: Institute for Multidisciplinary Research – IMSI, Department of Life Sciences, Kneza Višeslava 1, 11030 Belgrade, Serbia, Tel/Fax: +381 (11) 2636 061;

*Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia, Tel.: +381 (11) 3951 327, Fax: +381 (11) 3972 840,

Abstract

α-Glucosidase inhibitory activity (AGL) of fruit wine samples made from blueberry, black chokeberry, blackberry, raspberry and sour cherry cultivars grown in Serbia was studied using an microvinification procedure. More precisely, both sugar and enzyme were added to the fruit must

before fermentation for half of the samples. This increased the extraction of phenolic compounds. All the samples showed higher bioactivity compared to acarbose, the compound used as a positive control. Blueberry (IC_{50} ~27 ± 1 μg/ml) and black chokeberry (IC_{50} ~28 ± 1 μg/ml) wine samples had the highest values regardless of the vinification method. In addition to this, chlorogenic and caffeic acids were recognised as their key AGL bioactives. Taken all together, the fruit wine samples or their lyophilised extracts may be considered as complementary medicine supplements of potential interest for the control of postprandial hyperglycemia.

Keywords: Fruit wines, Blueberry, Black chokeberry, α-Glucosidase inhibitory activity, Chlorogenic acid, Caffeic acid

1. Introduction

The consumption of fruits and vegetables in regular diets may prevent some chronic diseases including diabetes mellitus (Costacou and Mayer-Davis, 2003; Hung et al., 2004). Indeed, WHO recommends a daily intake of 400 g of fruits and vegetables as a health prevention measure (WHO, 2015). α-Glucosidase is the enzyme located in the small intestine tract that is involved in

the final step of carbohydrate digestion – the breakdown of starch and disaccharides to glucose. Its optimum pH and temperature are 6-7.4 and 37ºC, respectively (Bailey, 2003). Berry fruits are rich sources of polyphenolics, bioactive compounds with health-promoting effects (Szajdek and Borowska, 2008). Both flavonoid such as apigenin, morin, myricetin and non-flavonoid compounds such as calystegines may show α-glucosidase inhibitory activity (AGL) (Jocković et al., 2013; Zeng et al., 2016). For example, α-glucosidase (α-Glu) inhibitors such as acarbose, miglitol and voglibose are able to suppress postprandial hyperglycemia, a prominent and early symptom of type 2 diabetes (Basha and Prasada Rao, 2017; Bailey, 2003; Potipiranun et al., 2017; Ramadhan et al., 2017). Most often natural α-glucosidase inhibitors show fewer and milder side effects (abdominal distention, flatulence and possibly diarrhea) than synthetic ones (Adisakwattana et al., 2009; Su et al., 2018; Wang et al., 2017; Vinholes et al., 2017). The overall effect of α-glucosidase inhibition is to reduce the flow of glucose from complex dietary carbohydrates into the bloodstream, diminishing the postprandial effect of starch consumption on blood glucose levels which may cause the development of diabetes (Bolen et al., 2007).

Previous studies with fruit wines used traditional procedures (Amidžić Klarić et al., 2011; Heinonen et al., 1998; Johnson et al., 2011). A new procedure, adding both sugar and enzyme before fermentation to increase phenolic extraction (Čakar et al., 2017), was used within the current work. The aim was to determine the possible effects on AGL of fruit wine samples using fruit cultivars from Serbia.

2. Materials and methods

2.1 Plant material

The fruits were purchased from commercial producers during 2014: blackberry (*Rubus caesius*) cultivar Čačanska bestrna was from Bojnik, Serbia; raspberry (*Rubus idaeus*) cultivar Meeker from Valjevo, Serbia; black chokeberry (*Aronia melanocarpa* Heynh.) and blueberry (*Vaccinium myrtilus*) were from the region of Rudnik mountain, Serbia; sour cherry (*Prunus cerasus* L.) cultivar Šumadinka was from the region of Grocka, Serbia. Fruit ripeness was

determined using a refractometer PAL-87S (Atago, Tokyo, Japan). As soon as the fruit was harvested, it was pressed into juice and the fermentation into the fruit wine begun. All fruits were free of mold and rotten fruits.

2.2 Chemicals and reagents

All chemicals and reagents of analytical grade were purchased from Sigma Aldrich (Steinheim, Germany). α-Glucosidase (lyophilised powder,) originated from the yeast *Saccharomyces cerevisiae* type I, containing ≥10 units/mg protein enzymatic activity (one unit liberates 1.0 μmol of D-glucose from *p*-nitrophenyl α-D-glucopyranoside per min at pH 6.8, 37 ºC) was used.

2.3 Preparation of wine samples

The fruit wine samples were produced from raspberry, blackberry, blueberry, black chokeberry and sour cherry cultivars. The fruits were pressed with the hand press RP-17 (Hromil, Kovilj, Serbia). Prior to fermentation, sour cherries were processed in two ways. The pits were removed from the fruit using a hand machine RM-1 (Hromil, Kovilj, Serbia) before the cherries were pressed or sour cherries were pressed together with the non-cracked pits. The experiments were divided in two sets, without and with additional sugar and enzyme into the fruit pomace, respectively. Total soluble solids (expressed in °Brix) were initially measured using the refractometer PAL-87S (Atago, Tokyo, Japan) in the fruit must of the first set. In the second set, sugar (sucrose) was added in the amount to increase total soluble solids of the must to 20.5 °Brix or 11% alcohol. The enzymatic preparation glycosidase Enartis Zym (Enartis, San Martino, Italy) was added at 2 g/100 kg in the second set. The final ethanol content was determined at the end of fermentation using an alcohol density meter DMA 35 (Anton Paar, Graz, Austria) after samples distillation (Table 1). The strength by volume (vol. %) was calculated using 20°C/20°C tables (OIV, 2009). In both cases, 10 g of $K_2S_2O_5$/100 kg was added to obtain 50 mg/kg of SO_2 in fruit must to inhibit bacterial growth. Both sets were divided into two subsets and were inoculated with a pure wine *Saccharomyces cerevisiae* strain Lievito Secco EZ FERM (Enartis) and ICV D254 (Lallemand, Montreal, Canada), respectively, at 20 g/100 kg. Both *Saccharomyces cerevisiae* strains had been successfully used. Specifically, 25 kg of fruit was fermented in 30 l barrels using

the pigeage system (Hromil, Kovilj, Serbia). Alcohol fermentation was done at 20°C over 7 to 10 days. During this process, the pomace was stirred twice a day. After fermentation, each fruit wine was separated from the pomace by sedimentation. Afterwards, they were racked off leaving the lees and kept at 12°C for the next 6 months, for further studies (Čakar et al., 2017). As an undesired ingredient for the screening of AGL, ethanol was removed using lyophilisation. The lyophilisation was carried out for 9 h at 0.30 mbar and -55°C using a laboratory freezedryer Christ Alpha 1-2/LD plus (Osterode am Harz, Germany) (main drying time: 8.5 h; final drying time: 30 min). The lyophilised wine samples were kept at −20ºC and used for further analysis within 5 day.

Table 1

2.4 Anti α-glucosidase assay

Lyophilised fruit wine samples were screened for AGL as described previously (McCue et al., 2005). Briefly, 0.4 units/ml of α-glucosidase was dissolved in 0.1 M phosphate buffer (monosodium phosphate, disodium phosphate, pH = 6.8). The lyophilised samples were dissolved in dimethyl sulfoxide (DMSO) at 5×10^{-2} g powder/ml. Afterwards, solutions were diluted in the phosphate buffer 0.1 M (pH = 6.8) so that the concentration in each sample well ranged from 8×10^{-8} to 1×10^{-3} g/ml. In each well, 50 µl of the sample solutions or 10% DMSO used as a blank were preincubated with 50 µl of the enzyme solution at 37°C for 15 min. Then, 50 µl of the substrate solution, *p*-nitrophenyl α-D-glucopyranoside (PNP-G in the phosphate buffer, 1.5 mg/ml) was added into each well. After measuring absorbance A_1 at 405 nm, on the ELISA reader Multiskan EX (Thermo Scientific, Waltham, MA, USA) the solution was incubated at 37°C for 5 min. The second absorbance A_2 was measured at 405 nm. The ΔA was obtained by the subtraction of A_1 from A_2 for the sample and the blank. ΔA for the samples and blank were obtained in the same way. Acarbose was used as a positive control.

The standards of epicatechin, protocatechuic acid, catechin, gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ellagic acid and rutin were dissolved in DMSO (shortly before their use) to 50 mmol/l, while stock solutions were diluted as previously described for the fruit wine samples. All the experiments were done in triplicate.

2.5 The Hill analysis

The dependence of the relative enzyme activity (REA; expressed as a percentage) for each phenolic compound of the concentration of each single phenolic compound was fitted to a sigmoid function. The relevant inhibitory parameters were obtained using the Hill analysis (Prinz, 2010) of the inhibitory curves, according to Eq. (1):

$$\log\left(\frac{\mathrm{REA}}{100-\mathrm{REA}}\right) = -\,n\log[\mathrm{I}] + n\log \mathrm{IC}_{50} \quad (1)$$

where [I] represents the concentration of a single phenolic compound, while n is the Hill coefficient of cooperativity.

Inhibitory curves for the fruit wine samples and standards of phenolic compounds were obtined using the Hill analysis. The abundance of these compounds was estimated on the basis of the quantitative analysis previously reported (Čakar et al., 2017).

The concentrations of the selected phenolics in the amount of the fruit wine samples inhibiting 50% AGL were used as a parameter for the estimation of their contributions to the IC_{50} values of the analysed wines prepared with or without (Tables 2 and 3) addition of sugar before fermentation (Čakar et al., 2017).

Table 2

Table 3

2.6 Statistical analysis

Statistical analysis was done using the software SPSS Statistic V22.0 (IBM, Chicago, IL, USA; 2014) and pair samples t-test was used to compare IC_{50} values of control and wines made with adition of sugar and enyzme. The $p<0.05$ were considered significant. The authors have also chosen to use 0.01 for some of the data to indicate the greater significance of the differences. Inhibition curve were prepared using the program Origin Pro 8 (OriginLab, Northampton, MA, USA; 2008).

3. Results and discussion

3.1 α-Glucosidase inhibitory activity of the fruit wine samples

Sigmoid-shaped inhibitory curves were obtained in all cases (Figures 1 and 2).

Figure 1

Figure 2

Table 4

The IC_{50} values obtained for the fruit wine samples had significant AGL (Table 4). Indeed, blueberry and black chokeberry wine samples (both without and with addition of sugar) were the most active. On the other hand, cherry with pit and raspberry were the least effective. Such results are in accordance with the previous ones for blueberry and raspberry acetic acid extracts (McDougall et al., 2005). A Norwegian study focussing on black chokeberry wine samples also supports the findings presented herein (Wangensteen et al., 2014). Another study on blackberry water and ethanol extracts has suggested that these beverages be used in diet as part of the therapeutic control of postprandial hyperglicemia (Sarkar et al., 2016). Methanol and acetone extracts of raspberries showed a strong AGL (Zhang et al., 2010). Furthermore, a study done in Poland has described the ability of cherry juice to inhibit α-glucosidase *in vitro* (Nowicka et al., 2016). Difference between AGL of the various kinds of fruits may be explained by different cultivars and/or growing conditions.

Indeed, the fruit wine samples produced with addition of sugar and enzyme before fermentation showed statistically higher AGL ($p<0.01$). The higher level of ethanol contributed to the increased extraction of total phenolics from the fruit pomace. The enzyme was also added to increase the content of total phenolics in the final product. However, the t-test indicated no significant statistical difference ($p\geq0.05$) for the IC_{50} values of the fruit wine samples prepared using different yeasts (Lievito Secco EZ FERM and ICV D254 yeasts). Previous work had suggested that biological activity of fruit wines was linked to the winemaking process and fruit used (Čakar et al., 2017). The wine lyophilisates (instead of the wine samples themselves) were used due to possible interference of the solvent (ethanol). However, it is well worth mentioning that that no AGL activity was found using the solvent (11% ethanol). AGL natural products are mainly distributed in the solid parts of the fruits, e.g., blueberry peel (McDougall et al., 2005;

Johnson et al., 2011; Wangensteen et al., 2014). In any case, their successful extraction represents a critical step for increasing the health-promoting effects of the fruit wines – the final products (Wang et al., 2012). The important thing is that they need to be in the liquid phase because solids are discarded.

3.2 α-Glucosidase inhibitory activity of the selected ingredients (phenolic compounds) from the fruit wine samples

Table 5

Figure 3

Inhibitory curves for the standards of the phenolic compounds are shown in Figure 3. In brief, the highest AGL were found for ellagic acid (7.9×10^{-6} M), chlorogenic acid (4.8×10^{-5} M) and catechin (5.6×10^{-5} M), while rutin and *p*-coumaric acid showed slightly lower inhibitory activities (Table 5). The Hill coefficient provides a way to quantify the degree of interaction between ligand binding sites. It actually describes the cooperativity of the ligand binding. The values of the Hill coefficients (n) (Table 5) for the majority of the analysed phenolics were below 1 ($n < 1$) which actually indicated a negative cooperative binding to the enzyme. On the other hand, the value of the Hill coefficient for ellagic acid ($n = 1.23$) suggested a positive cooperative binding. Finally, some phenolic acids including caffeic, protocatechuic and gallic acids were also found to inhibit α-glusosidase (Kwon et al., 2006).

3.3 Contribution of the selected phenolic compounds to the IC_{50} values of the fruit wine samples

Unlike ellagic acid, chlorogenic and caffeic acids significantly contributed to the inhibitory activity of the blueberry wine samples. Among hydroxybenzoic acid derivatives, protocatechuic and gallic acids were most effective. Similarly, chlorogenic acid mostly contributed to the inhibitory activity of the black chokeberry wine samples, followed by protocatechuic acid. On the

other hand, caffeic and gallic acids had less affect on the bioactivity of the same fruit wine samples (Tables 2 and 3). Such experimental data are in good agreement with the previous findings for the hypoglycemic action of both chlorogenic and caffeic acids (Hemmerle et al., 1997; Kwon et al., 2008).

Interestingly, protocatechuic acid mostly contributed to the IC_{50} values of the blackberry wines, both with and without added sugar. But gallic and ellagic acids also contributed. However, caffeic, gallic and protocatechuic acids predominantly contributed to the AGL of the raspberry wine samples. The existing data claiming that gallic acid has the ability to inhibit α-glucosidase are consistent with the current work (Oboh et al., 2016). As for the cherry wine samples with and without pits, chlorogenic and caffeic acids were most active. In contrast to the other samples, gallic acid did not contribute to their bioactivity (Tables 2 and 3).

The lack of contribution of certain phenolic compounds to the IC_{50} values of the studied fruit wine samples may be at least partially explained by their low or very low concentrations. In any case, the possibility of a synergistic effect should not be excluded. Such a case has been observed for other plants (Suraiya et al., 2018). Anthocyanins should be thoroughly studied for AGL (Braunlich et al., 2013). Additionally, tannins may also be of interest (Toda et al., 2001). Last but not least, the influence of the food matrix on AGL should be taken into consideration. Indeed, proteins may react with phenolics thus, decreasing their inhibitory activity (Lavelli et al., 2016). Furthermore, hydrogen bonding coupled with hydrophobic interactions between polyphenolics and proteins lead to the formation of soluble or insoluble aggregates (Granato et al., 2010; Zhao et al., 2013). Indeed, these aggregates were shown to release bioactive phenolics in an *in vitro* gastrointestinal model (Oliveira and Pintado, 2015).

While some hydroxycinnamic acid derivatives (such as chlorogenic and caffeic acid) significantly contributed to the enzyme inhibition, other compounds belonging to the same class were less active. A previous study has reported their health-promoting effects based on AGL (Adisakwattana et al., 2004) Thus, hydroxycinnamic acid derivatives might be used in combination with acarbose for the control of hyperglycemia, if additional studies would confirm the efficacy of such an approach. The fact that a daily dietary intake of these compounds can affect the

suppression of postprandial hyperglycemia is particularly encouraging (Adisakwattana et al., 2009; Arabbi et al., 2004; Hertog et al., 1992, 1993).

4. Conclusions

This study has shown that fruit wines supplemented with sugar and enzyme Enartis Zym before fermentation may have a greater ability to inhibit α-Glu *in vitro*. Specifically, blueberry and black chokebery wine samples had the highest AGL levels. In any case, all fruit wines and/or lyophilised extracts studied will be the subject of the further *ex vivo* and *in vivo* studies aiming to determine their impact with moderate use, i.e., 150 to 200 ml/day, for he control of postprandial hyperglycemia.

Acknowledgments

One of the authors (Uroš Čakar) gratefully acknowledges Dr. Tatjana Stanojković and Dr. Branislav Nastasijević for their precious help and support related to the experimental work. Author (Uroš Čakar) gratefully acknowledges Professor Thomas D. Zlatic for language editing assistance. Another author (Boris Pejin) dedicates this study to the memory of Diana, the beloved People's Princess.

Disclosure of Potential Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

References

Adisakwattana, S., Chantarasinlapin, P., Thammarat, H., & Yibchok-Anun, S. (2009). A series of cinnamic acid derivatives and their inhibitory activity on intestinal α-glucosidase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24, 1194–1200.

Adisakwattana, S., Sookkongwaree, K., Roengsumran, S., Petsom, A., Ngamrojnavanich, N., Chavasiri, W., Deesamer, S., & Yibchok-anun, S. (2004). Structure-activity relationships of *trans*-cinnamic acid derivatives on alpha-glucosidase inhibition. *Bioorganic & Medicinal Chemistry Letters*, 14, 2893–2896.

Amidžić Klarić, D., Klarić, I., & Mornar, A. (2011). Polyphenol content and antioxidant activity of commercial blackberry wines from Croatia: Application of multivariate analysis for geographic origin differentiation. *Journal of Food and Nutrition Research*, 50, 199–209.

Arabbi, P.R., Genovese, M.I., & Lajolo, F.M. (2004). Flavonoids in vegetable food commonly consumed in Brazil and estimated ingestion by the Brazilian population. *Journal of Agricultural and Food Chemistry*, 52, 1124–1131.

Bailey, C. J. (2003). New approaches to the pharmacotherapy of diabetes. In: J. C., Pickup, G. Williams (Eds.), *Textbook of Diabetes*. Oxford: Blackwell Science. Vol. 2, pp. 73.1–73.21.

Basha, S.A., & Prasada Rao, U.J.S. (2017). Bioactivities of fractions obtained from green gram (*Vigna radiata*) milled by-products. *Food Bioscience*, 19, 134–141.

Bolen, S., Feldman, L., Vassy, J., Wilson, L., Yeh, H.C., Marinopoulos, S., Wiley, C., Selvin, E., Wilson, R., Bass, E.B., & Brancati, F.L. (2007). Systematic review: Comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. *Annals of Internal Medicine*, 147, 386−399.

Braunlich, M., Slimestad, R., Wangensteen, H., Brede, C., Malterud, K.E., & Barsett, H. (2013). Extracts, anthocyanins and procyanidins from *Aronia melanocarpa* as radical scavengers and enzyme inhibitors. *Nutrients*, 5, 663–678.

Costacou, T., & Mayer-Davis, E.J. (2003). Nutrition and prevention of type 2 diabetes. *Annual Review of Nutrition*, 23, 147–170.

Čakar, U., Petrović, A., Janković, M., Pejin, B., Vajs, V., Čakar, M. & Djordjević, B. (2017) Differentiation of wines made from berry and drupe fruits according to their phenolic profiles. *European Journal of Horticultural Science*., In Press, DOI:10.17660/eJHS.2018/83.1.7

Granato, T.M., Piano, F., Nasi, A., Ferranti, P., Iametti, S., & Bonomi, F. (2010). Molecular basis of the interaction between proteins of plant origin and proanthocyanidins in a model wine system. *Journal of Agricultural and Food Chemistry*, 58, 11969–11976.

Hemmerle, H., Burger, H.J., Below, P., Schubert, G., Rippel, R., Schindler, P.W., Paulus, E., & Herling, A.W. (1997). Chlorogenic acid and synthetic chlorogenic acid derivatives: Novel inhibitors of hepatic glucose-6-phosphate translocase. *Journal of Medicinal Chemistry*, 40, 137–145.

Heinonen, I.M., Lehtonen, P.J., & Hopia, A.I. (1998). Antioxidant activity of berry and fruit wines and liquors. *Journal of Agricultural and Food Chemistry*, 46, 25–31.

Hertog, M.G.L., Hollman, P.C.H., & Katan, M.B. (1992). Content of potentially anticarcinogenic of flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry*, 40, 2379–2383.

Hertog, M.G.L., Hollman, P.C.H., & Van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusion, wines and fruit juices. *Journal of Agricultural and Food Chemistry*, 41, 1242–1246.

Hung, H.C., Joshipura, K.J., Jiang, R., Hu, F.B., Hunter, D., Smith-Warner, S.A., Colditz, G.A., Rosner, B., Spiegelman, D., & Willett W.C. (2004). Fruit and vegetable intake and risk of major chronic disease. *Journal of the National Cancer Institute*, 96, 1577–1584.

Jocković, N., Fischer, W., Brandsch, M., Brandt, W., & Dra□ger, B. (2013). Inhibition of human intestinal α-glucosidases by calystegines. *Journal of Agricultural and Food Chemistry*, 61, 5550−5557.

Johnson, M., Lucius, A., Meyer, T., & Gonzalez de Mejia, E. (2011). Cultivar evaluation and effect of fermentation on antioxidant capacity and *in vitro* inhibition of α-amylase and α-glucosidase by highbush blueberry (*Vaccinium corombosum*). *Journal of Agricultural and Food Chemistry*, 59, 8923–8930.

Kwon, Y.I., Vattem, D.V., & Shetty K. (2006). Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pacific Journal of Clinical Nutrition*, 15, 107–118.

Kwon, Y.I., Apostolidis, E., & Shetty, K. (2008). Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglyemia linked to type diabetes. *Journal of Food Biochemistry*, 32, 15–31.

Lavelli, V., Sri Harsha, P.S.C., Ferranti, P., Scarafoni, A., & Iametti, S. (2016). Grape skin phenolics as inhibitors of mammalian α-glucosidase and α-amylase – effect of food matrix and processing on efficacy. *Food & Function*, 7, 1655–1663.

McCue, P., Kwon, Y.I., & Shetty, K. (2005). Anti-amylase, anti-glucosidase and anti angiotensin I converting enzyme potential of selected foods. *Journal of Food Biochemistry*, 29, 278–294.

McDougall, G.J., Shpiro, F., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic compounds of soft fruits inhibit α-amylase and α-glucosidase. *Journal of Agricultural and Food Chemistry*, 53, 2760–2766.

Nowicka, P., Wojdyło, A., & Samoticha, J. (2016). Evaluation of phytochemicals, antioxidant capacity, and antidiabetic activity of novel smoothies from selected *Prunus* fruits. *Journal of Functional Foods*, 25, 397–407.

Oboh, G., Ogunsuyi, O.B., Ogunbadejo, M.D., & Adefegha, S.A. (2016). Influence of gallic acid on α-amylase and α-glucosidase inhibitory properties of acarbose. *Journal of Food and Drug Analysis*, 24, 627–634.

Oliveira, A., & Pintado M. (2015). Stability of polyphenols and carotenoids in strawberry and peach yoghurt throughout *in vitro* gastrointestinal digestion. *Food & Function*, 6, 3444–3453.

OIV, (2009). Density and Specific Gravity at 20°C. In: OIV, *Compendium of International Methods of Wine and Must Analysis*. Paris: Organisation Internationale de la Vigne et du Vin. 1−30

Potipiranun, T., Worawalai, W., & Phuwapraisirisan P. (2017). Lamesticumin G, a new α-glucosidase inhibitor from the fruit peels of *Lansium parasiticum. Natural Product Research*, In Press, DOI: 10.1080/14786419.2017.1354184

Prinz, H. (2010). Hill coefficients, dose-response curves and allosteric mechanisms. *Journal of Chemical Biology*, 3, 37–44.

Ramadhan, R., Kusuma, I.W., Amirta, R., Worawalai, W., & Phuwapraisirisan, P. (2017). A new 4–arylflavan from the pericarps of *Horsfieldia motleyi* displaying dual inhibitionagainst α-glucosidase and free radicals. *Natural Product Research*, In Press, DOI:10.1080/14786419.2017.1378204

Sarkar, D., Orwat, J., Hurburt, T., Woods, F., Pitts, J.A., & Shetty, K. (2016). Evaluation of phenolic bioactive-linked functionality of blackberry cultivars targeting dietary management of early stages type-2 diabetes using *in vitro* models. *Scientia Horticulturae*, 212, 193–202.

Su, N., Li, J., Ye, Z., Chen, T., & Ye, Ming (2018). Quality properties, flavor and hypoglycemia activity of kiwifruit-bitter gourd fermented milks. *Food Bioscience*, In Press, DOI: 10.1016/j.fbio.2018.02.002

Suraiya, S., Lee, J.M., Cho, H.J., Jang, W.J., Kim, D.G., Kim, Y.O., & Kong, I.S., (2018). *Monascus* spp. fermented brown seaweeds extracts enhance bio-functional activities. *Food Bioscience*, 21, 90–99.

Szajdek, A., & Borowska, E.J. (2008). Bioactive compounds and health-promoting properties of berry fruits: a review. *Plant Foods for Human Nutrition*, 63, 147–156.

Toda, M., Kawabata, J., & Kasai, T. (2001). Inhibitory effects of ellagi- and gallotannins on rat intestinal α-glucosidase complexes. *Bioscience, Biotechnology, and Biochemistry*, 65, 542–547.

Vinholes, J., Lemos, G., Lia Barbieri, R., Franzon, R. C., & Vizzotto, M. (2017). *In vitro* assessment of the antihyperglycemic and antioxidant properties of araçá, butiá and pitanga. *Food Bioscience*, 19, 92–100.

Wang, L., Yan, Y.S., Cui, H.H., Yin, Y.Q., Pan, J.T., & Yu, B.W. (2017). Three new resin glycosides compounds from *Argyreia acuta* and their α-glucosidase inhibitory activity. *Natural Product Research*, 31, 537–542.

Wang, S.Y., Camp, M.J., & Ehlenfeldt, M.K. (2012). Antioxidant capacity and α-glucosidase inhibitory activity in peel and flesh of blueberry (*Vaccinium* spp.) cultivars. *Food Chemistry*, 132, 1759–1768.

Wangensteen, H., Braunlich, M., Nikolic, V., Malterud, K.E., Slimestad, R., & Barsetta, H. (2014). Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: Antioxidant and enzyme inhibitory effects. *Journal of Functional Foods*. 7:746–752.

World Health Organization (WHO). (2015). Diet, nutrition and the prevention of chronic diseases. Technical Report Series No. 916, http://whqlibdoc.who.int/trs/who_trs_916.pdf. (accessed 10 February 2018).

Zeng, L., Zhang, G., Lin., S., & Gong, D. (2016). Inhibitory mechanism of apigenin on α glucosidase and synergy analysis of flavonoids. *Journal of Agricultural and Food Chemistry*, 64, 6939−6949.

Zhang, L., Li, J., Hogan, S., Chung, H., Welbaum, G.E., & Zhou, K. (2010). Inhibitory effect of raspberries on starch digestive enzyme and their antioxidant properties and phenolic composition. *Food Chemistry*, 119, 592–599.

Zhao, W., Iyer, V., Flores, F.P., Donhowe, E., & Kong, F. (2013). Microencapsulation of tannic acid for oral administration to inhibit carbohydrate digestion in the gastrointestinal tract. *Food & Function*, 4, 899–905.

Table 1. Total soluble solids of must and alcohol content.

Wine sample	Lievito Secco EZ FERM yeast		ICV D254 yeast	
	Total soluble solids must (º Brix)	Alcohol content (Vol %)	Total soluble solids must (º Brix)	Alcohol content (Vol %)
Black chokeberry control	11.5 ±0.2	6.6 ±0.1	11.9 ±0.1	6.9 ±0.1
Black chokeberry + sugar+ enzyme	18.8 ±0.4	11.1 ±0.1	19.1 ±0.2	11.2 ±0.1
Blueberry control	14.2 ±0.2	8.3 ±0.1	14.5 ±0.1	8.4 ±0.1
Blueberry + sugar+ enzyme	18.6 ±0.3	10.9 ±0.1	19.2 ±0.2	11.3 ±0.1
Blackberry control	13.4 ±0.2	7.8 ±0.1	13.7 ±0.2	7.9 ±0.1
Blackberry + sugar +enzyme	17.6 ±0.2	10.3 ±0.1	17.7 ±0.2	10.4 ±0.1
Raspberry control	12.8 ±0.1	7.4 ±0.1	12.6 ±0.1	7.3 ±0.1
Raspberry + sugar+ enzyme	16.7 ±0.2	9.8 ±0.1	16.5 ±0.2	9.7 ±0.1
Sour cherry control −pit	12.0 ±0.1	6.9 ±0.1	11.8 ±0.2	6.8 ±0.1
Sour cherry + sugar +enzyme −pit	18.4 ±0.2	10.8 ±0.1	18.2 ±0.3	10.7 ±0.1
Sour cherry control +pit	12.6 ±0.2	7.3 ±0.1	12.4 ±0.1	7.2 ±0.1
Sour cherry +sugar +enzyme +pit	18.9 ±0.2	11.2 ±0.1	18.8 ±0.2	11.0 ±0.1

Table 2. The concentrations of the selected phenolic compounds (M) and their contributions (%) to the IC_{50} values of the fruit wine samples made with Lievito Secco EZ FERM yeast.

Type of Fruit	Type of Vinification	Epicatechin		Protocatechuic acid		Catechin		Gallic acid		Chlorogenic acid		Caffeic acid		*p*-Coumaric acid		Ellagic acid	
		M	%	M	%	M	%	M	%	M	%	M	%	M	%	M	%
Black	contro	3.5	-	4.2	7.7	8.	-	3.	1.	2.	18	6.	5.6	2.	-	5.4	-

choke berry	l	2 ×10^{-9}		5 ×10^{-6}	1	44 × 10^{-9}		74 × 10^{-8}	47	16 ×10^{-6}	.9	73 ×10^{-7}	7	60 ×10^{-8}		0 ×10^{-8}	
Black choke berry	+ sugar + enzyme	6.52 ×10^{-9}	-	4.78 ×10^{-6}	8.35	2.48 × 10^{-8}	-	7.73 × 10^{-8}	1.81	2.32 ×10^{-6}	19.5	9.01 ×10^{-7}	6.15	7.33 ×10^{-8}	2.87	9.37 ×10^{-8}	-
Blueberry	control	2.40 ×10^{-7}	2.51	8.84 ×10^{-7}	1.17	1.90 × 10^{-7}	2.10	5.43 × 10^{-7}	3.37	3.64 ×10^{-6}	21.9	9.39 ×10^{-7}	6.38	1.97 ×10^{-8}	-	1.43 ×10^{-7}	1.37
Blueberry	+ sugar + enzyme	2.03 ×10^{-7}	2.02	7.47 ×10^{-7}	1.05	1.52 × 10^{-7}	1.87	4.09 × 10^{-7}	3.19	2.26 ×10^{-6}	19.0	6.74 ×10^{-7}	5.67	2.38 ×10^{-8}	-	1.28 ×10^{-7}	1.15
Black berry	control	5.77 ×10^{-9}	-	2.86 ×10^{-6}	6.45	1.26 × 10^{-7}	1.45	1.64 × 10^{-6}	5.86	3.21 ×10^{-8}	-	2.90 ×10^{-8}	1.17	1.92 ×10^{-8}	-	4.93 ×10^{-7}	3.30
Black berry	+ sugar + enzyme	1.52 ×10^{-8}	-	2.77 ×10^{-6}	6.27	1.62 × 10^{-7}	1.91	1.94 × 10^{-6}	6.15	3.96 ×10^{-8}	1.03	6.11 ×10^{-8}	1.37	3.18 ×10^{-8}	1.03	5.07 ×10^{-7}	3.32
Raspberry	control	9.52 ×10^{-9}	-	1.24 ×10^{-6}	3.96	1.49 × 10^{-7}	1.85	1.89 × 10^{-6}	6.11	7.97 ×10^{-9}	-	1.71 ×10^{-7}	1.63	2.64 ×10^{-8}	-	2.34 ×10^{-7}	2.31
Raspberry	+ sugar + enzyme	9.52 ×10^{-9}	-	9.91 ×10^{-7}	1.48	1.53 × 10^{-7}	1.87	1.44 × 10^{-6}	4.93	1.58 ×10^{-8}	-	2.11 ×10^{-7}	3.76	5.06 ×10^{-8}	2.44	2.31 ×10^{-7}	2.30
Sour cherry	control – pit	1.47 ×10^{-7}	1.57	2.75 ×10^{-6}	6.15	2.81 × 10^{-7}	3.03	8.55 × 10^{-9}	-	5.16 ×10^{-6}	29.3	1.42 ×10^{-6}	7.67	3.81 ×10^{-8}	1.23	0	-
Sour	+	2.6	2.	2.6	6.0	3.	3.	1.	1.	4.	27	1.	7.8	7.	3.35	0	-

cherry	sugar +enzyme /−pit	0 ×10^{-7}	60	8 ×10^{-6}	3	31 × 10^{-7}	27	58 × 10^{-8}	12	52 ×10^{-6}	.2	51 ×10^{-6}	7	60 ×10^{-8}			
Sour cherry	control + pit	1.65 ×10^{-7}	1.74	2.95 ×10^{-6}	6.57	3.12 × 10^{-7}	3.11	1.15 × 10^{-8}	-	5.46 ×10^{-6}	31.2	1.52 ×10^{-6}	7.90	4.85 ×10^{-8}	2.32	0	-
Sour cherry	+sugar +enzyme /+pit	2.47 ×10^{-7}	2.55	2.50 ×10^{-6}	5.52	3.31 × 10^{-7}	3.27	1.17 × 10^{-8}	-	4.18 ×10^{-6}	25.2	1.41 ×10^{-6}	7.65	7.19 ×10^{-8}	2.92	0	-

Table 3. The concentrations of the selected phenolic compounds (M) and their contributions (%) to the IC_{50} values of the fruit wine

Type of Fruit	Type of Vinification	Epicatechin		Protocatechuic acid		Catechin		Gallic acid		Chlorogenic acid		Caffeic acid		*p*-Coumaric acid		Ellagic acid	
		M	%	M	%	M	%	M	%	M	%	M	%	M	%	M	%
Black choke berry	control	3.80 ×10^{-9}	-	4.21 ×10^{-6}	7.59	1.06 × 10^{-8}	-	3.90 × 10^{-8}	1.57	2.17 ×10^{-6}	19.0	5.86 × 10^{-7}	5.15	2.02 ×10^{-8}	-	5.57 × 10^{-8}	-
Black choke berry	+ sugar + enzyme	3.63 ×10^{-9}	-	2.96 ×10^{-6}	6.55	1.61 × 10^{-8}	-	5.42 × 10^{-8}	1.68	1.47 ×10^{-6}	16.4	4.28 × 10^{-7}	4.78	4.21 ×10^{-8}	1.81	6.24 × 10^{-8}	-
Blueberry	control	2.84 ×10^{-7}	2.79	1.03 ×10^{-6}	3.71	2.24 × 10^{-7}	2.15	5.75 × 10^{-7}	3.51	4.17 ×10^{-6}	25.2	1.08 × 10^{-6}	7.13	2.11 ×10^{-8}	-	1.67 × 10^{-7}	1.43
Blueberry	+ sugar + enzyme	2.66 ×10^{-7}	2.62	9.07 ×10^{-7}	1.21	1.87 × 10^{-7}	2.07	5.02 × 10^{-7}	3.17	2.70 ×10^{-6}	19.6	8.18 × 10^{-7}	5.97	2.71 ×10^{-8}	-	1.60 × 10^{-7}	1.41
Blackberry	control	4.38 ×10^{-9}	-	3.12 ×10^{-6}	6.74	1.40 × 10^{-7}	1.83	1.75 × 10^{-6}	5.97	3.10 ×10^{-8}	-	2.88 × 10^{-8}	1.11	1.71 ×10^{-8}	-	5.33 × 10^{-7}	3.61

Blackberry	+ sugar + enzyme	1.61×10^{-8}	-	2.79×10^{-6}	6.18	1.70×10^{-7}	1.93	1.93×10^{-6}	6.15	3.63×10^{-8}	-	5.65×10^{-8}	1.21	2.78×10^{-8}	-	5.07×10^{-7}	3.32
Raspberry	control	1.00×10^{-8}	-	7.03×10^{-7}	0.97	8.65×10^{-8}	-	9.97×10^{-7}	4.45	3.69×10^{-9}	-	1.13×10^{-7}	1.47	1.51×10^{-8}	-	1.34×10^{-7}	-
Raspberry	+ sugar + enzyme	1.18×10^{-8}	-	1.14×10^{-6}	3.72	1.81×10^{-7}	2.06	1.64×10^{-6}	5.25	1.32×10^{-8}	-	2.60×10^{-7}	3.91	6.09×10^{-8}	2.56	2.65×10^{-7}	2.41
Sour cherry	control − pit	1.57×10^{-7}	1.61	2.76×10^{-6}	6.15	2.78×10^{-7}	2.98	8.33×10^{-9}	-	5.26×10^{-6}	29.8	1.44×10^{-6}	7.71	4.06×10^{-8}	1.55	0	-
Sour cherry	+ sugar + enzyme /−pit	2.30×10^{-7}	2.47	2.33×10^{-6}	5.31	2.95×10^{-7}	3.23	1.51×10^{-8}	1.07	3.97×10^{-6}	23.0	1.34×10^{-6}	7.47	7.08×10^{-8}	3.07	0	-
Sour cherry	control + pit	1.63×10^{-7}	1.73	2.70×10^{-6}	6.07	2.82×10^{-7}	3.18	1.28×10^{-8}	-	5.19×10^{-6}	29.3	1.40×10^{-6}	7.57	4.72×10^{-8}	2.17	0	-
Sour cherry	+sugar +enzyme /+pit	2.25×10^{-7}	2.37	2.38×10^{-6}	5.38	3.05×10^{-7}	3.30	1.64×10^{-8}	1.14	4.06×10^{-6}	23.1	1.39×10^{-6}	7.55	7.35×10^{-8}	3.25	0	-

samples made with ICV D254 yeast.

Table 4. The IC_{50} values of the fruit wine samples, along with the relevant lyophilised extract yield values.

Fruit type	Type of vinification	Lievito Secco EZ FERM yeast		ICV D254 yeast	
		IC_{50} (µg/ml)	Yield (%)	IC_{50} (µg/ml)	Yield (%)
Black chokeberry	control	49±2	4.7	50±2	4.9
Black chokeberry	+ sugar + enzyme	28±1[a*]	4.7	30±1[a*]	4.8
Blueberry	control	48±1	2.7	49±2	2.4
Blueberry	+ sugar + enzyme	27±1[a*]	2.8	29±1[a*]	2.5
Blackberry	control	50±1	2.3	53±2	2.3
Blackberry	+ sugar + enzyme	33±1[a*]	1.9	35±2[a*]	2.1
Raspberry	control	60±1	2.4	59±2	2.7
Raspberry	+ sugar + enzyme	41±1[a*]	2.8	43±1[a*]	2.7
Sour cherry	control − pit	73±1	2.8	71±2	2.7
Sour cherry	+ sugar + enzyme/−pit	57±2[b*]	2.7	55±2[b*]	2.9
Sour cherry	control + pit	72±1	2.6	71±2	3.0
Sour cherry	+sugar +enzyme/+pit	57±2[c*]	2.9	58±2[c*]	3.0

IC_{50} Acarbose 77.8±5.7 µg/ml

[a] - statistically significant different from control, *p<0.01

[b] - statistically significant different from control − pit, *p<0.01

[c] - statistically significant different from control + pit, *p<0.01

Table 5. α-glucosidase inhibitory activity of the standards of the selected phenolic compounds: IC_{50} values and the Hill coefficients.

Compound	IC_{50} (M)		n* value	p** value
	Sigmoidal analysis	The Hill analysis		
Epicatechin	$(1.6\pm0.2)\times10^{-4}$	$(1.2\pm0.04)\times10^{-4}$	0.56±0.02	0.52±0.03
Protocatechuic acid	$(2.9\pm0.2)\times10^{-4}$	$(2.9\pm0.07)\times10^{-4}$	0.57±0.03	0.56±0.05

Catechin	$(5.6\pm0.4)\times10^{-5}$	$(5.3\pm0.2)\times10^{-5}$	0.64±0.04	0.61±0.03
Gallic acid	$(2.0\pm0.2)\times10^{-4}$	$(2.3\pm0.1)\times10^{-4}$	0.58±0.02	0.59±0.04
Chlorogenic acid	$(4.8\pm0.4)\times10^{-5}$	$(5.0\pm0.2)\times10^{-5}$	0.43±0.02	0.40±0.03
Caffeic acid	$(3.5\pm0.3)\times10^{-4}$	$(3.7\pm0.1)\times10^{-4}$	0.43±0.03	0.44±0.03
p-Coumaric acid	$(7.6\pm0.6)\times10^{-5}$	$(7.3\pm0.3)\times10^{-5}$	0.36±0.01	0.31±0.02
Ellagic acid	$(7.9\pm0.5)\times10^{-6}$	$(7.8\pm0.3)\times 10^{-6}$	1.26±0.10	1.23±0.10
Rutin	$(6.1\pm0.5)\times10^{-5}$	$(6.4\pm0.2)\times10^{-5}$	0.54±0.03	0.55±0.03

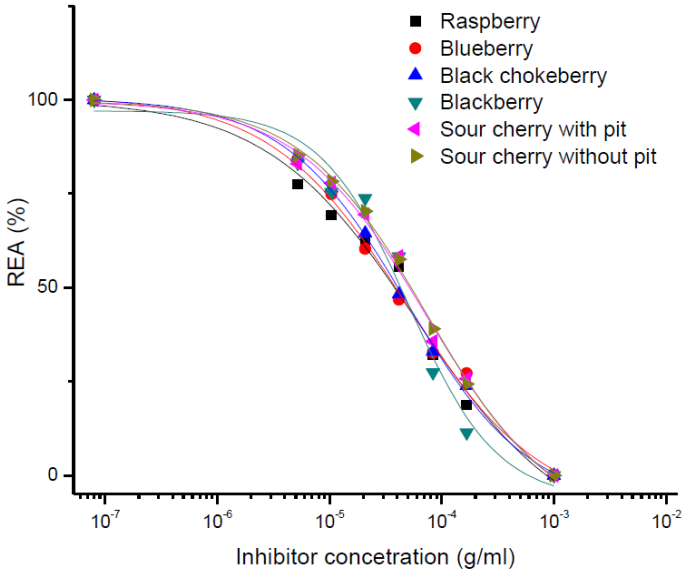
*the Hill coefficient
**parameter of sigmoid function

Figure captions

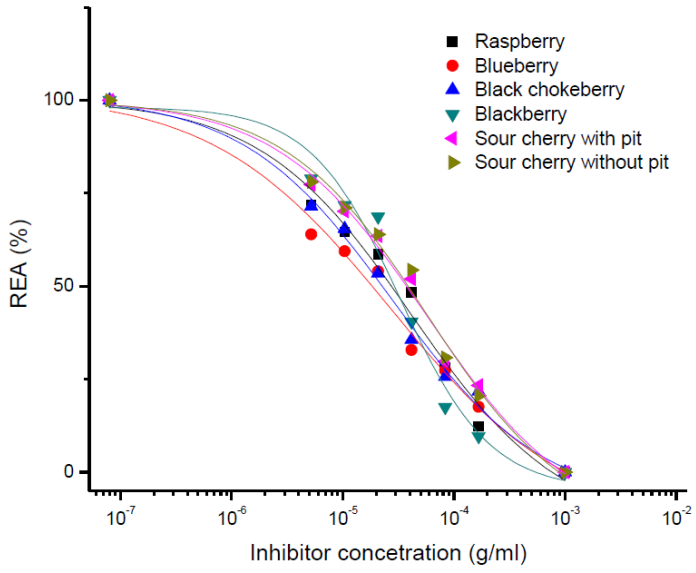
Figure 1. α-Glucosidase inhibitory activity induced by the lyophilisates of the fruit wine samples produced with Lievito Secco EZ FERM yeast, control and +sugar+enzyme (a and b, respectively) (REA - relative enzyme activity).

Figure 2. α-Glucosidase inhibitory activity induced by the lyophilisates of the fruit wine samples produced with ICV D254 yeast, control and +sugar+enzyme
(a and b, respectively) (REA - relative enzyme activity).

Figure 3. α-glucosidase inhibitory activity induced by phenolic compounds (a) and the Hill analysis of the inhibitory curves (b). The given values represent the mean of at least three experiments.

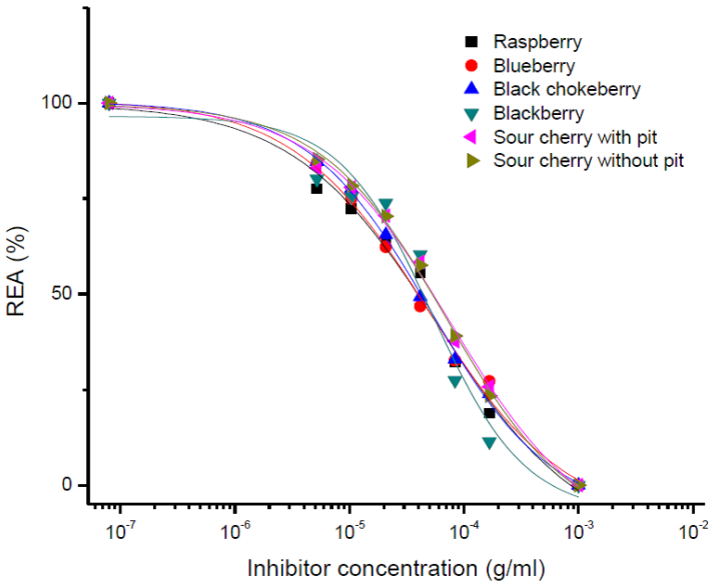


(a)

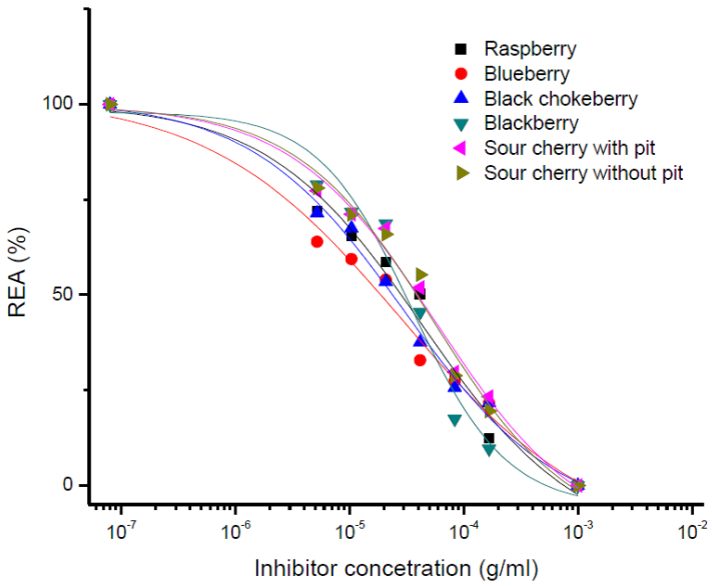


(b)

Figure 1a and 1b

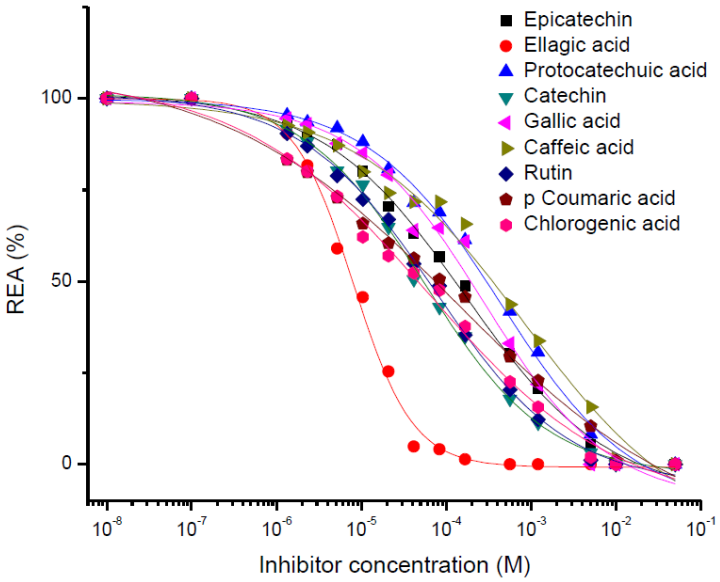


(a)

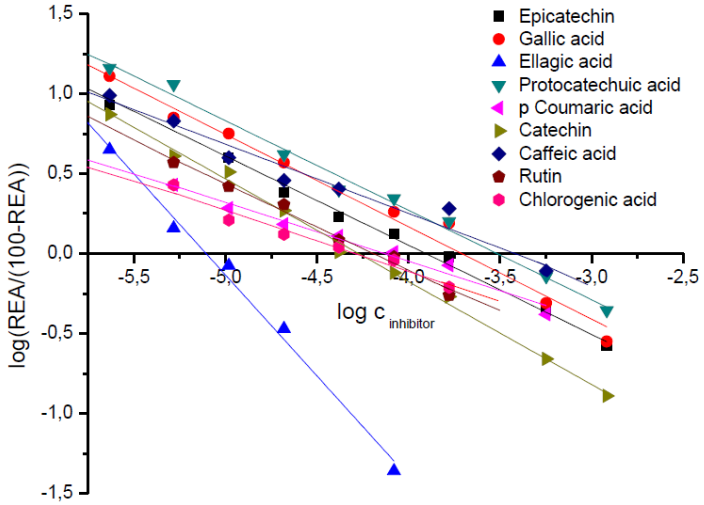


(b)

Figure 2a and 2b



(a)



(b)

Figure 3a and 3b

Graphical abstract