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ORIGINAL PAPER

Fruit juices are effective anti-amyloidogenic agents

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Abstracts

Amyloid fibril formation has been associated with a great variety of human diseases. Fruits contain different important bioactive molecules without causing various undesirable side effects, which are necessary for disease prevention and treatment. Here we report that various fruit juices inhibited the amyloid formation by α-chymotrypsin in aqueous ethanol at pH 7.0. Turbidity measurements, total phenolic content determination, as well as Congo red binding assay were used to analyse the inhibition of amyloid fibril formation. We showed that the black currant juice possessed the strongest inhibitory potential against protein aggregation because it contains the most polyphenolic compounds too and its effect was concentration dependent. Interestingly, white grapes, figs and bananas are relatively effective although they are not high in polyphenols. These fruits are typically sweet. The sugars in them also contribute to their effectiveness. Eating black currant can reduce the likelihood of formation of amyloid fibrils.

Keywords Amyloid fibrils · Black currant · Congo red · Polyphenols

Introduction

It has been found that proteins and peptides have a characteristic tendency to transform from their native state to amyloid aggregates. This phenomenon appears to be in the background of one of the some of the most frequent highly debilitating and currently incurable human disorders, such as Alzheimer's and Parkinson's disease (Zaman et al. 2019; Simon et al. 2012). Stabilizing protein structure may be important. There are various possibilities for this, such as bonding to a solid support, chemical modification and the use of additive molecules (Kotormán et al. 1986, 2003, 2009). Therefore, it is extremely important to find molecules that can inhibit the formation of unwanted amyloid fibrils (Panda and Jhanji 2019; Kasi and Kotormán 2019). Plants are rich in bioactive compounds, which deserve attention. Epidemiological studies suggest that the diet rich in plant polyphenols may decrease susceptibility toward osteoporosis, cardiovascular diseases, cancer, diabetes and neurodegenerative diseases (Pandey and Rizvi 2009; Rose et al. 2018). So far, more than 8000 natural polyphenols have been identified in plants such as flavonoids, phenolic acids and derivatives, tannins, coumarins, stilbenes, lignans and lignins (Freyssin et al. 2018; Kotorman et al. 2017). Since these natural molecules might have lower toxicity than synthetic chemical compounds, they may be useful in treating various incurable diseases (Dhouafli et al. 2018). Sylimarin, a mixture of flavonoids, can inhibit Aβ polymerisation (Guo et al. 2019). By consuming fruits rich in polyphenol compounds, the risk of developing Alzheimer's disease and dementia can be reduced (Rehman et al. 2019). Ferulic acid, also common in fruits, inhibited insulin fibrillation (Jayamani and Shanmugam 2014). Neuroprotective molecule resveratrol present in grape juice (Colica et al. 2018). In addition to resveratrol, grapes contain other anthocyanins, catechins, flavonols and polyphenols. Recent research shows that whole-grape products protect against aging-related diseases (Singh et al. 2015). Raspberries, cranberries and grapes have been found to be effective in inhibiting islet amyloid polypeptide amyloid aggregation (Kao et al. 2015). Anthocyanins are water-soluble phenolic compounds in plants (Siatka 2018). Blackberry, blueberry, cranberry, black raspberry, red raspberry and strawberry extracts containing anthocyanins have

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inhibited both methylglyoxal- and thermal-induced Aβ fibrillation (Ma et al. 2018). The chromatographic analysis has shown that the catechins are the major polyphenols in the sloe extract (Alejandre et al. 2019). The results of Fraternale et al. (2009) have shown that the three most representative anthocyanins of sloe juice are cyanidine-3-rutinoside, peonidine-3-rutinoside and cyanidine-3-glucoside. In addition to anthocyanides, sloe contains phenolic acids (neochlorogenic and caffeic acids) and flavonoids (quercetin and myricetin) (Veličković et al. 2014). Cyanidin-3-rutinoside has reduced insulin fibrillation (Saithong et al. 2018). Quercetin has inhibited amyloid fibrillation of bovine insulin and has destabilized preformed fibrils (Wang et al. 2011). Myricetin has inhibited islet amyloid polypeptide aggregation (Zelus et al. 2012). That is, the numerous bioactive components present in sloe are considered anti-amyloidogenic compounds. Black currant is a particularly rich source of biologically active compounds, such as large amounts of anthocyanins (delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside, and cyanidin-3-rutinoside), quercetin, myricetin, kaempferol, epicatechin, catechin (Paunović et al. 2017). The main ingredient of black currant is cyanidine-3-glucoside (Laczkó-Zöld et al. 2018). Delphinidin appears to be particularly important in inhibiting the formation of α-synuclein filament (Freyssin et al. 2018). Cyanidine-3-O-glucoside and cyanidin-3-rutinoside are neuroprotective compounds (Qin et al. 2013; Saithong et al. 2018). Black currant is a rich source of vitamin C in addition to polyphenols (Khan et al. 2014). Increased intake of vitamin C can protect against Alzheimer's disease-like pathologies (Kook et al. 2014).

Regular consumption of fruits rich in polyphenolic compounds is recommended for human nutrition as they contribute to reducing the risk of neurodegenerative diseases.

The aggregation of α-chymotrypsin in aqueous ethanol is non-physiological, but it can be used to detect the aggregation inhibitory efficiency of an agent (Kotormán and Bedő 2020).

Materials and methods

Materials

Three times crystallized, lyophilized α-chymotrypsin (EC 3.4.21.1) from bovine pancreas was purchased from Sigma-Aldrich Ltd. (Budapest, Hungary). Folin-Ciocalteu's phenol reagent was the product Merck Ltd. (Darmstadt, Germany). The fruits used were purchased at the local market.

Preparation of the fruit juices

The fruits were cut into small pieces and then squeezed with a hand fruit press. The resulting fruit juices were centrifuged (1 min, 13,000 rpm) and the supernatants were used for further testing.

In vitro formation of α-chymotrypsin fibrils

The α-chymotrypsin samples were incubated for 1 day at 24 °C in the presence of 55% ethanol/10 mM phosphate buffer at pH 7.0 in the presence and absence of different fruit juices.

Turbidity measurements

Turbidity of α-chymotrypsin aliquots in the absence and presence of various fruit juices were studied by using Cecil CE-5501 UV–visible spectrophotometer in a cuvette of 1 cm path length. The turbidities of the samples were determined by measuring the change in the absorption at 350 nm. Before the measurements samples were incubated at 24 °C for 24 h. The protein concentration was 0.15 mg/ml in 55% ethanol/10 mM phosphate buffer at pH 7.0. Prior to the experiments, blank corrections were made for the solutions without the enzyme.

Aggregation kinetics

The aggregation kinetics assays were performed in 55% ethanol/10 mM phosphate buffer (pH 7.0) at 0.15 mg/ml α-chymotrypsin concentration in the presence and absence of black currant juice. The increase of the absorption at 350 nm was followed for 1200 s using Hitachi U-2000 spectrophotometer.

Determination of total phenolic content

The total phenol contents of the fruit juices were measured by the Waterhouse method with Folin Ciocalteu reagent (Waterhouse 2002). The absorptions of the samples were measured at 765 nm against the reagent blank. All polyphenol contents are given in mg GAE/l. The calibration line was prepared with gallic acid in the range 0–50 mg/l.

Congo red (CR) binding assay

Upon the binding of CR to amyloidogenic proteins, an increase in absorption intensity and a characteristic red shift of the absorption maximum can be observed (Kotormán et al. 2015). CR solution was prepared in a 5 mM phosphate buffer (pH 7.0) containing 150 µM NaCl. Then, 200 µl aliquot of the aggregated sample was mixed with 800 µl CR solution. The absorption spectra were then recorded using a Hitachi U 2000 UV–visible spectrophotometer after 15 min incubation time between 400 and 600 nm in a 1 cm pathlength cuvette.

Statistical analysis

All experimental data were determined as the mean ± standard error (SEM) of the mean of three independent measurements. Significance was determined by one-way analysis of variance (ANOVA).

Results

α-Chymotrypsin aggregation

The aggregation of α-chymotrypsin in 55% ethanol at pH 7.0 was measured by using turbidity measurements at 350 nm. The turbidity measurements were performed in the presence and absence of 21 different fruit juices after 24 h incubation at 24 °C as shown in Fig. 1. Maximum absorption in the absence of fruit juice indicated the formation of protein aggregates. Lower absorption at 350 nm in the presence of fruit juice indicated the effectiveness of the inhibitory agent. The total polyphenol contents of white grapes, figs and bananas were relatively low, but they had significant inhibitory effect. This was because these fruits were sweet and polyhydroxy compounds were also able to stabilize protein structure (Simon et al. 2002). The more stable the protein structure was the less prone it was to fibril formation. All the investigated fruit juices were efficient, but the best inhibitory agent was the black currant juice. It reduced fibril formation by 58.5% at 50 fold dilution. The black currant juice contained the most total phenolic content too among the fruit juices tested: 4361.3 ± 222.1 mg GAE/l.

We found that the aggregation inhibiting effect of black currant juice depended on the concentration as suggested by reduced absorption intensity with a maximum reduction seen at the highest concentration: 25 times diluted juice (Fig. 2). The statistical results of the different dilutions (diluted 25, 50, 100, and 200 times) were as follows [$F(1,4) = 211.20$, $p < 0.001$], [$F(1,4) = 136.46$, $p < 0.001$], [$F(1,4) = 19.48$, $p < 0.05$], [$F(1,4) = 8.47$, $p < 0.05$].

The aggregation kinetics assays were performed in the absence and presence of black currant juice diluted 200 and 100 times (Fig. 3). These measurements also support the concentration-dependent effect of black currant juice.

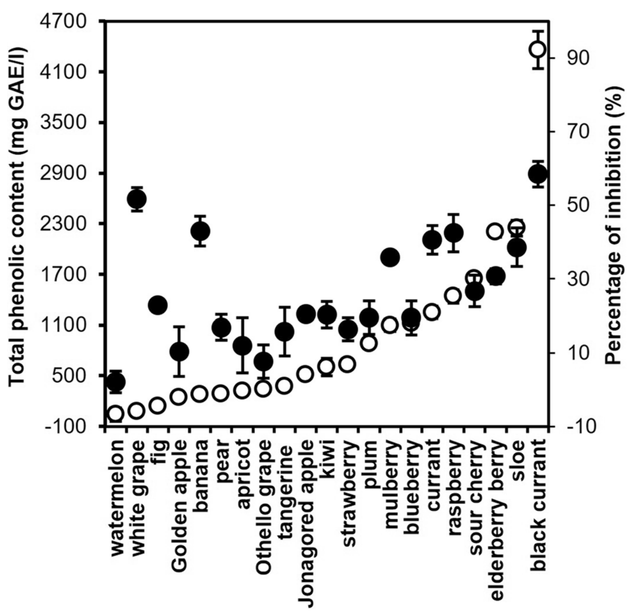

Fig. 1 Change in the percentage of inhibition in 55% ethanol (●) with the total phenolic content (o). Fruit juices were diluted 50 times in distilled water. Protein concentration was 0.15 mg/ml. All data were presented as mean ± standard error of the mean (SEM) from three independent measurements

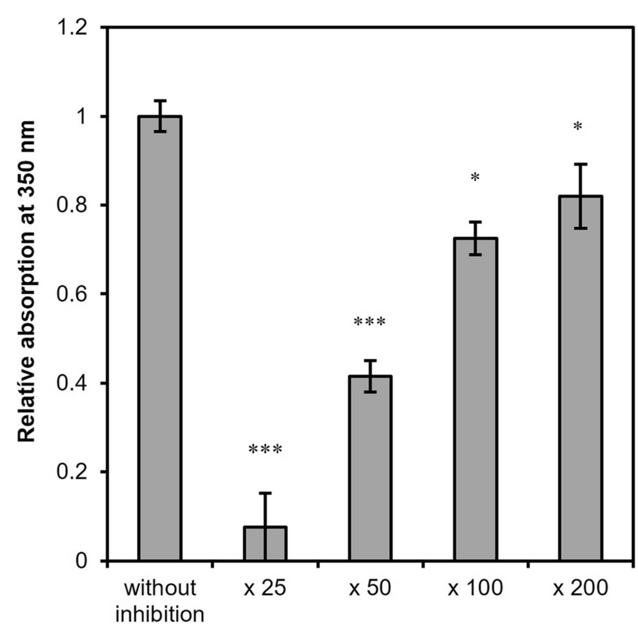

Fig. 2 Turbidity measurements in the absence and presence of different concentration of the black currant juice by recording the absorption at 350 nm at 0.15 mg/ml α-chymotrypsin concentration in 55% ethanol at pH 7.0. Each bar represents the average of at least three independent measurements. All data are presented as mean ± standard error of the mean (SEM). Significance was defined as $^{***}P < 0.001$ and $^{*}P < 0.05$

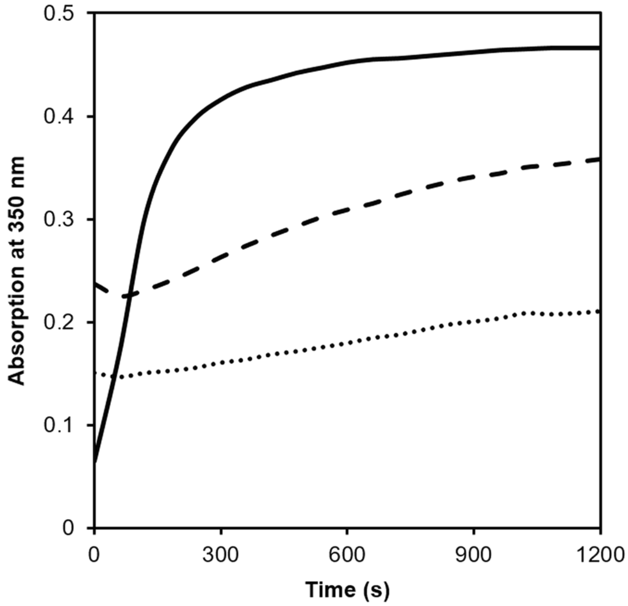

Fig. 3 Kinetics of the aggregation of α-chymotrypsin without black currant juice (solid line) in 55% ethanol at pH 7.0, or in the presence of black currant juice diluted 200 times (dashed line), or 100 times (dotted line). Protein concentration was 0.15 mg/ml

CR binding assay

The inhibition activity of the black currant juice on amyloid-like fibrillation was characterized by CR binding assay (Fig. 4). Without the black currant juice the characteristic changes in CR spectra indicated the presence of a significant amount of amyloid aggregates and its was also apparent that the inhibitory effect of the black currant juice was concentration-dependent.

Discussion

The fibril-forming and toxicity-reducing effects of plant biophenols has been demonstrated (Ayaz et al. 2019; Omar 2017). A fruit-rich diet helps prevent Alzheimer's disease (Ravi et al. 2019). The detection of amyloid growth is usually done by measuring the turbidity of the solution (Zhao et al. 2016; Kotormán et al. 2018). The efficacy of the inhibitor can be supported when turbidity is reduced in the presence of an inhibitor (Ali et al. 2019). Using the CR dye attached to the β plates, the presence of fibrils can be detected (Sohail et al. 2018). CR binding assay has been extensively utilized to investigate the anti-fibrillation activity of different inhibitors too (Awasthi and Saraswathi 2016).

A comparative study of the total phenolic content and the protein aggregation inhibitory effect may be useful in selecting a more efficient fruit. Out of the tested 21 fruit juices, black currant juice has been shown to be the most effective

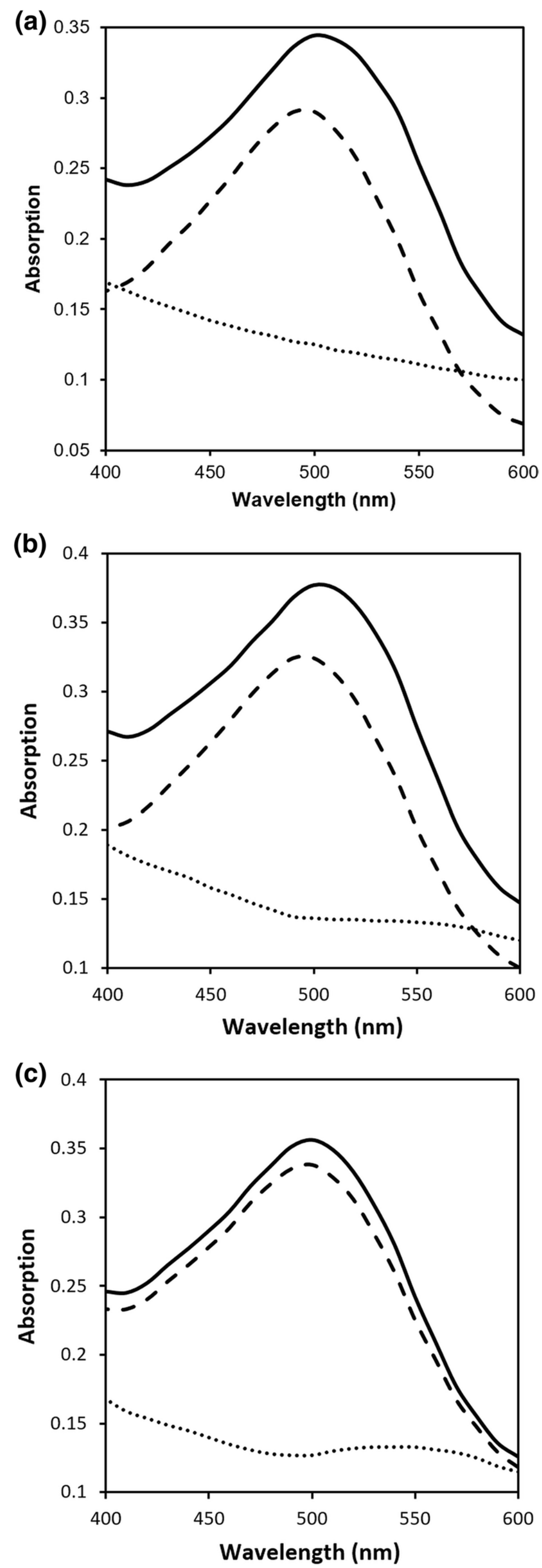

Fig. 4 CR absorption spectra of α-chymotrypsin in the absence (**a**) and presence of the black currant juice diluted 100 times (**b**) and 50 times (**c**): α-chymotrypsin + CR (solid line), CR alone (dashed line), α-chymotrypsin alone (dotted line)

in inhibiting aggregation, using both methodologies. The fact that black currant juice has a significant effect even at a 200-fold dilution proves that consuming this fruit can help prevent the formation of fibrils, which also contains the most polyphenolic compounds.

Conclusion for future biology

Fruits contain several bioactive compounds that can help prevent protein conformational diseases, therefore their consumption is beneficial. Among the 21 fruits examined, black currant has been found to be particularly effective.

Authors' contributions DR, BA, OP and KM carried out the experimental work, the manuscript was written by MK.

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Data accessibility The data sets supporting this article have been uploaded as part of the Supplementary Material.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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