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Avocado Juice Prevents the Formation of Trypsin Amyloid-Like Fibrils in Aqueous Ethanol

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Abstract

In this work fruit and vegetable juices were analyzed for their ability to prevent the aggregation of trypsin using turbidity measurement. Fruit and vegetable juices are capable of inhibiting the aggregation of PMS-trypsin in aqueous ethanol. Among the juices examined, avocado was found to be the most effective. Choline bitartrate was investigated for its ability to inhibit the fibrillation of PMS-trypsin. We have found that avocado juice and choline bitartrate have an inhibitory effect on the formation of trypsin amyloid-like fibrils using Congo red-binding assay.

Keywords

amyloid-like fibrils, avocado, choline bitartrate, Congo red, inhibitory effect, trypsin

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The formation of amyloid fibrils that have high β-sheet contents are associated with many human diseases such as Alzheimer's disease (AD), Huntington's disease, Parkinson's disease, Creutzfeldt-Jacob disease, and amyotrophic lateral sclerosis.[1-3] Alzheimer's disease is the most common neurodegenerative disease in aging caused by the aggregation of amyloid-β-peptide into amyloid fibrils.[4] Almost all the medications tested for AD showed hardly any efficacy. There is a great need for therapies that prevent and/or slow AD progression.[5] Many plant extracts have been investigated for effectiveness against AD.[6] There is an aspiration to seek compounds with antifibrillation activity.[7-10] Protein conformational diseases worldwide make millions of lives more difficult. There are many antioxidants and/or anti-inflammatory nutrients of food that can either help alleviate or delay the onset of the disease. Inadequate nutrition patterns can help the disease develop, while a healthier style such as the Mediterranean Diet can improve the body's mechanism to prevent AD.[11,12] Dietary fatty acids and antioxidants can contribute to reducing the risk of dementia.[13] Natural products isolated from fruit extracts have many positive health benefits that show features of anticancer, antiaging, and antiobesity properties. Earlier studies have shown that many fruits can inhibit protein aggregation.[14] Ferulinic acid, found in many vegetables and fruits, has been reported to inhibit effectively the formation of numerous amyloid fibrils.[15] More than 80% of the population in developing countries use traditional herbs to treat various diseases. The avocado (*Persea americana*) is consumed for its high nutritional value and favorable health effects.

Avocado is an easy-to-access fruit with many essential nutrients and valuable phytochemicals. Avocado is a rich source of different vitamins such as A, B_6, C, E, K_1, and choline (B_8). There are various phytosterols and phytostanols, phenolics and polyphenolic compounds, seven-carbon sugars, minerals, carotenoids, fatty acids, and proteins in it.[16,17] The phenolic compounds in avocado pear, such as epicatechin, epigallocatechin, lupeol, ferulic acid, epigallocatechin-3-*O*-gallate, *p*-hydroxybenzoic acid, apigenin, and naringenin, could have contributed to the observed medicinal properties of the plant.[18] Sixty-one phenolic and other polar compounds were identified in avocado peel.[19] A number of studies have shown that polyphenolic compounds inhibit the fibril formation.[20-24] The antihypertensive, lipid-lowering, antiobesity, antidiabetic, antiatherosclerotic, antithrombotic, and cardioprotective effects of avocado have been demonstrated in many studies.[16] The avocado fruit phytochemicals also can provide a beneficial nutrition strategy for cancer prevention.[25] Avocado is a source of unique antioxidants with potential neuroprotective effect.[26]

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B6 vitamin and choline are neuroprotective.[27] Plant phytosterols are naturally occurring cholesterol equivalents that are able to pass through the blood-brain barrier. The most widespread plant steroids are the biologically active stigmasterol, sitosterol, brassicasterol, and campesterol. Stigmasterol reduces amyloidogenic processing and, therefore, may be advantageous in AD.[28] The diversity of bioactive nutrients present in avocados plays a key role in the prevention and treatment of various neurodegenerative diseases.[29] Natural compounds can be a good starting point for discovering protein misfolding inhibitors.[30]

In our experiments, trypsin modified with phenylmethylsulfonyl fluoride (PMSF) was used as a model protein. Phenylmethylsulfonyl-trypsin amyloid-like fibrils were prepared as previously reported by Kasi et al.[31] Earlier we had showed that PMS-trypsin forms amyloid-like fibrils in 60% ethanol/10 mM phosphate buffer (pH 7.0).[32] The detection of amyloid growth is usually done by measuring the turbidity of the solution.[33] PMS-trypsin was incubated for 24 hours in 60% ethanol in either the absence or presence of different juices diluted 50 times at pH 7.0. Among the fruit and vegetable juices examined, avocado was found to be the most effective in inhibiting trypsin aggregation. In the presence of a 50-fold dilution of avocado juice, the amount of aggregates decreased to 20% relative to the sample not containing it (Figure 1).

It was found that the amount of amyloid-like fibril formation was greatly reduced with increasing concentration of avocado juice as demonstrated by turbidity measurements at 350 nm. Based on our findings, we determined that the antiamylodigenic effect of avocado juice was dependent on its concentration (Figure 2).

The absorption spectrum of the amyloid-specific Congo red (CR) aqueous solution shows a maximum absorption at 490 nm. If the CR molecules bind to β-sheet-rich amyloid fibrils, this results in a characteristic increase in absorption and a red shift of absorption maximum from 490 to 540 nm.[34] When the sample contains some amyloid material, the resulting difference spectrum shows a peak at 540 nm, indicating a red shift characteristic of amyloid-bound CR.[35] Congo red binding experiments showed that the antiamyloidogenic activity of avocado juice is concentration dependent. Avocados contain a

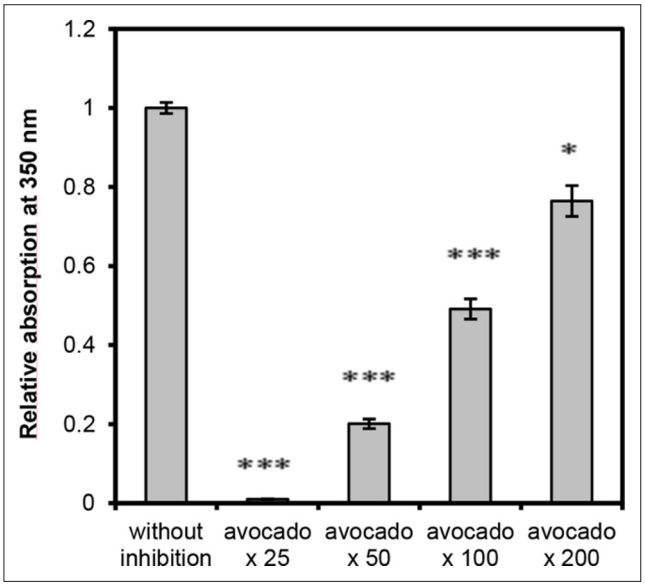

Figure 2. Turbidity at 350 nm of PMS-trypsin in 60% ethanol in the absence and presence of different concentrations of avocado juice. Protein concentration: 0.13 mg/mL. All data are presented as mean ± standard error of the mean from 3 independent measurements. Significance was defined as ***$P < 0.001$ and *$P < 0.05$.

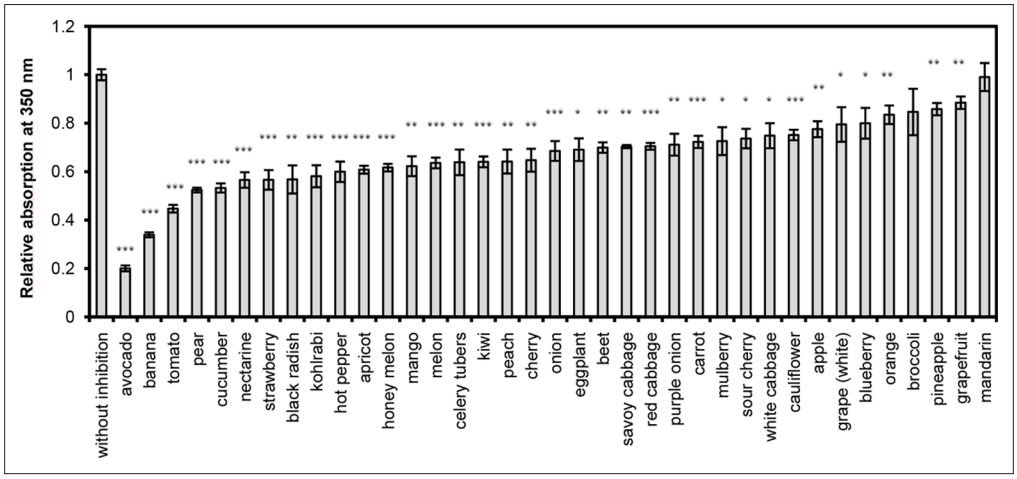

Figure 1. Turbidity measurements of fruit and vegetable samples by recording the absorption at 350 nm. PMS-trypsin (0.13 mg/mL) was incubated in 60% ethanol in either the absence or presence of different juices diluted 50 times at pH 7.0. Each bar represents the average of at least 3 independent measurements. All data are presented as mean ± standard error of the mean. Significance was defined as ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$.

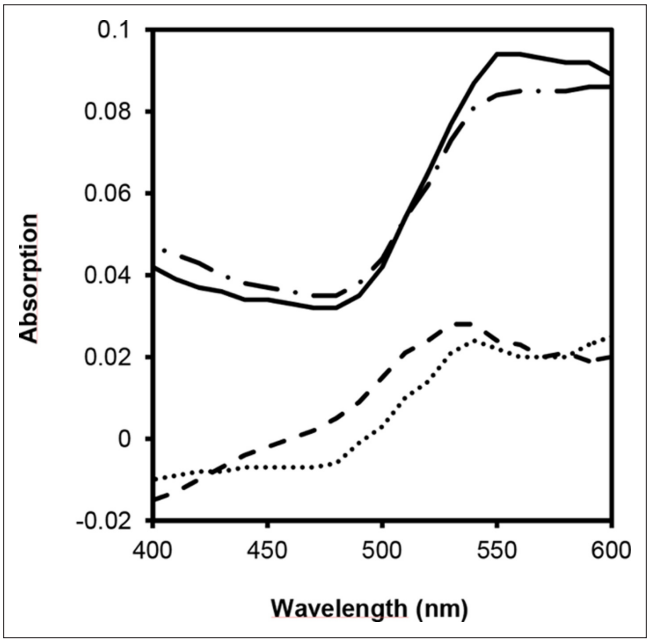

Figure 3. Congo red absorption difference spectra of PMS-trypsin in the absence (solid line) and presence of avocado juice diluted 100 times (dashed line) and 200 times (dotted line), and presence of 9.3 μM choline bitartrate (dashed-dotted line).

very high amount of choline (14.2 mg/100 g),[16] so we also examined the effect of commercially available choline bitartrate on the formation of PMS-trypsin amyloid-like fibrils. We showed that it inhibits fibril formation (Figure 3).

Fuentes et al's experimental results showed that many natural product sources potentially inhibit amyloid aggregation.[36] Data of Mellott et al suggest that dietary supplementation of choline reduces amyloidosis, which may be a preventive strategy for AD during fetal development and early postnatal life.[37] Flicker et al found that B vitamins can lower Aβ40 plasma levels and play a role in AD prevention.[38] According to the present study, avocado juice and choline bitartrate significantly inhibited the formation of PMS-trypsin amyloid-like fibrils, so both could be used effectively as antiamyloidogenic agents.

Experimental

General Experimental Procedures

The absorption of the samples was monitored using a Cecil CE 5501 UV-vis spectrophotometer. The CR absorption spectra were recorded with a UV-vis spectrophotometer (Hitachi U 2000).

Materials

The bovine trypsin (EC 3.4.21.4; from bovine pancreas) was the product of Sigma-Aldrich Ltd. (Budapest, Hungary). The choline bitartrate was purchased from Vital-Trend Ltd. (Budapest, Hungary). All other reagents were of analytical quality. The fruits and vegetables used were bought on the local market.

Preparation of the Juices

The fruits and vegetables were chopped, and then were squeezed with a hand fruit press. The juices were centrifuged for 1 minute at 13 000 rpm and the supernatants used for further studies. The samples were kept in the freezer until used. The supernatants were diluted in distilled water as required prior to the measurements.

In Vitro PMS-Trypsin Fibril Formation

Amyloid-like fibrils were prepared using catalytically inactive PMS-trypsin. The soluble protein samples in the presence of 60% ethanol/10 mM phosphate buffer at pH 7.0 were first mixed by vortexing and then incubated for 24 hours at 24°C to induce amyloid-like fibril formation.

Turbidity Measurements

The turbidity of the fruit and vegetable juice samples was monitored by measuring the absorption at 350 nm. In these experiments, 0.13 mg/mL PMS-trypsin concentration was used. Significance was determined by one-way analysis of variance.

Congo Red-Binding Assay

Congo red-binding assay was used to detect amyloid formation.[39] Two hundred microliters of 1-day-aged samples containing 0.13 mg/mL PMS-trypsin in 60% ethanol/10 mM phosphate buffer at pH 7.0 were mixed with 800 μL of CR (disodium-3,3'[[1,1-biphenyl]-4,4'-diylbis(azo)] bis(4-amino-naphthalin-1-sulfonate)) solution in 5 mM phosphate buffer/150 mM NaCl at pH 7.0, and incubated at room temperature for at least 15 minutes prior to absorption measurement. The absorption spectrum of each sample was recorded from 400 to 600 nm using 1 cm path length quartz cuvettes. The difference spectra were obtained by subtracting the absorption spectrum of CR and the protein alone from the absorption spectrum of CR in the presence of PMS-trypsin amyloid-like fibrils.

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Declaration of Conflicting Interests

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