Nigerian Journal of Biochemistry and Molecular Biology
Volume 26, Number 2, 2011, pp. 116-125 (Printed in Nigeria)
0189-4757/96 $3.0 + 0.00
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NJBMB-009-11

Effects of Aqueous Extracts of Some Citrus Peels on key Enzymes Linked With Type-2 diabetes

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ABSTRACT
An approach to controlling blood glucose levels in individuals with type-2 diabetes is the inhibition of enzymes involved in the breakdown of starch and absorption of glucose, such as α-amylase and α-glucosidase. This study therefore, sought to characterize the interaction of aqueous extracts from some Nigerian citrus peels [orange (*Citrus sinensis*), grapefruit (*Citrus paradisii*) and shaddock (*Citrus maxima*)] with α-amylase and α-glucosidase *in vitro*. The total phenolic, flavonoid, vitamin C content and antioxidant activities were also determined. The results revealed that orange peels had the highest total phenol content (8.33mg/g) and flavonoid content (0.58mg/g) as compared to grapefruit [total phenol (6.50mg/g), flavonoid (0.29mg/g)] and shaddock peels [total phenol (5.00mg/g), flavonoid (0.15mg/g)]. Also, orange peels' aqueous extracts showed the highest antioxidant potential as typified by a higher reducing power, ABTS radical scavenging ability and inhibition of Fe^{2+} - and sodium nitroprusside- induced lipid peroxidation in rat's pancreas – in vitro. The aqueous extracts from the citrus peels inhibited α-glucosidase and α-amylase activities, though with a stronger inhibition of α-glucosidase [orange (37.50%), grape (50.0%), shaddock (37.50%)] than α-amylase activities [orange (25.32%), grape (29.29%), shaddock (21.34%)]. The phenolic contents, vitamin C contents, antioxidant activities and inhibition of key enzymes linked with type 2 diabetes suggests the potential use in the control of blood glucose levels associated with type-2 diabetes.

Keywords: Orange; Grapefruit; Shaddock; Diabetes; Amylase; Glucosidase

INTRODUCTION

Diabetes mellitus affects approximately 135 million people in the world and is projected to affect about 300 million individuals by the year 2025. However, Type-2 diabetes is the most common form of diabetes, accounting for 90% of all cases, and is a metabolic disorder primarily characterized by insulin resistance, relative insulin deficiency, and an abnormal rise in blood sugar, right after a meal, called postprandial hyperglycemia (IDF, 2001; Choukem *et al*., 2007). Drugs involved in diabetes management include α-amylase and α-glucosidase inhibitors, which are oral anti-diabetic drugs used for diabetes mellitus type 2 and work by preventing the digestion of complex carbohydrates (such as starch). The digestion of starch (a major source of carbohydrate in the diet of most humans) starts in the mouth, where salivary α-amylase hydrolyzes the internal glycosidic linkages of starch, producing short polysaccharide fragments or oligosaccharides. However, salivary α-amylase is inactivated by the low pH of the stomach, but pancreatic α -amylase continues the breakdown process. In the intestine, α-glucosidases break down disaccharides into monosaccharides which are readily absorbed through the epithelia cells of the small intestine into blood stream.

Hence, inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates resulting in decreased glucose absorption because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of these drugs therapies is to decrease current blood glucose levels: the long term effect is a small reduction in hemoglobin A1c level. However, high inhibition of pancreatic *α*-amylase could result in the abnormal bacterial fermentation of undigested starch in the colon, and thus the usefulness of mild *α*-amylase inhibitory activity (Horii *et al.,* 1987). Therefore, natural inhibitors from dietary plants are important as they have lower inhibitory activity against *α*-amylase and a stronger inhibitory activity against *α* -glucosidase and can be explored as effective therapy for postprandial

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hyperglycaemia with minimal side effects (Kwon *et al.,* 2006).

Free radicals have been implicated in the development and complications of diabetes in a number of ways; the white blood cell production of reactive oxygen species mediates the immune destruction of the beta cells in the islets of Langerhans in the pancreas (Oberley, 1988). Furthermore, abnormalities in transition metal metabolism are postulated to result in the establishment of diabetes (Wolff, 1993). Diabetes-associated hyperglycemia causes intracellular oxidative stress, which contributes to vascular dysfunction (Baynes, 1991).

Citrus fruits have a small edible portion and large amounts of waste materials such as peels and seeds. Belitz and Grosch (1999) reported that peels contain more total phenol content than the peeled citrus fruits. Citrus fruits' peels contain significant amount of phenolic compounds, especially phenolic acids and flavonoids (Gorinstein *et al.*, 2001), which are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Alia *et al.,* 2003; Amic *et al.*, 2003). More so citrus peels are used in folk medicine, in the management of diabetes, though there is very limited information on the mode of action of these peels in the management of the disease. It is therefore expedient to investigate the possible mechanism of action of the aqueous extracts of some citrus peels in the management /prevention of type-2 diabetes.

MATERIALS AND METHODS

Sample collection

The peels of three citrus fruits; Orange (*Citrus sinensis*), Grapefruit (*Citrus paradisii*) and Shaddock (*Citrus maxima*) were bought from the Akure main market. The peels were sun dried for 7 days and ground to fine powder.

Preparation of aqueous extract

The aqueous extract of the peels were subsequently prepared by soaking the powdered samples in water for 24 h at 37 ^{o}C. The resulting mixture was filtered and later centrifuged at 357.80 x g for 10 minutes to obtain a clear supernatant which was then stored in the refrigerator for subsequent use (Oboh *et al*., 2007).

METHODS

Determination of total phenol content

The total phenol content was determined according to the method of Singleton *et a.* (1999). Briefly, appropriate dilutions of the extracts were oxidized with 2.5ml of 10% (v/v) Folin-Ciocalteau's reagent and neutralized with 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45^{o}C and the absorbance was read at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method described by Meda *et al* (2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml of methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1M potassium acetate and 1.40 ml of water, and allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixture was subsequently read at 415 nm. The total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

In vitro antioxidant studies

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging abilityof the extracts

The ABTS· scavenging ability of the extracts were determined according to the method described by Re *et al* (1999). The ABTS· was generated by reacting an (7 mmol/l) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/l, final concentration) in the dark for 16 hours and adjusting the Absorbance at 734 nm to 0.700 with ethanol. A known volume (0.2 ml) of appropriately diluted extract was added to 2.0 ml of ABTS· solution and the absorbance were read at 734 nm after 15 minutes. The trolox equivalent antioxidant capacity was subsequently calculated.

Determination of reducing property of the extracts

The reducing property of the extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution as described by Oyaizu (1986). Briefly, a known volume (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml

of 1% potassium ferricyanide. The mixture was incubated at 50 ^{o}C for 20 minutes and then 2.5 ml of 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 minutes. A known volume (5 ml) of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was read at 700 nm and the ferric reducing antioxidant property calculated.

Preparation of tissue homogenates

The rats were decapitated under mild diethyl ether anaesthesia and the pancreas was rapidly isolated and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1:10 w/v) with about 10-up-and-down strokes at approximately 1200 rev/minute in a Teflon glass homogenizer. The homogenate was centrifuged at 3000 x *g* for 10 min to yield a pellet that was discarded, and a low-speed supernatant (S1) was kept for lipid peroxidation assay (Belle *et al*., 2004).

Assay for lipid peroxidation

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al* (1979). Briefly, 100 µl of S1 fraction was mixed with a mixture containing 30 µl of 0.1M pH 7.4 Tris-HCl buffer, extract (0-100 µl) and 30 µl of 250 µM freshly prepared $FeSO_4$ (the procedure was also carried out using 5µM sodium nitroprusside). The volume was made up to 300 µl with water before incubation for 1hour at 37^{o}C. The colour of the reaction was developed by adding 300 µl of 8.1% Sodium doudecyl sulphate (SDS) to the reaction mixture containing S1. This was subsequently followed by the addition of 600 µl of acetic acid/HCl (pH 3.4) mixture and 600 µl of 0.8% Thiobarbituric acid (TBA). This mixture was incubated for 1 hour at 100^{o}C. TBARS (Thiobarbituric acid reactive species) produced were read at 532 nm and the absorbance was compared with that of calibration curve for malondialdehyde.

Enzyme inhibition assays

α-Amylase

Appropriate dilution of the phenolic extracts (500 µl) and 500 µl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) (0.5mg/ml) were incubated at 25°C for 10 minutes. Then, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures was incubated for 10 minutes at 25°C and stopped with 1.0 ml of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 minutes, and cooled to room temperature. The reaction mixture was diluted by adding 10 ml of distilled water, and absorbance read at 540 nm. The EC_{50} (the extract concentration inhibiting 50% of the α-amylase activity) of the phenolic extracts was calculated (Worthington Biochemical Corp. 1978).

α-Glucosidase

Appropriately diluted phenolic extracts (50 µl) and 100 µl of α-glucosidase solution (1.0 U/ml) in 0.1 M phosphate buffer (pH 6.9) was incubated at 25 °C for 10 minutes. Then, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated for 5 minutes at 25 °C, before reading the absorbance at 405 nm in the spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition. The EC_{50} (the extract concentration inhibiting 50% of the α-glucosidase activity) of the phenol extracts was calculated (Apostolidis *et al.*, 2007).

Data analysis

The results of the three replicates were pooled together and expressed as mean ± SEM. Student t-test, one-way analysis of variance and the least significance difference were carried out (Zar, 1984). Significance was accepted at $p \leq 0.05$. EC_{50} was determined using linear regression analysis.

RESULTS AND DISCUSSION

The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs (Kiselova *et al.,* 2006; Kedage *et al*., 2007; Klimczak *et al*., 2007; Jayaprakasha *et al*., 2008; Oboh and Rocha, 2008; Oboh and Shodehinde, 2009). Antioxidant activity of fruits and vegetables significantly increases with the concentration of total polyphenols (Ghasemi *et al*., 2009).

Orange peels had the highest total phenol content (8.33mg/g) and flavonoid content (0.58mg/g) as compared to grapefruit peels [total phenol (6.50mg/g), flavonoid (0.29mg/g)] and shaddock peels [total phenol (5.00mg/g), flavonoid (0.15mg/g)] (Table 1). The orange peels had the highest vitamin C content of 10.49 mg/g, while grapefruit and shaddock peels had vitamin C content of 6.58 mg/g and 7.32 mg/g respectively (Table 1). The high total phenolic and flavonoid content of the peels agrees with earlier reports on the phenolic contents of some citrus peels (Oboh and Ademosun, 2006). In addition, phenolic contents are usually higher in peels of citrus fruits than in tissues and juices (Belitz and Grosch, 1999; Gorinstein *et al.,* 2001). Phenolics are capable of scavenging free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (Alia *et al.,* 2003; Amic *et al.*, 2003). The vitamin C content of the peels is higher than that of peppers (Oboh and Rocha, 2007a & b) and vegetables (Oboh, 2005). Vitamin C is found in fruits, juices and green leafy vegetables where it exhibit antioxidant properties, protects the body against cancer and maintains the flexibility of the blood vessels (Block *et al*., 1992).

Free radicals are involved in the development and complications of diabetes in a number of ways; the white blood cell production of reactive oxygen species mediates the autoimmune destruction of the beta cells in the islets of Langerhans in the pancreas (Oberley, 1988). Abnormalities in transition metal metabolism are postulated to result in the establishment of diabetes (Wolff, 1993). Diabetes-associated hyperglycemia causes intracellular oxidative stress, which contributes to vascular dysfunction (Baynes, 1991). Diabetes can be induced in animals by the drugs alloxan and streptozotocin; the mechanism of action of these two drugs is different, but both result in the production of reactive oxygen species. Scavengers of oxygen radicals are thus effective in preventing diabetes in these animal models (Obereley, 1988). The free radical scavenging ability of the aqueous extracts of the citrus peels was studied using moderately stable nitrogen-centred radical species -ABTS radical (Re *et al*, 1999). The ABTS· scavenging ability reported as trolox equivalent antioxidant capacity (TEAC) as presented in Figure 1 shows that orange peels had the highest antioxidant capacity, whereas there was no significant difference in the antioxidant capacity of the grapefruit and shaddock peels. The trend in the results agree with the phenolic content in the citrus peels and many previously published works, where correlation were reported between phenolic content and antioxidant capacity of some plant foods (Chu *et al*., 2002; Sun *et al.,* 2002; Amic *et al.,* 2003).

There was no significant difference ($P>0.05$) in the ferric reducing antioxidant properties (FRAP) of the aqueous extracts of the orange and shaddock peels, but was significantly higher ($P<0.05$) than that of grapefuit peels (Figure 2). Fe^{2+} catalyzes the decomposition of hydrogen peroxide to give OH· via the Fenton reaction, a mechanism through which iron induces lipid peroxidation, characterized by increase in Malondialdehyde (MDA) content (Bayir *et al.,* 2006; Oboh *et al.*, 2007). The incubation of pancreas of rats in the presence of 25 µM of Fe^{2+} caused a significant increase in the MDA content of the pancreas (Figure 3). Nevertheless, the aqueous extracts significantly ($P<0.05$) inhibited MDA production in the pancreas in a dose-dependent manner, though as shown in Table 2, the EC_{50} of orange and grapefruit peels suggest stronger inhibitory effect on the production of MDA in the pancreas (*in vitro*) than that of shaddock peels. The reason for the higher inhibitory ability of the orange peels cannot be categorically stated, but it could be due to other antioxidant mechanisms, since the orange peels had the highest phenolic contents, vitamin c content, ABTS· scavenging ability and ferric reducing antioxidant property. Likewise, the incubation of the pancreas of the animals in the presence of 5µM of sodium nitroprusside (Figure 4 and Table 3, respectively) caused a significant ($P<0.05$) increase in the MDA content. Sodium nitroprusside, a component of antihypertensive drugs causes cytotoxicity through the release of cyanide and/or nitric oxide (Oboh and Rocha, 2008).

Table 1: Total Phenol, Total Flavonoid and Vitamin C content of some citrus peels (mg/g)

Sample	Orange Peels	Grapefruit Peels	Shaddock Peels
Total Phenol	8.33±0.91	6.50±0.74	5.00±0.62
Total Flavonoid	0.58±0.12	0.29±0.08	0.15±0.09
Vitamin C	10.49±0.62	6.58±0.96	7.37±0.62

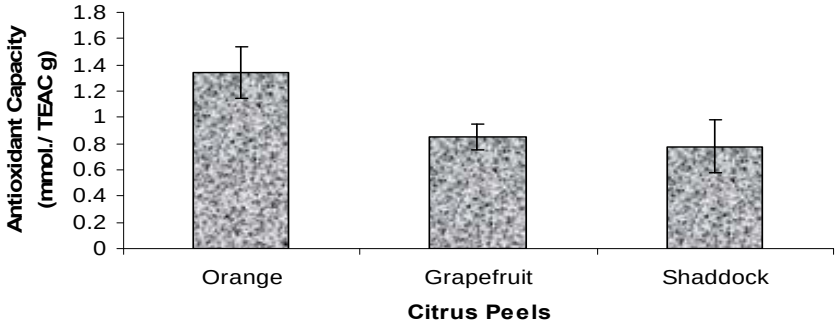

Fig. 1: Antioxidant capacities of aqueous extracts of some citrus peels

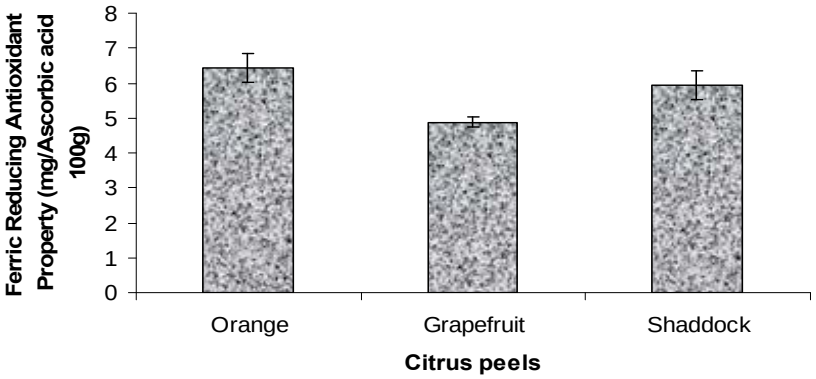

Fig. 2: Ferric reducing antioxidant properties of aqueous extracts of some citrus peels

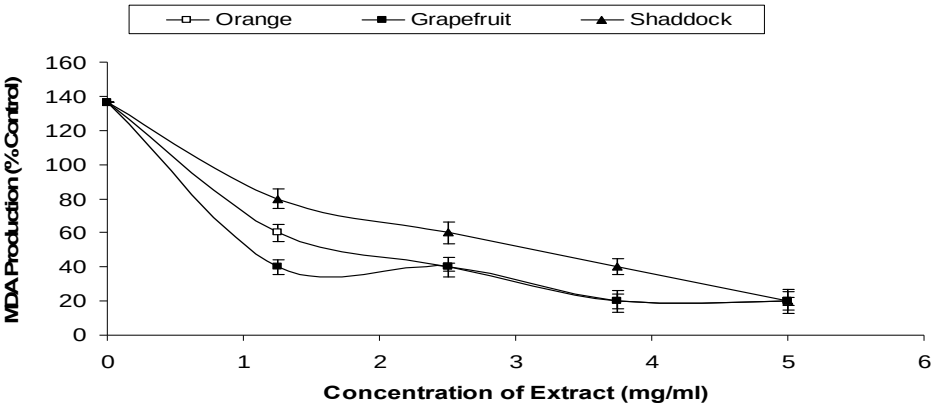

Fig. 3: Inhibition of Fe^{2+} induced lipid peroxidation in the pancreas of rats by aqueous extracts of some citrus peels

Table 2: EC_{50} of aqueous extracts of citrus peels and inhibition of Fe^{2+} induced lipid peroxidation in pancreas (mg/ml)

Sample	**EC_{50}**
Orange peels	2.99 ± 0.20
Grapefruit peels	2.94 ± 0.10
Shaddock peels	3.36 ± 0.40

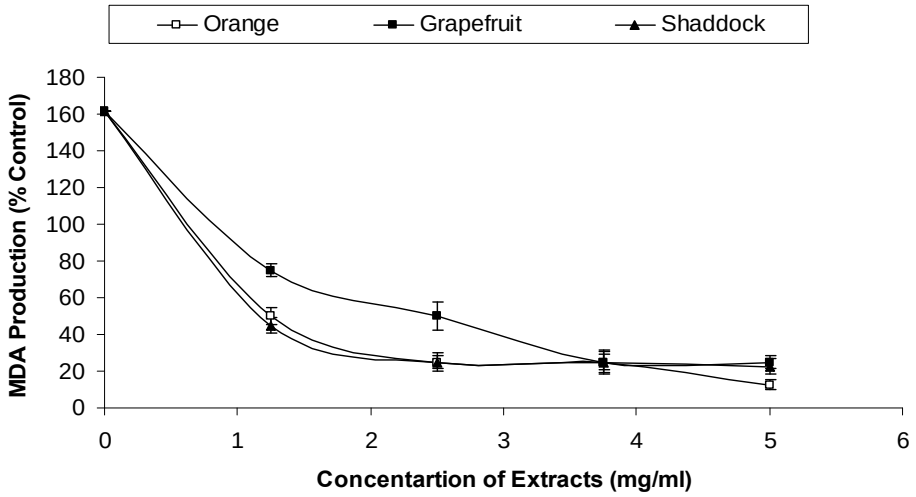

Fig. 4: Inhibition of sodium nitroprusside induced lipid peroxidation in the pancreas of rats by aqueous extracts of some citrus peels

The aqueous extracts caused dose-dependent significant ($P<0.05$) inhibition of the sodium nitroprusside-induced oxidative stress in the pancreas, and similar to the inhibition of MDA production in Fe^{2+}-induced lipid peroxidation in the pancreas; orange peels showed the strongest inhibitory effect on sodium nitroprusside-induced MDA production in the pancreas (*in vitro*) compared to the grapefruit peels and shaddock peels. It could be speculated that the higher inhibitory ability of the aqueous extracts of the orange peels is due to higher $NO\cdot$ scavenging ability.

Inhibition of enzymes involved in the metabolism of carbohydrates such as α-amylase and α-glucosidase is one of the therapeutic approaches for reducing postprandial blood glucose values ina bid to prevent / manage diabetes (Shim *et al*., 2003). α -amylase is the enzyme that hydrolyzes starch to maltose and consequentially higher postprandial hyperglycemia, while the enzymes summarized as α-glucosidase are responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. The inhibition of these enzymes leads to a decrease of blood glucose level, because the monosaccharides are the form of carbohydrates which is absorbed through the mucosal border in the small intestine. The interaction of the aqueous extracts with α-amylase at a concentration of 150 µg/ml, as shown in Figure 5, revealed that the extracts from the peels inhibited α-amylase activity. However, there was no significant ($P>0.05$) difference in the inhibitory effect of the three peels. These inhibitions of α-amylase by the extracts could be due to the phenolics as previously reported (Oboh and Ademosun, 2010a, 2010b, inhibited pancreatic α-amylase, while phenolics from green and black tea were reported to inhibit salivary α-amylase activity (Zhang and Kashket, 1998). Therefore, these could explain part of the reason for the use of these peels in the management of diabetes.

It is noteworthy, however, that isolated phenolics from some citrus species, such as hesperidine and naringenin had been reported to lower blood glucose in male mice (Mouly *et al.,* 1994; Schieber *et al.,* 2001) and since, the mechanisms involved in this anti-hyperglycemic effect of citrus peels are not clear, it is possible that their phenolics (flavonoid and non-flavonoid) inhibit α -amylase activity *in situ*.

The extracts from the peels inhibited α-glucosidase activity, with grapefruit extracts showing the highest inhibition, compared to orange and shaddock peels, where there was no significant ($P>0.05$) difference in their inhibitory ability (Figure 6). Inhibition of α-glucosidase enzyme systems reduces the rate of digestion of complex carbohydrates, and less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of this inhibition is to decrease current blood glucose levels: the long term effect is a small reduction in hemoglobin A1c level (Kwon *et al*, 2006, 2007). The ability of the peels to inhibit α-glucosidase activity could also be linked to the phenolics of the peels, as phenolics from orange and grapefruit peels have been shown to inhibit α-glucosidase activity *in vitro* (Oboh and Ademosun, 2010a and b).

Furthermore, a comparison of the α-amylase and α-glucosidase inhibition by the extracts from the peels at the same concentration (150 µg/ml) shows a stronger inhibition of α-glucosidase activity, but mild inhibition of α-amylase activities and this could address the main drawback of currently used α-glucosidase and α-amylase inhibitors drugs such as acarbose with side effects such as abdominal distention, flatulence, meteorism and possibly diarrhoea (Bischoff, 1994). It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Horii *et al*., 1987; Bischoff, 1994). Therefore, this result confirms the claim that natural inhibitors from dietary plants have lower inhibitory effect against α -amylase activity and a stronger inhibitory activity against α-glucosidase and can be used as effective therapy for postprandial hyperglycemia with minimal side effects (Kwon *et al*., 2006, 2007). The inhibitory effect of citrus peels aqueous extracts could provide some biochemical in-sight for the use of these citrus peels in the

management of type 2 diabetes, in addition to the already identified insulin-like compound in citrus peels - emulin (Shpigel, 2008).

In conclusion, the inhibition of key enzymes linked with type-2 diabetes (α – amylase and α-glucosidase) and oxidative stress; and other antioxidant activities exhibited by the aqueous extracts of the citrus peels could be part of the mechanism by which citrus peels manage and/ or prevent type-2 diabetes. Moreover, the strong α-glucosidase inhibition, but mild inhibition of α-amylase makes it a good nutraceutical for the management of type-2 diabetes with minimal side effects currently observed with some of the drugs presently used for type-2 diabetes.

Table 3: EC_{50} and inhibition of sodium nitroprusside induced lipid peroxidation in pancreas (mg/ml) of rats by aqueous extracts of citrus peels

Sample	**EC_{50}**
Orange peels	3.01 ± 0.40
Grapefruit peels	3.30 ± 0.20
Shaddock peels	3.12 ± 0.50

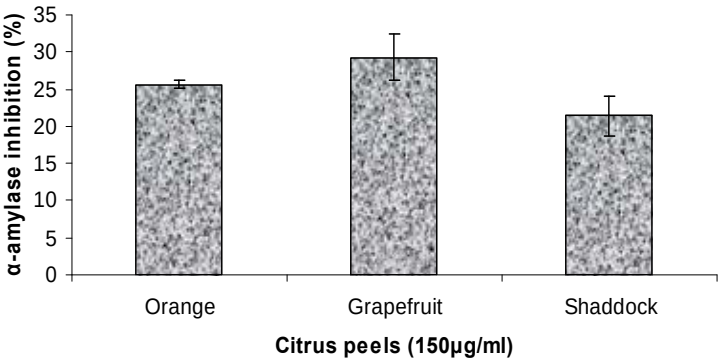

Fig. 5: Interaction of aqueous extracts of some citrus peels with α-Amylase

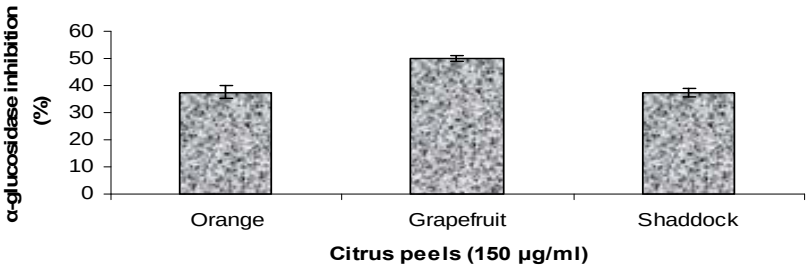

Fig. 6: Interaction of aqueous extracts of some citrus peels with α-Glucosidase

REFERENCES

Alia, M., Horcajo, C., Bravo, L. and Goya L. (2003). Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutrition Research 23: 1251–1267

Amic, D., Davidovic-Amic, D., Beslo, D. and Trinajstic, N. (2003). Structure-related scavenging activity relationship of flavonoids. Croatia Chemical Acta 76: 55–61

Apostolidis, E., Kwon, Y. I. and Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science and Emerging Technologies 8: 46-54.

Bayır, H., Kochanek, P. M. and Kagan, V. E. (2006). Oxidative stress in immature brain after traumatic brain injury. Developmental Neuroscience 28: 420–431.

Baynes, J. W. (1991). Role of oxidative stress in the development complications in diabetes, Diabetes 40: 405-412.

Belitz, H. D. and Grosch, W. (1999). Fruits and fruit products In. Hadziyev, D. (Ed.). *Food Chemistry*, Berlin, Heidelberg: Springer Verlag. pp. 748-799.

Belle, N. A. V., Dalmolin, G. D., Fonini, G., Rubim, M. A. and Rocha, J. B. T (2004). Polyamines reduces lipid peroxidation induced by different pro-oxidant agents. Brain Research 1008: 245-251.

Bischoff, H. (1994). Pharmacology of alpha-glucosidase inhibition. European Journal of Clinical Investigation 24: 3-10.

Block, G., Patterson, B and Subar, A. (1992). Fruits, vegetables and cancer prevention. A review of epidemiological evidence. Nutrition and Cancer 18: 1-29.

Choukem, S. P., Kengne, A. P, Dehayem, Y. M., Simo, N. L. and Mbanya, J. C. (2007). Hypertension in people with diabetes in sub-Saharan Africa:Revealing the hidden face of the iceberg. Diabetes Research and Clinical Practice 77: 293-299.

Chu, Y., Sun, J., Wu, X. and Liu, R. H. (2002). Antioxidant and antiproliferative activity of common vegetables. Journal of Agriculture and Food Chemistry 50: 6910-6916.

Ghasemi, K., Ghasemi, Y. and Ebrahimzadeh M. A. (2009). Antioxidant Activity, Phenol and Flavonoid Contents Of 13 Citrus Species Peels And Tissues. Pakistan Journal of Pharmaceutical Science 22: 3: 277-281

Gorinstein, S., Martin-Belloso, O., Park, Y., Haruenkit, R., Lojek, A. and Ciz, M. (2001). Comparison of some biochemical characteristics of different citrus fruits. Food Chemistry 74: 309-315.

Horii, S., Fukasse, K., Matsua, T., Kameda, K., Asano, N. and Masui, Y. (1987). Synthesis and a-D-glucosidase inhibitory activity of N-substituted valiolamine derivatives as potent oral antidiabetic agents. Journal of Medicinal Chemistry 29: 1038-1046.

International Diabetes Federation (2001). www.idf.org

Ipinjolu, J. K. (2000): Performance of juvenile orange koi carp (*Cyprinucarpio leanneanus*) fed diets supplemented with sweet orange peel meal: Body composition, nutrition, utilization and skin pigmentation. Sokoto Journal of Veterinary Science 228-229.

Jayaprakasha, G. K., Girennavar, B. and Patil, B. S. (2008). Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different *in vitro* model systems. Bioresource Technology 99(10): 4484-4494.

Kedage, V. V., Tilak, J. C., Dixit, G. B., Devasagayam, T. P. A. and Mhatre, M. A (2007). Study of antioxidant properties of some varieties of grapes (*Vitis vinifera* L.). Critical Rev. Food Science and Nutrition 47: 175-185.

Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B. and Yankova, T. (2006). Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytotherapy Research 20(11): 961- 965.

Klimczak, I., Malecka, M., Szlachta, M. and Gliszczynska-Swiglo, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. Journal of Food and Compositional Analysis 20: 313-322.

Kwon, Y. I., Vattem, D. A. and 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., Kim, Y. C. and Shetty, K. (2007). Health benefits of traditional corn, beans and pumpkin: *In vitro* studies for hyperglycemia and hypertension management: Journal of Medicinal Food 10: 266–275.

Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. Food Chemistry 91: 571-577

Mouly, P. P., Arzouyan, C. R., Gaydou, E. M. and Estienne, J. M. (1994). Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavonone glycosides. Journal of Agriculture and Food Chemistry 42: 70-79

Oberley, L. W. (1988): Free radicals and diabetes. Free Radicals in Biology and Medicine 5: 113 - 124

Oboh, G. and Ademosun, A. O., (2006). Comparative studies on the ability of crude polyphenols from some Nigerian citrus peels to prevent lipid peroxidation – In vitro. Asian Journal of Biochemistry 1: 169 - 177

Oboh, G and Ademosun, A.O. (2010a). Phenolic extracts from Grapefruit Peels (Citrus paradisi) Inhibit Key Enzymes Linked to Type-2 Diabetes and Hypertension. Journal of Food Biochemistry, USA – In Press

Oboh, G and Ademosun, A.O. (2010b). Phenolics from Orange Peels (Citrus sinensis) Inhibit Key Enzymes Linked to Non-Insulin Dependent Diabetes Mellitus (NIDDM) and Hypertension. La Rivista Italiana Delle Sostanze Grasse , Italy – In Press

Oboh, G. and Rocha, J.B.T., (2007a). Antioxidant in foods: A new challenge for food processors. In: Panglossi, H. V. (Ed), *Leading Edge Antioxidants Research,* Nova Science Publishers Inc. New York US, pp. 35-64.

Oboh, G. and Rocha, J. B. T. (2007b): Polyphenols in Red Pepper [*Capsicum annuum var. aviculare* (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. European Food Research and Technology 225: 239-247

Oboh, G. and Rocha, J. B. T. (2008). Antioxidant and neuroprotective properties of sour tea (*Hibiscus sabdariffa*, calyx) and green tea (*Camellia sinensis*) on some pro-oxidants induced lipid peroxidation in brain - *In vitro*. Food Biophysics. 3: 382-389.

Oboh, G., Puntel, R. L. and Rocha, J. B. T. (2007). Hot pepper (*Capsicum annuum, Tepin and Capsicum chinese, Habanero*) prevents Fe^{2+} - induced lipid peroxidation in brain – *In vitro*. Food Chemistry 102: 178-185.

Oboh, G. and Shodehinde, S. A. (2009). Distribution of nutrients, polyphenols and antioxidant activities in the Pilei and Stipes of some commonly consumed edible mushrooms in Nigeria. Bulletin of Chemical Society of Ethiopia 23 (3):

Oboh, G. (2005). Effect of blanching on the antioxidant property of some tropical green leafy vegetables. Lebensm Wiss Technology 38: 513–517.

Ohkawa, H., Ohishi, N, and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Annals in Biochemistry 95: 351-358.

Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition.,* **44**: 307–315

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radicals in Biology and Medicine. 26: 1231-1237.

Schieber A, Stintzing, F. C. and Carle, R. (2001). Byproducts of plant food processing as a source of functional compounds - recent developments. Trends in Food Science and Technology 12: 401-413.

Shim, A., Young-Jun, A., Doo, F., Ho-Kyung, B., Ahn, D., Se-Young, B., Kim, S. and Yong-Suk, C. (2003). Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose Journal of Ethnopharrmacology 85:283–287.

Shpigel, J. R. (2008). Citrus peel compound Emulin beneficial to people with diabetes. Focus Express Mail Pharmacy Inc.

Singleton, V. L., Orthofor, R. and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocaltau reagent. Methods in Enzymology**. 299**: 152-178

Sun, J., Chu, Y. F., Wu, X. and Liu, R. H. (2002). Antioxidant and antiproliferation activities of common fruit. Journal of Agricultural and Food Chemistry 50: 7449-7454.

Wolff, S. P. (1993). Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology mellitus and complications. British Medical Bulletin 49: 642-652

Worthington Biochemical Corporation (1978). Worthington, enzyme and related biochemicals. Worthington Biochemical Corp., Freehold, N. J.

Zar, J. H. (1984). Biostatistical analysis, Prentice-Hall, Inc., USA, pp. 620.

Zhang, J. and Kashket. S. (1998). Inhibtion of salivary amylase by black and green teas. Caries Research 32:233-238.