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Antiglycation, Antioxidant and Antidiabetic Activity of Strawberry (*Fragaria × ananassa*) Fruits during Ripening Stages.

Sandhya Rani[#], Pallavi Mandave[#], Aniket Kuvalekar*, and Prabhakar Ranjekar.

Interactive Research School for Health Affairs, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India.

[#] These authors contributed equally to the work reported in this paper

ABSTRACT

In present study, antiglycation, anti-oxidant and anti-diabetic potential of three different stages [Green (I), Greenish White (II), Whitish Red (III)] of strawberry was investigated. Time bound antiglycation activity of the extract was assayed by incubating guanosine with glucose or fructose with or without extract. To determine the anti-oxidant activity, antioxidant enzyme assays (Catalase, Peroxidase, Polyphenol oxidase) and free radical scavenging assays (Xanthine oxidase, superoxide anion free radical scavenging) were performed while anti-diabetic assays were carried out through measurement of inhibition of α-glucosidase and α-amylase activities. Maximum UV absorbance for glycation reactions was observed for 24 hrs. The superoxide scavenging activity of 100 µg fruit extract of stage I, II and III was found to be 4.35%, 18.74% and 16.28% respectively. Xanthine oxidase inhibitory activity showed a maximum percent inhibition of 60.68% at concentration of 702.46 µg ± 1.53 at stage III. IC_{50} value for α-glucosidase and α –amylase activity at stage III was found to be 154.27 µg ± 33.12 and 195.28 µg ± 13.30 respectively. Present study demonstrates that strawberry during ripening stages exhibits potent antiglycation, antioxidant and anti-diabetic activities. Ripening stages of strawberry should further be investigated for mechanisms of antiglycation, antioxidant and anti-diabetic activities for management of diabetes.

Keywords: Antiglycation, antioxidant, anti-diabetic, strawberry

***Corresponding author email*: kuaniket@gmail.com**

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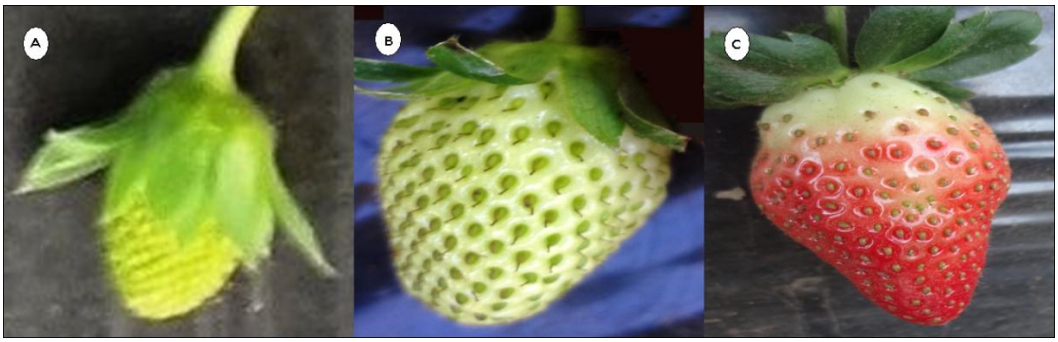
INTRODUCTION

Strawberry is the most popular fragile, perishable fruit of desirable taste and flavor and is consistently ranked among the top food sources of polyphenols and antioxidants [1]. Strawberries contain an incredible combination of phytonutrients including anthocyanin, ellagitanins, flavonoids, terpenoids and phenolic acids [2]. Among these phytonutrients, anthocyanins are the most important polyphenols found in strawberries and possess anti-inflammatory, antioxidant and anticancer properties [3, 4]. Strawberries also protect pancreatic beta cells from glucose induced oxidative stress [5]. Vitamin C, flavonoids and ellagic acid, present in strawberry, have the potential against many metabolic disorders. Flavonoid consumption triggers an antioxidant mechanism that functions at cellular level to fight free radical production and lowers the risk of heart disease, cancer and diabetes. Strawberries also contain high amount of folic acid which plays a key role in healthy fetal development.

Incorporating polyphenols in diet is extremely beneficial for health as it lowers the risk of type 2 diabetes and increases activity of insulin [6]. Fisetin, a naturally occurring flavonoid found in strawberry, increases the production of an enzyme glyoxalase1 which helps in removing advanced glycation end products (AGEs) from blood. Animal studies have also reported that reduced AGEs in fisetin-treated mice [7]. AGEs are formed with consistently high blood glucose levels, and are found in vessels, nerves, eyes, kidneys which lead to chronic complications of diabetes such as neuropathy, retinopathy, and nephropathy.

Naturally occurring antioxidants present in fruits and vegetables stabilize or deactivate free radicals, before they attack targets in biological cells. They also act as AGEs inhibitors by slowing down the formation of free radicals in many chronic diseases. Strawberries possess a 10 fold greater antioxidant capacity than several other fruits and have attracted special interest because of their free radical scavenging abilities [8]. In view of these health promoting effects of strawberry, the present study was undertaken to determine the antiglycation, antioxidant, anti-diabetic potential of strawberries at progressive pre-ripening stages (namely: Green (I), Greenish White (II) and Whitish Red (III) (Figure 1). Though our previous studies reported promising antiglycation, antioxidant and antidiabetic activities of ripened strawberries (Red stage) [9] there is no data available about these activities during ripening stages of strawberry fruit.

Figure 1: Strawberry fruits at different maturation stages. A: Green (Stage I), B: Greenish white (Stage II), C: Whitish Red (Stage III)



MATERIAL AND METHODS

Chemicals

All chemicals used were of analytical grade. Quercetin, guanosine, ascorbic acid (Vitamin C), p-nitrophenyl-alpha-d-glucopyranoside (PNPG), riboflavin, nitrobluetetrazolium (NBT) were obtained from Sigma-Aldrich (USA). D-glucose, D-fructose and hydrogen peroxide were purchased from SRL (India). Guaiacol, monopotassium phosphate, monosodium phosphate, alpha-amylase, starch, dinitrosalicylicacid (DNS), alpha-glucosidase, xanthine oxidase, ammonium sulphate, xanthine, aluminium chloride, sodium hydroxide, sodium nitrite, ethylenediaminetetraacetic acid (EDTA), sodium carbonate, commassie blue, allopurinol, and hypoxanthine were obtained from Hi-Media.

Plant Material

Fruitsof *Fragaria×ananassa*Duch.cv. *Sweet Charlie* (Sc) were harvested from commercial plantation located at Mahabaleshwar, Maharashtra, India, 1438 m above the mean sea level (MSL). The fruits were collected during pre-ripening developmental stages namely Green (Stage I), greenish-white (Stage II) and Whitish-red (Stage III). They were immediately frozen in liquid nitrogen and were stored at -80°C till further use.

Extraction

Fruits from the selected stages were homogenized in liquid nitrogen to prepare fine powder, which was extracted using phosphate buffer (100 mM, pH 7.0). Briefly, 1 gm of fruit powder was added to 20 ml of solvent. Filtration was done using fine Muslin cloth and Whatman filter paper no. 1. Filtrates were centrifuged at 10,000 rpm, 4°C for 15 min and clear supernatant was used for assays. The extracts were stored at -20°C until analysis. All assays were performed in triplicate, unless otherwise mentioned.

Antiglycation assay

In-vitro antiglycation activity of strawberry

Guanosine (100 µg) was incubated with 100 µg fruit extract of strawberry, 600 mg glucose or fructose under sterile conditions for 24, 48, 72 and 96 hours at 37°C. Solution of guanosine without sugar and fruit extract served as control. Absorbance was read at 254nm in spectrophotometer (PerkinElmer's LAMBDA 25/35/45).

In-vitro antiglycation activity of strawberry under oxidative stress

Hydrogen peroxide was used for generation of Reactive Oxygen Species (ROS). Guanosine (100 µg) was incubated with 100 µg fruit extract of strawberry, 600 mg glucose/fructose and 100mM hydrogen peroxide for 24, 48, 72 and 96 hours at 37°C. After incubation, the tubes were irradiated under 254nm UV light for 30 minutes. Absorbance of the solution was measured using spectrophotometer at 254 nm (PerkinElmer's LAMBDA 25/35/45).

Antioxidant enzymes assays

Catalase (CAT) Assay

The activity of catalase was assayed according to the method described by Bergmeyer [10]. The assay mixture for the catalase activity contained 1.5ml 25mM Hydrogen peroxide, 1.5 ml 75mM sodium phosphate buffer (pH 7.0), and 400 µg fruit extract of strawberry. Phosphate buffer (pH 7.0) served as control. Enzyme activity was detected by decrease in the absorbance at 240nm for 3 min in a spectrophotometer (PerkinElmer's LAMBDA 25/35/45). The protein concentration from the enzyme extract was determined using Bradford's method [11]. The activity of CAT was expressed in terms of the enzyme units. Unit activity of the enzyme was defined as that activity which changes the absorbance of the reaction mixture by 0.001 in 1 min/mg protein under assay conditions.

Peroxidase (POX) Assay

The assay of POX was carried out as per the method described Yuan and Jiang [12]. The reaction mixture contained 3 ml 100mM phosphate buffer (pH 7.0), 400 µg fruit extract of strawberry, 3 ml 20 mMguaiacol and 30µl 1.7mM hydrogen peroxide. Enzyme activity was detected by increase in the absorbance at 436nm for 3 min in a spectrophotometer (PerkinElmer's LAMBDA 25/35/45). The protein concentration from the enzyme extracts was determined using Bradford's method [11]. The activity of POX was expressed in terms of the enzyme units. Unit activity of the enzyme was defined as that activity which increases the absorbance of the reaction mixture by 0.001 in 1 min/mg protein under assay conditions.

Polyphenol oxidase (PPO) assay

The extraction and assay of PPO was carried out using a method described by Jiang [13]. The reaction mixture contained 1 ml 100 mM methyl catechol , 2 ml 100 mM phosphate buffer (pH 7.0), and 400 µg fruit extract of strawberry. The progress of the reaction was detected by the increase in the absorbance at 495nm for 3 minutes using spectrophotometer (PerkinElmer's LAMBDA 25/35/45). The protein concentration from the enzyme extracts was determined using Bradford's method [11]. The activity of PPO was expressed in terms of enzyme units. Unit activity of the enzyme was defined as that activity which increases the absorbance of the reaction mixture by 0.001 in 1 min/mg protein under assay conditions.

Antioxidant assays

Xanthine oxidase (XO) inhibition assay

Xanthine oxidase activity was determined as per the method of Cinmanga [14]. The assay mixture contained various concentrations of fruit extracts (0, 10, 20, 50, 100, 500 µg) and 1.5 ml 0.15 mM xanthine solution as the substrate. Samples were incubated at 30°C for 10 min. The reaction was initiated by adding 0.28U xanthine oxidase dissolved in phosphate buffer (66.67 mM, pH 7.5). Change in the absorbance/min was measured at 295nm for 10 min. against enzyme blank. The % inhibition of this enzyme with plant extracts was calculated using the formula:

$$\% \text{ Inhibition} = [(\text{OD Control} - \text{OD Sample})/ (\text{OD Control})] \times 100$$

Superoxide anion scavenging assay

Superoxide scavenging activity was determined by NBT reduction method as described by McCord and Fridovich [15]. The reaction mixture contained 200 µl 0.11M EDTA prepared in phosphate buffer (66.67mM, pH 7.5), 100 µl 0.53 mM riboflavin, 100 µl 1.2 mM NBT, 3 ml 51.5 mM phosphate buffer (pH 7.4) and different concentrations fruit extracts (0, 10, 20, 50, 100, 500 µg). Samples were kept under bright light for 15 minutes and the absorbance was read at 560nm. The % scavenging activity of plant extracts was calculated using the formula:

$$\% \text{ Scavenging} = [(\text{OD Control} - \text{OD Sample})/ (\text{OD Control})] \times 100$$

Total flavonoid content

Total flavonoid content was determined as per the method described by Ordonez [16], using Quercetin (50µg/ml) as standard. Reaction mixture contained 50 µg fruit extract, 75 µl 5% $NaNO_2$,150 µl 10% $AlCl_3$ and 500 µl 1M NaOH. Final volume of the reaction was adjusted to 2.5 ml with distilled water. After 5 minutes of incubation, 500 µl 1M NaOH was added to the reaction mixture and the absorbance was monitored at 510 nm. Flavonoid content was calculated using the following formula:

$$\% \text{ Flavonoids} = [\{(\text{OD extract} * 0.05)/ \text{OD Quercetin}\}/ \text{Extract concentration}] * 100$$

Antidiabetic activity assay

α- amylase inhibition activity

The α-amylase inhibitory activity was determined by an assay from the Worthington Enzyme Manual [17]. Various concentrations of fruit extract (0, 10, 20, 40, 60, 80 and 100 µg) were used for the assay. The reaction mixture containing 50µl of 0.05U α-amylase and desired concentration of fruit extract in 20mM phosphate buffer (pH 6.9), was incubated at 25°C for 10 minutes. The reaction was initiated by adding 500µl 1% starch solution in 20 mM phosphate buffer. The reaction was stopped with 1ml 90 mMdinitrosalicylicacid (DNS), and the mixture was placed in a water bath at 85°C for 5 min.

The reaction mixture was diluted by addition of 8ml distilled water and the absorbance was measured at 540nm using microplate reader (BIO-RAD iMark). Acarbose (1mg/ml), anα-amylase inhibitor, was used as a positive control. The % α-amylase inhibitory activity was calculated using the following formula:

% Inhibition= [(OD Control – OD Sample)/ (OD Control)] x 100

α -glucosidase inhibition assay

Alpha-glucosidase inhibition activity was analysed as described by McCue [18]. The reaction mixture contained 100µl 100 mM phosphate buffer (pH 6.8), 5U alpha-glucosidase enzyme and fruit extract of varying concentrations (2, 4, 6, 8, 10, 20, 50, 100µg). The reaction was pre-incubated for 5 min at 37°C, and then 50µl 5mM PNPG was added to the mixture as a substrate. Reaction mixture was further incubated at 25°C for 5 min. Acarbose (1mg) was used as a positive control. The intensity of yellow colour was measured at 405 nm with microplate reader (BIO-RAD iMark). The % inhibition was calculated using the formula:

% Inhibition= [(OD Control – OD Sample)/ (OD Control)] x 100

Statistical analysis

All samples were analyzed in triplicates and the experimental data are presented as mean ± standard deviation. The dose effect curve for each extract was linearized by regression analysis and used to derive the IC_{50} values for each stage.

RESULTS

Antiglycation assays

Fruit extract of strawberry was evaluated for the antiglycation activity. Table 1 summarizes the absorbance of control (guanosine + water), glycated, fructated and glycation/fructation reaction with ROS and UV. Maximum absorbance was observed at 24 hours for glycated and fructatedguanosine and it decreased consecutively from 48 to 96 hours. Glycation and fructation reactions of guanosine under oxidative stress resulted in an increase in absorbance from 24 hours to 96 hours. Tables 2 and 3 depict the inhibition activity of glycation/fructation with guanosine by fruit extract from stage I to stage III respectively. Increase in the inhibition of glycation/fructation from stage I to stage III was observed. Maximum inhibition of 16% at stage III and minimum inhibition of 9% at stage I was reported after 96 hours for guanosineglycation. Whereas, maximum inhibition of 18% at stage III and minimum inhibition of 10% at stage I was observed after 96 hours for guanosinefructationby strawberry extract.

Tables 4 and 5 show inhibition of glycation/fructation of guanosine under ROS and UV. Decrease in absorbance from 24 to 96 hours and consecutive increase in the percent inhibition from stage I to stage III was observed as a result of oxidative stress for various time periods (24 to 96 hours). Maximum inhibition of 22% and 25% for guanosineglycationand fructation respectively, was observed at stage III under ROS and UV. Addition of fruit extract to these reactions resulted in increase in inhibition of nascent glycation and fructation of guanosine as well as glycation and fructation reactions under oxidative stress. The results from our study suggest that all the three stages (stage I to III) of strawberry possess a significant antiglycation activity.

Antioxidant enzymes, Antioxidant assays and Flavonoid content

The unit enzyme activity of three antioxidant enzymes catalase, peroxidase and polyphenol oxidase are depicted in Table 6. It was observed that catalase and peroxidase activity decreased from stage I to III. Maximum catalase and peroxidase activity of 1.21×10^2 units and 9.21×10^2 units were observed at stage I respectively while polyphenol oxidase activity was found to be increased from stage I to III where maximum activity of 0.72×10^2 units was observed at stage III.

To assess the antioxidant activity of strawberry during fruit ripening stages two antioxidant assays Xanthine oxidase and superoxide radical scavenging were carried out (Table 7). The superoxide scavenging activity of 100 µg fruit extract of stage I, II and III was found to be 4.35%, 16.28% and 28.74% respectively.

Standard quercetin showed a maximum inhibition of 41.86% at a concentration of 100 µg. Xanthine oxidase assay showed a maximum percent inhibition of 60.68% at a concentration of 702.46 µg ± 1.53 at stage III by fruit extract of strawberry. Standard allopurinol showed a maximum inhibition of 62.78% at a concentration of 420 µg ± 0.912. In summary, fruit extract at stage III demonstrated significant xanthine oxidase inhibitory activity and effective capacity to scavenge free radicals.

Flavonoids, as an antioxidative agent help in slowing down the oxidation reactions and possess anti-mutagenic and anti-carcinogenic properties. Wetherefore, determined the flavonoid content of the three stages of strawberry. Flavonoid content in terms of quercetin equivalent was found to be 120, 260 and 300.1 mg of Quercetin/g for stage I, II and III respectively indicating that all the three stages of strawberry during maturation possess significant flavonoid content.

Antidiabetic Activity Assays

Table 8 shows the anti-diabetic activity of fruit extract of strawberry during ripening stages from stage I to III. IC_{50} for α-glucosidase activity for all the stages from I to III was found to be 328.58 µg ± 36.26, 270.94 µg ± 42.49 and 154.27 µg ± 33.12 respectively. IC_{50} for α –amylase activity for all the stages from I to III was found to be 2192.75 µg ± 204.75, 304.56 µg ± 22.66 and 195.28 µg ± 13.30 respectively. Standard acarbose showed an IC_{50} of 45.49 µg ± 0.78 and 15.58 µg ± 0.80 for α-glucosidase and α-amylase respectively.

Table 1: Absorbance of glycated, fructated and ROS-modified glycated and fructatedguanosine at 260nm for various incubation time regimens

Incubation Time (Hrs)	Glycatedguanosine	Fructatedguanosine	Glycation reaction ofguanosine under oxidative stress	Fructation reaction of guanosine under oxidative stress	Guanosine + Water
24	3.1	3.2	3.2	3.3	1.2
48	2.8	3.0	3.3	3.6	1.1
72	2.7	2.7	3.4	3.69	0.9
96	2.5	2.6	3.52	3.8	0.6

Table 2: Percent inhibition of glycation of guanosine by strawberry fruits at different stages of maturation

Time Period (Hrs)	Stage I (%)	Stage II (%)	Stage III (%)
24	3	6	9
48	6	9	13
72	8	11	14
96	9	12	16

Table 3: Percent Inhibition of fructation of guanosine by strawberry fruits at different stages of maturation

Time Period (Hrs)	Stage I (%)	Stage II (%)	Stage III (%)
24	4	7	10
48	6	10	14
72	9	12	16
96	10	14	18

Table 4:Inhibition of glycation of guanosine with ROS and UV modification by strawberry fruits at different stages of maturation

Time Period (Hrs)	Stage I (%)	Stage II (%)	Stage III (%)
24	5	8	14
48	8	11	17
72	10	13	20
96	14	17	22

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Table 5: Percentage inhibition of fructation of guanosine with ROS and UV modification by strawberry fruits at different stages of maturation

Time Period (Hrs)	Stage I (%)	Stage II (%)	Stage III (%)
24	7	9	16
48	9	13	18
72	14	16	23
96	16	19	25

Table 6: Enzyme activities of Catalase, peroxidase and polyphenol oxidase during fruit maturation stages

Assay	Stage I	Stage II	Stage III
Catalase	1.21×10^2	0.79×10^2	0.68×10^2
Polyphenol oxidase	0.14×10^2	0.53×10^2	0.72×10^2
Peroxidase	9.21×10^2	7.27×10^2	4.58×10^2

Table 7: Xanthine oxidase inhibition and Super oxide free radical scavenging of strawberry fruits at different stages of maturation

Assay	Positive Control	Positive Control		Stage I		Stage II		Stage III	
		IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)	IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)	IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)	IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)
XOIA	Allopurinol	128.96 ± 2.1	62.78 (420± 0.91)	4509.7 ± 10.78	32.73 (843.1 ± 1.24)	927.96 ± 18.97	53.28 (746.7 ± 0.98)	791.04 ± 20.38	60.68 (702.4 ± 1.53)
SAFRS	Quercetin	174.41 ± 11.2	5.62 (486± 0.28)	3987.5 ± 351.77	16.62 (943.2 ± 43.91)	699.32 ± 19.53	62.00 (849.4 ± 39.03)	528.35 ± 26.37	63.42 (986.9 ± 9.86)

XOIA: Xanthine oxidase inhibition activity, SAFRS: Superoxide anion free radical scavenging

Table 8: α –glucosidase and α –amylase inhibition activity of strawberry fruits at different stages of maturation

Anti-diabetic Assays	Std (Acarbose)		Stage I		Stage II		Stage III	
	IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)	IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)	IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)	IC_{50} (µg/µl)	% Max. inhibition (Extract conc. in µg)
α -glucosidase	45.49 ± 0.78	96 ± 0.62	328.58 ± 36.26	60.32 (480.7 ± 16.7)	270.94 ± 42.49	64.54 (432.09 ± 18.29)	154.27 ± 33.12	54.47 (188.36 ± 10.78)
α -amylase	15.58 ± 0.80	72.10 (91.05 ± 11.18)	2192.7 ± 204.7	50.81 (975.2 ± 19.7)	304.56 ± 22.66	53.84 (492.96 ± 7.20)	195.28 ± 13.30	49.46 (194.29 ± 8.63)

DISCUSSION

It has been reported that glycation plays a significant role in the development of diabetic complications [19, 20]. Glycation is a condensation reaction in which reducing sugars and amino group of proteins undergo rearrangements to form stable ketoamines, which lead to formation of Advanced Glycation End Products (AGEs) [21]. AGEs may alter structure, functions and stability of peptides and there are many reports available on accumulation of AGEs in diabetes and its complications [22]. Under *in-vitro* and *in-vivo* conditions nucleotides from advanced glycation end products have been reported [23]. Guanosine, a DNA nucleoside, reacts with sugars in a similar way as with proteins and forms DNA-AGEs complex [24]. These nucleotide AGEs have been known to play a critical role in complications related to diabetes [25]. Synthetic AGE inhibitors aminoguanidine [26] and pimagedine [27] have been shown to be associated with side effects. Therefore, anti-glycating agents from plant and vegetables have received considerable attention.

In the present study, inhibition of glycation/fructation with guanosine by the fruit extract of strawberry has been studied. Non-enzymatic glycationreactions mightblock the free -NH_2 group present in guanosine resulting in decrease in the absorbance of glycation and fructation reaction.

Oxidative stress is usually associated with increased formation of reactive oxygen species (ROS). ROS reacts with many macromolecules causing damage to DNA, lipids and proteins. AGEs formed with ROS tend to form cross-links by binding to cell surface receptors and are associated with diabetes and its complications [28, 29]. Fruits and vegetables are rich sources of polyphenols which bestow on them the ability to remove free radicals from AGEs [30]. Strawberries are among the top dietary sources of polyphenols in addition to being a fruit with a low caloric value. The present studies also indicate the strong antiglycation activity of strawberry. Maximum inhibition is observed incase of fructation of guanosine, as formation of AGEs is slowest with glucose as compared to fructose [29] and fructose derived AGEs are formed 10 times faster as compared to glucose derived AGEs [31].

Maturation (ripening) has been described as an oxidative process in climateric fruits. Studying the activity of antioxidant enzymes is important as it protects the fruit tissues from toxic ROS. In present study, antioxidant enzyme activity of catalase, peroxidase and polyphenoloxidase is evaluated. Catalase is found majorly in the peroxisome and is readily involved in the photorespiration metabolism. Whereas, PPO and POX are the most studied enzymes in fruits and vegetables as these enzymes are primary defense mechanisms in host-parasite interaction. Peroxidase is involved in many metabolic processes such as defense mechanism and lignifications [32, 33]. The variation in peroxidase activity during tissue/fruit maturation has been studied in *Capsicum annuum* [34]. Kaus [35] reports that peroxidase activity is decreased during maturation. Our results of ripening stages of strawberry during maturation also show a decrease in activity during ripening stages. The enhancement in POX activity with maturation of fruit might defend the cells against harmful concentrations of hydroperoxides. Increase in the activity may be due to phenols which get utilized during fruit development for the generation of antioxidant compounds by enzymes, resulting in decreased activity of peroxidase. It is well known that the photorespiration is related with the chlorophyll pigment and is found abundantly in the green stage (I). However, as the fruit matures, anthocynidins, proanthocyanidins and condensed tannins get accumulated resulting in chlorophyll degradation of fruit leading to decreased activity of catalase from stage I to III.

Free radicals are constantly generated in living systems and play a critical role in various pathological manifestations especially degenerative diseases [36]. It is well known that antioxidants fight against free radicals and exert their action by scavenging ROS [37]. Synthetic drugs that protect against oxidative damage have various side effects. Hence, consuming natural antioxidants from food supplements and vegetables is considered as a better alternative [38]. Superoxides form hydroxyl radicals which contribute to oxidative stress and are the most destructive reactive oxygen species [39]. Xanthine oxidase is an enzyme that generates reactive oxygen species and contributes to the oxidative damage to living tissues. It is involved in medical condition known as gout, which is characterized by hyperuricemia, leading to serious disorders like renal failure [40, 41]. Allopurinol is the only xanthine inhibitor available in the treatment of gout [42, 43] but has many side effects such as allergic reactions and nephropathy [44]. Considering this scenario, search for novel inhibitors from natural sources is extremely important. In this context, we have demonstrated the antioxidant potential of ripening stages of strawberry indicating that its consumption could be useful to human health.

Herbal extracts have been reported for their anti-diabetic activities and screening of two enzymes namely alpha-glucosidase and alpha-amylase has received attention. Inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase is one of the best alternatives to impair glucose absorption for the treatment of diabetes [45, 46]. Synthetic inhibitors like acarbose and voglibose are used for the treatment of diabetes but they are costly and have adverse side effects [47]. Therefore, herbal medicines as an anti-diabetic drug which should be less toxic, more effective, cheap and easily available, are given more attention. It has been reported that glucose-lowering effects[48] of medicinal plants may be due to phenolics [49], flavonoids [50] and triterpenoids [51]. Our observation that strawberries possess a significant anti-diabetic activity provides another natural source for possible control of diabetes.

CONCLUSION

The present study has demonstrated that strawberry fruit during ripening stages possess potent antiglycation, antioxidant and anti-diabetic activities which might be derived from the compounds such as flavonoids and phenols. Consumption of strawberries can provide a good source of antioxidants and nutrients, emphasizing their potential for use in the development of nutraceuticals or as functional food for the benefit of human health.

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