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Original Article

Anti-glycation Activity of Various Fruits

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Abstract

Background: The antioxidant potential of fruit is well known. Here, we evaluated the anti-glycation effect of several fruits and investigated their potential use as Anti-Aging treatments in cosmetic and food industries.
Objectives: To test flesh and peel fruit extracts for inhibition of albumin and collagen glycation.
Methods: Sample of fruits were dried ground and extracted at 80°C for 1 hour; then added to two *in vitro* models of glycation; glucose and human serum albumin (HSA); and glucose and bovine skin collagen type I. The extract mixtures were incubated at 60°C and the fluorescence (excitation 370nm/ detection 440nm) measured after 40 hours and 10 days using the ARVO^{TM} MX 1420 ARVO series multilabel counter, Perkin-Elmer.
Result: We found *Punica granatum* (pomegranate) and *Garcinia mangostana* (mangosteen) peels displayed higher anti-glycation activity ($IC_{50} \leq 0.04$ mg/mL) than aminoguanidine in the HSA models; the activity of *Citrus aurantifolia* (lime) flesh and *Pseudocydonia sinensis* (karin/Chinese quince) flesh was lower than pomegranate and mangosteen. We found pomegranate peel, *Stauntonia hexaphylla* (mube) peel, mangosteen peel, and *Malus pumila* (apple) variety "*san fuji*" peel displayed the highest anti-glycation activity in the collagen model. The apple varieties; "*san jonagold*", "*alps otome*", "*sekai ichi*" were more active than other apple varieties. Peel samples were twelve times more active than flesh samples in the collagen model, and seventeen times more active in the HSA model.
Conclusion: Peel samples returned higher anti-glycation activity than flesh samples, and apple peel may be a low-cost raw material for cosmetic and food industries with potential to reduce glycation stress.

KEY WORDS: glycation stress, glucose, albumin, collagen, aminoguanidine

Introduction

Glycation stress is one of the risk factors for aging inside and outside of the body. Aging can most often be seen in skin, where protein glycation and glycoxidation end product formation cause oxidative stress that damages cell membranes. Protein glycation occurs when blood sugar reacts with protein, such as collagen, to form AGEs, which degrade the collagen in skin [1-4].

Our recent research has focused on ways to inhibit AGEs formation with the objective of treating degenerative changes, promoting health, and mitigating the effect of lifestyle-related disease. Several companies in the cosmetic and food industries have funded research into glycation-inhibiting ingredients with the goal of maintaining a youthful skin and discovering new Anti-Aging treatments. One approach is by searching for anti-glycation and antioxidant agents from natural compounds that inhibit AGEs formation.

Fruits and vegetables are known to contain high concentration of antioxidants and a diet high in these foods should help prevent oxidative stress, and slow the aging process. Several authors have identified anti-glycation and antioxidant agents in plant species; for example: Pomegranate fruit juice (PFE). PFE can be used to delay or prevent onset of diabetes and aging complications [5]. Studies on polysaccharides extract from Longan (*Dimocarpus longan*) pericarp showed 60.4% anti-glycation activity [6]. A complex mixture of blueberry(*vaccinium sp*) polyphenols increased the lifespan and slowed aging related declines in *Caenorhabditis elegans*. Other research found robust and reproducible evident that the polyphenolic compounds in blueberries benefited aging separately from any antioxidant effects [7]. An 8-week program that provided dietary supplements of spinach, strawberry or blueberry extracts effectively reversed age-related deficits in neuronal function and behavior in 19 month F344 rats [8]. This research demonstrated that natural compounds from blueberries can provide Anti-Aging benefits *in vivo* in live animal. Other research suggests that a combination of antioxidant/anti-inflammatory polyphenol compounds found in fruits and vegetables may effectively reverse aging [9].

Fruit peels are known to be richer in phenolic compounds with good antioxidant activities than the fruit flesh. *Actinidia chinensis* (gold kiwi fruit) peel contains high concentrations of phenolic and flavonoid compound that produce high antioxidant

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activity; kiwi fruit extracts have potential as anti-skin aging agent. Similarly, pomegranate peel (*Punica granatum*) has been reported to possess antioxidant and inhibit tyrosinase [4, 10].

Here, we evaluated the anti-glycation effects of seventy-four fruit species and varieties to assess their potential as anti-glycation products. We focused on apple varieties because Apple is a worldwide commoditiy; you can find it almost everywhere and not to expensive. Also while some other fruit's peel cannot be consume, apple's peel can. And previous research found the antioxidant activity of apple peel was approximately 83 µmol vitamin C equivalents. This means that the antioxidant activity of 100 g apples (about one serving of apple) is equivalent to about 1,500 mg of vitamin C, although, apples only contain about 5.7 mg vitamin C per 100 g fruit [11,12]. Apple diet reduced aging in fruit fly models and Sunagawa et al found that apple procyanidins could extend the mean lifespan of *Caenorhabditis elegans* by 8%-12% [13,14].

Apple peels also contain two to six times (depending on the variety) more phenolic compounds, and two to three times more flavonoids than the flesh. The antioxidant activity of the peel was two to six times greater than the flesh, depending on the variety of the apple [12,15-16]. Leontowicz et al found that lipid peroxidation inhibition and plasma antioxidant capacity were higher in rats fed peel compared to rats fed apple flesh [12,17].

Methods

In vitro models of glycation using glucose and human serum albumin (HSA), and glucose and bovine collagen type-I, were used to test the inhibition of AGEs formation by fruits. The fluorescence was measured using the ARVO™ MX 1420 ARVO series multi-label counter, Perkin-Elmer [18].

Extract preparation

Seventy-four fresh fruits, including citrus fruits and apple varieties were collected from a local supermarket. Samples were dried at 65°C for 72 hours, then ground and extracted with distilled water at 80 °C in a water bath for one hour. The concentration of each sample was estimated from the weight difference, before and after incubation of 5ml sub-samples, dried in aluminum trays at 120 °C for 1.5 hours.

Glycation models

The HSA model was prepared by incubating HSA with and without glucose at 60 °C for 40 hours as previously reported [18]. Similar methods were also reported at 37 °C for 3-14 days [19-21] and the amount of AGEs generation was approximately as same as the amount generated after incubation at 60 °C for 40 hours [22]. The glucose (+) reaction solution contained 0.1M phosphate buffer (pH 7.4), 40 mg/mL HSA (Sigma-Aldrich Chemical Ltd, MO, USA), 2.0M glucose solution, and distilled water at a 5:2:1:1 volume ratio. The glucose (–) reaction solution contained 0.1M phosphate buffer (pH 7.4), 40 mg/mL HSA, and distilled water at a 5:2:2 volume ratio.

100µL of each test sample (fruit samples of 1 hour extract, aminoguanidine, or water (control)) were added to 900 µL of glucose (+) or Glucose (–) HSA solution. After 40 hours incubation, sample solution (200 µL), distilled water (200 µL), and 5µg/mL quinine sulfate (200 µL), were dispensed into a black micro-plate; the fluorescence (excitation 370nm/ detection 440nm) was measured using ARVO™ MX 1420 ARVO series multi-label counter, Perkin-Elmer [2,18].

The inhibitory activity of each sample was calculated from:

Inhibitory activity against fluorescence AGEs (%) = (1-Glu (+) sample-Glu (–) sample) / (Glu (+) control-Glu (–) control) ×100

the 50% inhibitory concentration (IC_{50}) against fluorescence AGEs was calculated from a regression curve of the inhibitory activity at three concentrations for each sample (n = 3).

The glycation of bovine skin collagen type-I was modeled by incubating collagen with and without glucose at 60 °C for 10 days. The glucose (+) reaction solution contained 0.1M phosphate buffer (pH 7.4), 3 mg/mL bovine skin collagen type-I (Nippi, Adachi-ku, Tokyo, Japan), and 2.0M glucose solution at a 5:2:2 volume ratios. The glucose (–) reaction solution contained 0.1M phosphate buffer (pH 7.4); 3 mg/mL bovine skin collagen type I, and distilled water at a 5:2:2 volume ratio.

The activity of the extracts in the collagen model was measured using the same formula as the HSA model. The activity of each extract was compared with the activity of aminoguanidine [2].

Statistical analysis

Mann-Whitney U test were calculated with SPSS Statistics 21 statistical analysis software (IBM, Somers, NY), with a two-sided significance level of 5%.

Results

Inhibition activity of rind and pulp samples

The IC_{50} of aminoguanidine was 0.063 mg/mL in the HSA and 0.232 mg/mL in the collagen model (***Table 1***). In the HSA model, pomegranate and mangosteen peels were the most active, followed by lime and Karin. The flesh and peel samples of pomegranate, buntan, sudachi, and hassaku were more active against glycation activity than other fruits. This result was not compared with the fruit that do not have peel sample.

In the bovine skin collagen type 1 model, pomegranate, mube, mangosteen peels, apple "*sanfuji*"peel, and karin all inhibited glycation. The flesh and peel of pomegranate, persimmon "*kinokawagaki*", sudachi, mube, and yuzu were more active against glycation activity than the other fruit samples.

Cherry (sato nishiki), pear (kosui), sweet tamarind and some other fruit were more active against HSA glycation than collagen glycation.

Table 1 Inhibition of formation of fluorescence AGEs by fruit samples. The results ranked in order of the inhibitory activity (IC_{50}) of the flesh samples in the HSA model.

No	sample name : variety	scientific name	IC_{50} HSA		IC_{50} collagen		detail
			flesh	Peel	flesh	peel	
1	aminoguanidine		0.063	*1)	0.232	*	
2	Lime	*Citrus aurantifolia*	0.147	*	0.435	*	
3	Karin	*Pseudocydonia sinensis*	0.202	*	0.149	*	
4	star fruit	*Averrhoa carambola*	0.211	*	0.376	*	flesh sample contain also peel
5	passion fruit	*Passiflora edulis*	0.227	*	2.871	*	flesh sample contain seed
6	feijoa (pineapple guava)	*Feijoa sellowiana*	0.231	*	0.264	*	
7	strawberries	*Fragaria × ananassa*	0.288	*	0.607	*	flesh sample contain seed+peel
8	blueberries	*Vaccinium corybosum*	0.293	*	0.207	*	flesh sample contain also peel
9	cherry : sato nishiki	*Prunus avium*	0.355	*	60.417	*	
10	Banana	*Musa sp.*	0.358	*	0.427	*	
11	Fig	*Ficus carica*	0.390	*	3.118	*	
12	melon : ars	*Cucumis melo var cantalupensis*	0.417	*	7.758	*	
13	pear : kosui	*Pyrus pyrifolia*	0.444	*	23.471	*	kikusui × wasekozo
14	Pineapple	*Ananas comosus*	0.450	*	1.253	*	
15	Buntan	*Citrus grandis*	0.462	0.421	2.149	0.424	
16	Lemon	*Citrus × limonium*	0.464	*	0.942	*	
17	grapefruit (yellow)	*Citrus × paradise*	0.490	*	1.405	*	
18	Orange	*Citrus sinensis*	0.491	*	7.388	*	
19	miracle fruit	*Synsepalum dulcificum*	0.511	*	0.810	*	flesh sample contain also peel
20	Sudachi	*Citrus sudachi*	0.527	0.453	0.394	0.214	
21	Kabosu	*Citrus sphaerocarpa*	0.535	*	1.583	*	
22	Yuzu	*Citrus junos*	0.539	0.837	0.376	0.555	
23	kiwi fruit green	*Actinidia diliciosa*	0.550	*	0.588	*	
24	makuwa (korean melon)	*Cucumis melo*	0.576	*	4.436	*	
25	Plum	*Prunus domestica*	0.679	*	3.304	*	
26	grape : delaware	*Vitis vinifera*	0.690	*	1.058	*	
27	mangosteen	*Garcinia mangostana*	0.697	0.040	1.224	0.074	flesh sample contain seed
28	Papaya	*Carica papaya*	0.727	*	1.291	*	
29	pomegranate_	*Punica granatum*	0.750	0.005	0.322	0.033	flesh sample contain seed
30	sweet tamarind	*Tamarindus indica*	0.750	*	25.482	*	
31	Hassaku	*Citrus haisaku*	0.764	0.230	1.835	0.181	
32	shiikwaasaa	*Citrus depressa*	0.775	*	0.453	*	
33	snake fruit	*Salacca zalacca*	0.906	*	0.575	*	
34	watermelon	*Citrullus lanatus*	0.949	*	3.354	*	
35	grape : taiho	*Vitis vinifera*	0.965	*	8.519	*	
36	persimmon	*Diospyros kaki*	1.031	1.142	0.827	0.289	
37	Mango	*Mangifera indica*	1.067	*	1.804	*	
38	apple : fuji	*Malus domestica*	1.077	0.520	37.605	0.560	kokko × dericious
39	zabon (pomelo)	*Citrus maxima*	1.101	0.568	2.828	0.378	
40	kiwano (horned melon)	*Cucumis metuliferus*	1.122	3.548	0	none	flesh sample contain seed
41	Sweetie	*Citrus grandis × C. paradise*	1.134	0.531	62.947	0.238	
42	apple : san-jyonagold	*Malus domestica*	1.136	0.641	1.172	0.339	growing without bags
43	apple: alpsotome_	*Malus domestica*	1.195	0.288	1.001	0.536	fuji × kogyoku
44	Peach	*Prunus persica*	1.327	*	54.357	*	
45	muscat_	*Vitis vinifera*	1.328	1.912	1.849	4.070	
46	pomegranate	*Punica granatum*	1.347	*	2.034	*	juice
47	kiwi fruit (gold)	*Actinidia chinensis*	1.450	*	7.752	*	
48	kinkan(kumquats)	*Citrus japonica*	1.464	*	1.194	*	flesh sample contain also peel
49	coconut_	*Cocos nucifera*	1.466	*	0.181	*	
50	apple : sekaiichi_	*Malus domestica*	1.620	0.445	0.645	0.564	dericious × golden dericious
51	white sapote	*Casimiroa edulis*	1.872	*	1.379	*	
52	pear : aurora	*Pyrus communis*	2.087	*	10.186	*	
53	apple : shinanosweet	*Malus domestica*	2.528	2.028	2.292	*	fuji × tsugaru
54	apple : kogyoku	*Malus domestica*	2.595	0.609	1.967	0.527	
55	apple : jyonagold_	*Malus domestica*	2.876	0.529	4.162	0.995	golden dericious × kogyoku
56	apple : san-mutsu_	*Malus domestica*	2.962	0.701	2.826	0.747	growing without bags
57	Mube	*Stauntonia hexaphylla*	3.200	0.293	0.803	0.052	Flesh sample contain seed
58	persimmon : kinokawa	*Diospyros kaki*	3.447	1.167	0.236	0.215	
59	grapefruit (red)	*Citrus × paradise*	3.451	0.432	1.893	1.070	
60	Coconut	*Cocos nucifera*	4.093	*	0.218	*	water
61	Iyokan	*Citruc iyo*	4.157	0.875	2.222	0.900	
62	unshiu (mikan) : arida	*Citrus unshiu*	6.567	0.974	1.042	0.301	
63	grape : kaiji	*Vitis vinifera*	6.587	0.942	4.807	1.004	
64	apple : toki_	*Malus domestica*	6.808	0.427	1.981	0.268	orin × fuji
65	apple : yoko	*Malus domestica*	7.108	0.527	17.766	0.671	
66	apple : akibae_	*Malus domestica*	7.192	0.643	3.461	0.495	sensyu × tsugaru
67	apple : san-fuji	*Malus domestica*	7.742	0.262	3.233	0.098	growing without bags
68	apple : meigetsu	*Malus domestica*	7.899	1.101	67.213	5.069	akagi × fuji
69	pear : hosui	*Pyrus.pyrifolia*	8.658	2.590	35.035	1.191	(kikusui × yakumo) × yakumo
70	apple : orin	*Malus domestica*	8.743	0.979	3.276	1.461	golden dericious × india
71	apple : hokuto	*Malus domestica*	9.139	0.562	20.118	0.826	fuji × mutsu
72	akebia	*Akebia quinata*	12.974	0.218	1.026	0.244	
73	apple : mutsu	*Malus domestica*	18.112	0.489	2.556	0.529	golden dericious × india
74	dragonfruit	*Hylocereus undatus*	33.910	0.253	10.996	3.600	flesh sample contain seed
75	pear : atago__	*Pyrus communis*	45.531	4.055	9.971	1.324	
76	melon : raiden	*Cucumis melo*	47.176	*	8.011	*	
77	apple : koutoku/komitsu	*Malus domestica*	109.568	1.620	3.513	0.524	fuji × rom16

(*) samples are not available

IC_{50}; 50% inhibitory concentration expressed in mg/mL.

Inhibition activity of apple varieties

Several apple varieties presented higher inhibitory activity than others. In the HSA model the activity of the peel from; "*san fuji*", "*alps otome*", "sekai ichi",and "*toki*" was higher than that in other varieties, although four to seven times lower than the anti-glycation activity of aminoguanidine (***Fig. 1***). In the bovine skin collagen type I model, the activity of the peel from; "*sanfuji*" was two times higher than aminoguanidine. "*toki*", and "*sanjonagold*" peels also showed high anti-glycation activity similar to aminoguanidine.

The inhibitory activity of flesh was not as strong as the activity of the peel samples (***Fig. 2***), and the inhibitory activity of "*alps otome*" and "*sekai ichi*" peels was three to four times greater than the activity of flesh from the same varieties. The results from the collagen model were similar and the inhibitory activity of the peel was about one to two times greater than activity of flesh samples from the same apple variety. The average ratio of flesh: peel IC_{50} was17:1 in the HSA model ($p < 0.001$) and 12:1 in the collagen model ($p < 0.001$).

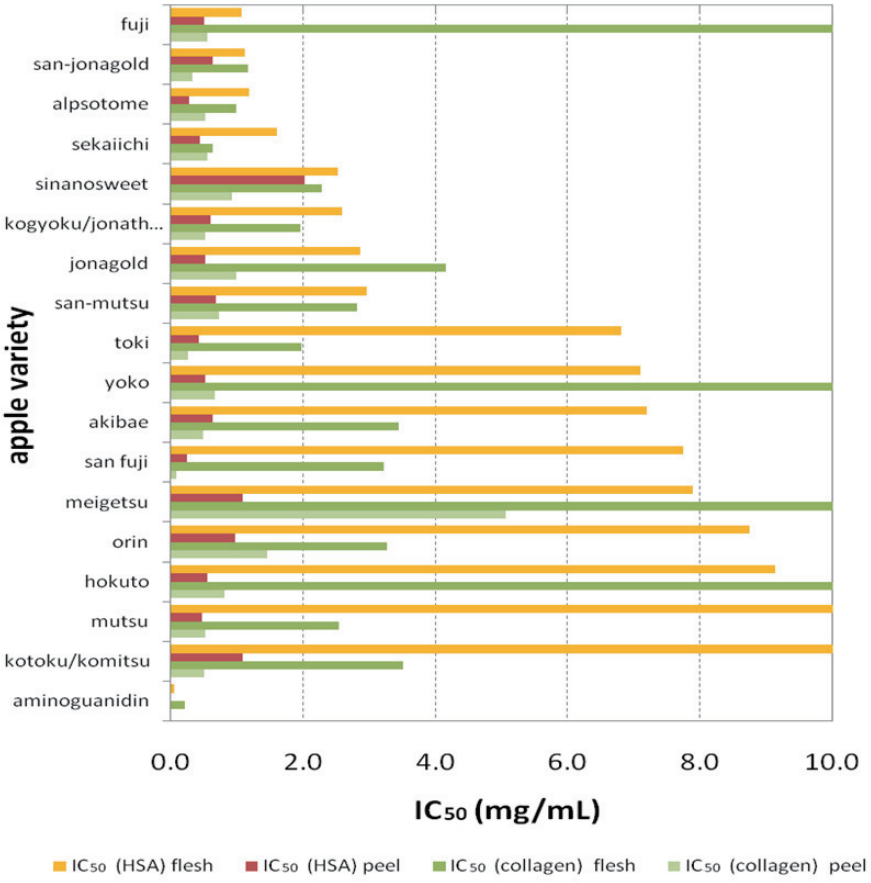

Fig. 1. Inhibitory activity of different apple variety against fluorescence AGEs formation in HSA and bovine skin collagen type I. IC_{50} = 50% inhibitory concentration expressed in mg/mL. HSA = human serum albumin. AGEs = advanced glycation end product. (n = 3).

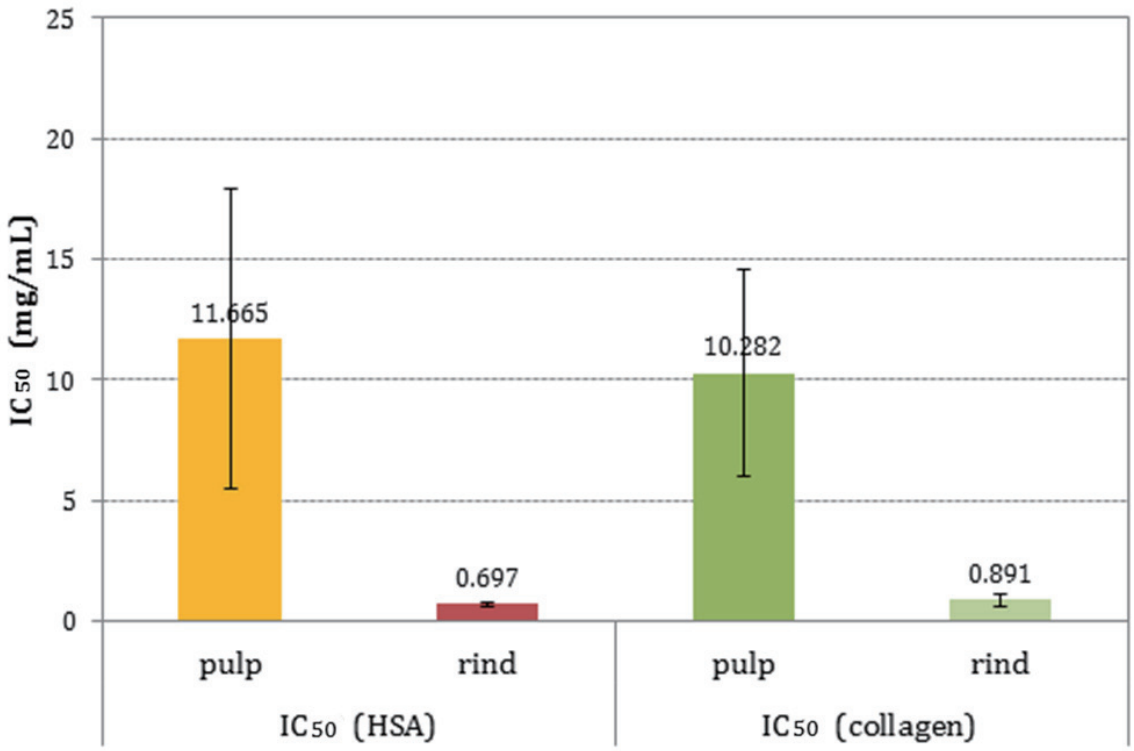

Fig. 2. Inhibitory activity against fluorescence AGEs formation of flesh and peel sample. IC_{50} = 50% inhibitory concentration expressed in mg/mL. HSA = human serum albumin. AGEs = advanced glycation end product. The peel IC_{50} average was17:1 (HSA); 12:1 (collagen). (n = 17, average ± standard error mean).

Discussion

These findings suggest that some fruit, fruit varieties, and some part of the fruit contain different concentrations of glycation activity inhibitors. Pomegranate, sudachi and mangosteen strongly inhibited HSA and collagen glycation; the apple varieties" alps otome", "sekai ichi", and "san jonagold", showed higher anti-glycation activity than other varieties in both glycation models. We have to pay attention to the fact that the *in vitro* experimental results are different from the *in vivo* reaction. However, these *in vitro* glycation models are useful for screening the anti-glycation ingredients from natural compounds as previously reported [22-24].

Several fruit like cherry, pear and sweet tamarind inhibited HSA glycation better than inhibited collagen glycation, this probably due to the difference in amino acid content in each protein or the difference in polyphenol content of the samples.

Kokila *et al.* found Pomegranate exhibited glycation inhibition potential because of its free radical scavenging properties and may also inhibit fructosamine formation by modifying the amino or carbonyl groups in the Maillard reaction. Pomegranate fruit extract (PFE) may act as an antioxidant by supplying hydrogen; hydrogen combines with radicals to terminate the radical chain reactions, although the exact mechanism is unknown [5].

Sudachi, lime, lemon (even though the peel samples are not provided), and yuzu exhibited high anti-glycation activity. These fruits are considered potential anti-glycation agents because they contain high level of antioxidant compounds, such as flavanones, flavones, flavonols, phenolic acids, *etc.* The dominant rutinosyl flavanones include eriocitrin, narirutin and hesperidin, which are abundant in lemon, lime, mandarin and sweet orange [25].

Strawberries and blueberries also possess high anti-glycation activity. Strawberries contain the flavonoid fisetin, which is known to reduce AGEs in mice [26]. These decreases were accompanied by increased activity of the enzyme glyoxalase-1, which promotes removal of toxic AGEs precursors. Fisetin is also found, although in 5- to 10-fold lower concentrations, in apples, persimmons and even smaller amounts in kiwi fruit, peaches, grapes, tomatoes, onions, and cucumbers [26]. The blueberries had high levels of anthocyanidins and proanthocyanidins, which is known for their strong antioxidant activity [27], and these compounds maybe responsible for blueberries anti-glycation activity.

Glycation is a major source of reactive oxygen species (ROS) and reactive carbonyl species (RCS) are generated by oxidative (glycoxidative) and non-oxidative pathways [28]. Reactive dicarbonyl species, such as methylglyoxal (MGO) and glyoxal (GO), have received extensive attention because these compounds are highly reactive and can form AGEs with proteins, phospholipids, and DNA. Dietary flavonoids may inhibit AGEs formation by trapping reactive dicarbonyl compounds; The major bioactive apple polyphenols, phloretin and its glucoside, phloridzin can trap reactive MGO or GO to form mono- and di-MGO or GO adducts under physiological conditions (pH 7.4, 37 °C) efficiently. Scientists report that flavonoids containing vicinyl dihydroxyl groups, such as quercetin and myricetin, could significantly decrease the level of GO during the auto-oxidation of glucose *in vitro* [29, 30].

Apples are major source of polyphenols such as catechin, epicatechin (EC), procyanidins, quercetin glycosides, chlorogenic acid, phloretin, and phloridzin may be able to trap the reactive dicarbonyl species MGO and GO, therefore can inhibit the formation of AGEs. Another type of important dietary flavonoid, chalcone (phloretin and phloridzin) which is major component of dihydrochalcone found in apples also had the potential to prevent AGEs formation [29].

The concentration of these polyphenols differs between apple varieties and tissue (peel or flesh). Procyanidins, catechin, epicatechin, chlorogenicacid, phloridzin, and the quercetin conjugates are found in much higher concentrations in the peel than in the apple flesh [31], which explains why apple peel exhibits higher anti-glycation activity than the apple flesh. The different in hybridization and in polyphenols content and concentration are considered as the cause of different glycation inhibition level in apple varieties.

Although the type and concentration of polyphenol differs in each of these fruits we suggest the polyphenol concentration is largely responsible for fruit anti-glycation activity. Most of the phenolic compounds are found in fruit peels, so the anti-glycation activity of fruit is greater in the fruit peel than in the fruit flesh. Especially for apples, the data indicate that it is reasonable to eat them with peel in order to reduce the glycation stress.

Here we discovered that fruits possessed high anti-glycation activity, however, fruits also high in fructose, which contribute greatly in glycation process more than glucose; thus cause aging and disease. Further research may identify other natural anti-glycation compounds with potential as an Anti-Aging treatment and study its bioavailability *in vivo* is considered important to explore the potential of fruits as an Anti-Aging treatments for food and cosmetic industries and its impact on the prevention of aging and disease.

Conclusions

Pomegranate, sudachi and mangosteen exhibit higher anti-glycation activity in HSA and collagen than other fruits, and pomegranate and mangosteen peels displayed greater anti-glycation activity than aminoguanidine. Citrus fruits (lime, lemon, and yuzu), two berries (strawberry and blueberry), and several apple varieties ("*alps otome*", "*sekai ichi*", and "*san jonagold*") also displayed high anti-glycation activity.

Conflicts of Interest

The authors declare no financial or other conflicts of interest in the writing of this paper.

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