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Evaluation of the interaction of maize fiber gum with α-amylase and α-glucosidase enzymes and its effect on enzymes inhibition activity

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ABSTRACT

Maize bran is the most common by-product of maize milling process and it is mainly used as animal feed. In this study, antioxidant and anti-diabetic activities of two types of maize fiber gum, FAX (fiber with phenolic compounds) and Y (fiber without phenolic compounds), were examined. In addition, intrinsic and extrinsic fluorescence intensity was assessed to explore the inhibitory mechanism of two enzymes, α-amylase and α-glucosidase. The results revealed that FAX had the highest DPPH radical scavenging property at 39.74 ± 0.399 µmolTE/g, whereas Y had 3.73 ± 0.257 µmolTE/g. Furthermore, the ABTS cationic radical scavenging activity in FAX was 137.10 ± 2.99 µmolTE/g, whereas Y was 29.68 ± 1.17 µmolTE/g. FAX had a higher inhibition rate of porcine α-amylase enzyme activity than Y, and the difference was significant ($p < 0.05$). FAX inhibited rat intestinal α-glucosidase activity the highest (26.15%), whereas Y had no enzyme inhibition property at the concentration used. In addition, applying different concentrations of both fibers to α-amylase and α-glucosidase enzymes resulted in a decrease in fluorescence intensity; however, this intensity was higher for FAX. Both fibers were able of inhibiting both enzymes by changing the third structure of the enzyme via non-covalent bonds. Overall, the results showed that high phenolic fiber from maize bran can be consider as a natural source of antioxidant activity and inhibition of α-amylase and α-glucosidase enzymes, and that it can be used in the production of functional foods.

1. Introduction

As human age increases, the probability of chronic diseases such as cardiovascular diseases, neurological diseases, type 2 diabetes and various types of cancer increases.[1]. Diet helps a lot in curing a wide range of diseases[2]. Due to the presence of bioactive compounds with health-giving benefits, super-beneficial foods and nutritional supplements are the key to maintaining health and preventing chronic diseases related to diet and reducing health care costs. On the other hand, the development of safe natural and bioactive compounds from different sources with less risk potential than synthetic substances that can be used to prevent or treat several diseases has attracted more attention. Therefore, there is a lot of interest in researching compounds that can be used as useful food, food or medicine.[3].

Carbohydrates are the most important source of food energy, which make up a significant part of the total food consumed. Currently, the focus of research is on the role of dietary carbohydrate consumption and its relationship with obesity, insulin resistance, metabolic syndrome, heart function and blood circulation. In fact, the consumption of diets with a low glycemic index is related to reducing the risk of chronic diseases such as diabetes and cardiovascular diseases and maintaining an optimal body weight. Therefore, finding foods with a low glycemic index and also finding factors that can reduce the glycemic index of food have been considered.[4]. Diabetes is a set of metabolic diseases characterized by high blood sugar (hyperglycemia), disturbances in carbohydrate, fat and protein metabolism, defects in insulin secretion, insulin action, or both.[5]. One of the important approaches to reduce blood sugar after eating is to reduce or slow down the digestion and absorption of dietary carbohydrates [6]. The role of α-amylase enzyme becomes more colorful after consuming food as it converts polysaccharides in food such as glycogen, starch, etc. into glucose by breaking the beta bond (1 and 4) and causes an increase in blood sugar in the body. Today, inhibiting the activity of this enzyme is one of the challenges in dealing with diabetes [7]. In a study, the inhibitory effect of Elang tea polyphenols, epigallocatechin gallate (EGCG) and 3-O epigallocatechin gallate (EGCG3Me) on pancreatic α-amylase enzyme was investigated by absorption of ultraviolet spectrum and fluorescence. Based on the obtained results, the polyphenolic compounds of Elang tea, EGCG and EGCG3Me all have inhibitory properties towards α-amylase enzyme and the inhibitory half-concentration value $(IC)_{50}$) they were equal to 0.375, 0.350 and 0.572 mg/ml, respectively. Examining the fluorescence diagram showed that the inhibition pattern of Alang tea polyphenols and EGCG is competitive, but the inhibition of EGCG3Me is non-competitive. The difference in the type of inhibition of EGCG and EGCG3Me can be attributed to the structural difference of these two compounds[8]. According to the study of Zahraldin et al. (2018), the extracts obtained from various edible seaweeds, alginate obtained from brown algae and phenolic compounds have the potential to inhibit α-amylase enzyme. In this study, the inhibition kinetics of the enzyme was compared with acarbose. Extract obtained from algae*Laminaria digita* And*Andaria pinnatifida* It has an inhibitory effect and, respectively, with IC_{50} 0.74 and 0.81 mg/ml of both extracts had a combined inhibitory effect. The phenolic compound of 2 and 5 dihydroxybenzoic acid also has the ability to inhibit α-amylase enzyme and has IC_{50} It is 0.046. Alginate obtained from brown algae also has IC_{50} It was 0.074 mg/ml and its inhibitory mechanism was combined. Based on the results obtained from this research, crude extracts of seaweed, phenolic compounds and alginates are strong α-amylase inhibitors. As a result, they potentially delayed the release of glucose from starch and caused a momentary decrease in blood sugar after eating [9].

Fibers have many nutritional properties and their binding to digestive enzymes such as α-amylase is perhaps one of their most important features. In this study, the inhibitory effect of soluble (SDF) and insoluble (IDF) fibers extracted from ripe kiwi fruit on α-amylase enzyme was investigated. In equal concentrations of both types of fibers, insoluble fibers had more inhibition. The analysis of Mikael-Menten kinetics showed that the mode of inhibition of insoluble fibers was combined, so that the equilibrium constant of inhibition was

competitive and the inhibition of the enzyme was equal to 4 mg/ml. The enzyme inhibition of soluble fibers is non-competitive and the enzyme inhibition rate was 12.5 mg/ml [10]. In the study of Dital et al. (2015), the inhibitory effect of cellulose on α-amylase enzyme was investigated. The presence of cellulose decreased the rate of starch digestion. Analyzing the kinetics of α-amylase enzyme in the digestion of corn starch in the presence of cellulose showed that the inhibition type of cellulose fiber was mixed type and the inhibition level of cellulose enzyme was equal to 3 mg/ml. All the results showed the potential of cellulose as an inhibitory compound of starch digestion [11].

According to Rose et al.'s study (2008), about 25.6 million tons of corn are produced annually by wet milling and 6 million tons by dry milling, of which about 2.43 million tons of corn fiber and 0.341 million tons are produced. It is bran. The only use of these by-products produced is as animal feed. The main compound in fiber and corn bran is dietary fiber. Corn fiber itself consists of 280 g/kg of cellulose, 700 g/kg of hemicellulose and only a small fraction of about 10 g/kg of lignin [12]. Hemicellulose extracted from corn bran by alkaline extraction is also called corn fiber gum (MFG). The produced corn fiber gum contains ferulic acid groups in an esterified form (even after alkaline treatment). Nowadays, ferulic acid is of interest due to its antioxidant properties, and the ferulic acid compounds in the extracted MFG can be used to deal with gastrointestinal diseases. In fact, when the fiber is without ferulic acid, it is quickly absorbed in the upper part of the digestive tract. While phenolic bonds are released in the lower part of the digestive tract by microbial esterases, and because of this, they acquire the ability to absorb free radicals in this part of the intestine [13]. The antioxidant property of corn fiber gum can be related to the hydrocinnamic acids present in the fiber [14].

According to previous studies, there has been no research on the effect of corn fiber gum containing phenolic compounds on inhibiting the activity of α-amylase and α-glucosidase enzymes. Therefore, the purpose of this research is to investigate the degree of inhibition of α-amylase and α-glucosidase enzymes by corn fiber gum and to investigate the interaction between this fiber and two enzymes.

2- Materials and methods

2-1- Materials

The raw material needed to carry out this research is corn bran, which was obtained from Zar Fructose Factory (Karaj, Iran). The purchased corn bran was obtained by wet milling method. After sampling, the bran was separated by a 20 mesh sieve and kept at room temperature. Sodium hydroxide, hydrogen peroxide, hydrochloric acid were obtained from Majalli Co., Iran. Ethanol (non-toxic) from Bidestan Alcohol Company, DPPH (Diphenyl-1-picrylhydrazyl-2-2) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) from Sigma, Trolox , methanol, Folin-Ciocalto reagent, gallic acid and potassium persulfate from Merck, Germany, and commercial α-amylase enzyme from Novozyme, Denmark, PAHBAH (4-Hydroxybenzhydrazide), PNPG (4-Nitrophenyl α-D-glucopyranoside), α-enzyme Porcine amylase and mouse intestinal powder were obtained from Sigma.

2-2- Corn fiber gum extraction

The primary corn bran was crushed by a grinder and then passed through a 40 mesh sieve. Chopped bran was first mixed with distilled water to remove starch, then it was treated with thermomyl (α-amylase) enzyme. Bran starch was separated from bran by filtering and washing with distilled water, and the remaining material was placed in a 50-degree oven to dry overnight. After starching, corn fiber gum was extracted using the following two methods:

1- Fiber extraction by alkaline method + hydrogen peroxide (for ease of recognition, the fiber produced by this method was named Y) [13].

2- Fiber extraction by alkaline method without hydrogen peroxide (for ease of identification, the fiber produced by this method was named FAX) [15].

2-3- Total phenol and antioxidant properties of corn fiber gum

2-3-1- check the amount of total phenol

Folin-Ciocalto method was used to measure the total amount of phenolic compounds. According

to this method, 20 microliters of the sample is mixed with 100 microliters of Folin's solution and 1.16 ml of distilled water, and after about 8-3 minutes, about 300 microliters of 20% (W/V) sodium carbonate solution is added to the correct solution. has been added The resulting solution was transferred to test tubes with lids and the tubes were placed in a hot water bath at 40 degrees Celsius. After 30 minutes, the absorbance of the samples against the control sample (the original sample was replaced with distilled water) was read by a spectrometer at a wavelength of 765 nm.

The standard curve of gallic acid was drawn in concentrations (80-500 ppm) and the amount of total phenolic compounds in the sample was calculated in mg of gallic acid per gram of dry matter [16].

2-3-2- DPPH free radical inhibitory power

To perform the DPPH test, a solution of this compound was first prepared with a concentration of 0.1 mM (1.97 mg of DPPH powder was carefully dissolved in 50 ml of methanol). Then the samples were made with different concentrations and 100 microliters of the samples were mixed well with 900 microliters of DPPH solution in a suitable container and placed in a dark environment for half an hour at room temperature. After half an hour, the absorbance of the desired solution was read at 517 nm. The inhibition percentage of the desired substance was calculated according to the following equation:

Inhibition %= Attracting absorption* - Soluble absorption / Attracting absorption ×100

* Control absorption: In the control sample, instead of using the sample, the same amount of water is used [17].

The ability to inhibit the created radicals was expressed using the Trolox standard curve equation in different concentrations (0-350) as millimolar Trolox per gram of dry matter.

2-3-3- ABTS radical cation test

The basis of this test is the radical decolorization of ABTS cations by persulfate ions produced by the desired antioxidant. The maximum absorption of ABTS radicals produced by persulfate ions is at 734 nm, the higher the strength of the antioxidant compound, the more colorless the radical solution will be. 20 microliters of samples with different concentrations were mixed well with 980 microliters of ABTS solution and kept for 3-10 minutes in a dark environment at 37°C, and then the absorbance of the solution was read at 734 nm. The control sample was also prepared by adding 980 microliters of ABTS with 20 microliters of distilled water and the inhibitory percentage of the desired sample was calculated with the following equation:

Inhibition %= Attracting absorption* - Soluble absorption / Attracting absorption ×100

The radical scavenging power of ABTS cations was expressed using the Trolox standard curve equation in different concentrations (50-1100) as millimolar Trolox per gram of dry matter [18].

4-2- Anti-diabetic tests

2-4-1- Porcine α-amylase inhibition test

The basis of this reaction is based on the production of reducing sugar in an alkaline environment. The color of reducing sugar produced in combination with a dye called 4-hydroxybenzoic acid hydrazine turns yellow in an alkaline environment, and by increasing the color of the resulting solution at a wavelength of 410 nm, the activity of the enzyme can be calculated. To calculate the inhibitory activity of corn fiber gum, first different concentrations (10-30) mg/ml of fiber were prepared and equal proportions of enzyme and fiber (100 microliters) were mixed together, and then starch solution was added to the resulting mixture at 37°C. and was placed in a greenhouse for 20 minutes. The resulting solution was placed under a temperature of 100 degrees Celsius to deactivate the enzyme. Then, the desired solution was centrifuged at 13,000 rpm for 2 minutes in order to separate the digested starch. Then, 20 microliters of the desired solution was mixed with 3 ml of colored solution and placed under 70°C temperature for 10 minutes. The absorbance of the resulting compound was read at a wavelength of 410 nm [7]. The inhibition was calculated from the following equation:

Inhibition %= Control absorption- Soluble absorption / Control absorption×100

2-4-2- rat intestinal α-glucosidase inhibition test

First, the relevant enzyme was extracted from the mouse intestine. The extracted extract was diluted to the amount of Mu98/ml. Two hundred microliters of α-glucosidase enzyme was incubated with one hundred microliters of the sample for 5 minutes at 37°C. Then 100 microliters of precursor (p-nitrophenol α-diglucopyranoside) was added to the reaction mixture. The amount of inhibition of the enzyme at the wavelength of 405 nm was calculated for each sample from the following equation:
*In the positive control sample, acarbose was used instead of the compound [19].
Inhibition %= Control absorption- Soluble absorption / Control absorption×100

5-2-Extrinsic and intrinsic fluorescence test

Specific amounts of α-amylase enzyme (3 mL, 0.20 mg/mL) and α-glucosidase enzyme (3 mL, 0.25 mg/mL) with 0.3 mL of fiber gum extracted by both methods with concentrations of different (0.25, 0.50, 1, 2 and 4 mg/ml) were combined and kept at 37°C for 5 minutes. The fluorescence intensity of the enzymes was measured with an excitation wavelength of 290 nm and an emission wavelength of 310 to 500 nm [20]. The quenching effect due to molecular collision was evaluated using the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

where F_0 The maximum peak value of enzyme fluorescence intensity and F was the maximum peak value of enzyme fluorescence intensity in the presence of one of the fibers extracted from corn bran. The slope of the graph (KSV) in the Stern-Volmer equation was considered as an index of quenching effect of the collision on the intrinsic fluorescence of α-amylase and α-glucosidase [21].

6-2-Analysis of data analysis

To analyze the data, analysis of variance (ANOVA) was performed using MiniTab statistical software in the form of a completely randomized design. Tukey's test was used to check the difference between the means if the effect of the factors was significant (P<0.05). All results are expressed as the average of 3 repetitions ± standard deviation. Microsoft Excel software was used to draw the figures.

3. Results and Discussion

3-1- Extraction efficiency

The efficiency of fiber extraction produced by both methods used, namely FAX (without the presence of hydrogen peroxide) and Y (using hydrogen peroxide), is shown in Figure 1.

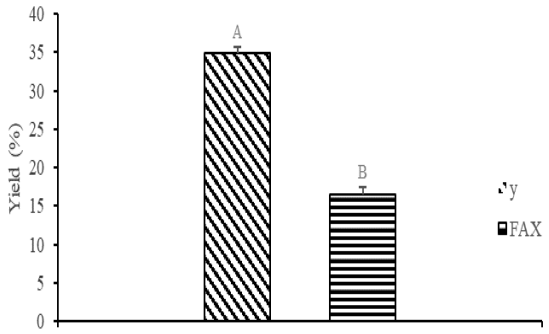


Fig 1 Yield of MFGs (FAX and Y) recovered from destarched maize bran
Different letters denote significant difference between treatments (p < 0.05).

The highest efficiency is related to Y fiber with 34.86%, followed by FAX
It was 16.54 percent. Results of fiber efficiency obtained from bran
Corn obtained from fermented corn and corn bran obtained from wet milling with fiber are reported as 13.01 and 19.02% (wt/wt), respectively [13]. In another study, the efficiency of fiber extracted from corn bran obtained by wet milling was equal to 19.5% (w/w) [15]. Therefore, the results of the present study were consistent with the aforementioned studies.

2-3- Chemical composition of fiber

The compounds in the structure of the extracted fibers were investigated, and the results are shown in Table 1. According to the study of Yadav et al (2010), the amount of protein, moisture and ash in the extracted fiber (with hydrogen peroxide) is 1.97, 6.32 and 6.05, respectively, which was consistent with the results of the present study [13].

Table 1 Proximate composition of maize fiber gums

Ash(%)	Protein(%)	Moisture (%)	sample
0.56^{a}± 6.71	0.08^{b} ± 1.40	0.54^{a} ± 6.52	**AND**
0.21^{a} ± 6.92	0.03^{a} ± 4.73	0.70^{a} ± 6.78	**FAX**

Different letters in each column denote significant difference between samples ($p < 0.05$).

3-3-Total phenol and antioxidant activity of corn fiber

3-3-1- Measuring the total amount of phenol in the extracted fibers

The total amount of phenolic compounds extracted from Y and FAX fiber is equal to 27.73 ± 0.66 and 16.28 ± 0.947 mg of gallic acid per gram of fiber, respectively. The antioxidant capacity of corn fiber gum is dependent on the amount of ferulic acid attached to the arabinoxylan structure in the fiber structure, and ferulic acid may change due to the extraction method [13]. Ayala-Soto et al. (2017) expressed the total phenol content of corn fiber gum extracted from corn bran obtained from fermented corn between 1.95 and 3.55 mg/g of gallic acid [14].

3-3-2 - ABTS cation radical inhibitory activity

Table 2 shows the cationic radical scavenging properties of ABTS. Based on the obtained results, the fiber extracted by both methods has antioxidant properties. The inhibitory activity of the FAX sample (extracted without using hydrogen peroxide) is equal to 137.10 ± 2.99 µmolTE/g, and the Y sample (extracted using hydrogen peroxide) is 29.68 ± 1.17 µmolTE/g. Are. In general, the obtained results show that with the addition of hydrogen peroxide, the amount of ABTS cationic radical inhibition activity in the fiber was reduced. Hydrogen peroxide is not a very reactive compound on its own, but it can sometimes be harmful to cells by increasing hydroxyl radicals. Hydrogen peroxide by sticking to one side of the cell membrane and reacting with Fe^{2+} و $With^{2+}$ And converting these metals into hydroxyl radicals causes destructive reactions in the cell [22].

3-3-3- DPPH radical inhibitory activity

DPPH radical inhibitory activity in terms of micromol Trolox/mg fiber is listed in Table 2. Based on the obtained results, both fiber samples have inhibitory properties. The highest inhibitory property is related to FAX fiber (without using hydrogen peroxide) with 39.74 ± 0.399 µmolTE/g, and then extracted Y fiber with 3.73 ± 0.257 µmolTE/g. By adding hydrogen peroxide in the extraction method, the inhibitory property of fiber decreased so that Y had the lowest inhibitory rate compared to the other extraction method, which can be attributed to the oxidizing property of hydrogen peroxide. In the study of Wang et al. (2010) IC_{50} Corn fiber increased from 0.1 to 1 with the presence of hydrogen peroxide, which well indicates the oxidizing property of hydrogen peroxide [23]. The more the amount of IC_{50} An increase in a compound indicates a lower antioxidant power of that compound. IC_{50} Corresponding to corn fiber gum is about 0.1 mg per ml, compared to ascorbic acid which has IC_{50}, it is 0.04 mg/ml, it is less. If the antioxidant property (IC_{50}) guar gum, sulfates derived from guar gum, oligosaccharides extracted from the hemicellulose of the woody parts of the plant and rice husk are around 4-5 mg/ml, which have weaker antioxidant properties compared to corn fiber gum. So, this gum has a higher antioxidant power compared to natural fibrous and gum compounds [24]. According to Herrera et al.'s (2020) study, the DPPH radical inhibitory activity in FAX (without using hydrogen peroxide) was reported to be 31.69 ± 0.75 µmolTE/g [15]. The DPPH radical scavenging activity of water-extracted corn fiber gum has been reported between 59.9 and 1.71 micromol Trolex/g fiber [25]. In a study, the antioxidant capacity of corn fiber extracted from corn bran obtained from fermentation waste was reported to be 16.39 micromol trolox per gram of fiber [26]. The method of extraction, the type of drying and the variety of the corn plant used are all influencing factors on the antioxidant properties of the produced corn fiber gum [13].

Table 2 Antioxidant activity of maize fiber gums

ABTS (molTE/gμ)	DPPH(manyTE/gμ)	Sample
1.71^{b}± 29.68	1.60^{b} ± 4.73	**AND**
2.99^{a} ± 137.10	0.39^{a} ± 39.74	**FAX**

Different letters in each column denote significant difference between samples ($p < 0.05$).

3-4-test to determine the inhibition of porcine α-amylase

In this research, the ability of fibers extracted from corn bran to inhibit the activity of α-amylase enzyme was investigated in order to delay starch hydrolysis. The obtained results showed the ability of both extracted fibers to inhibit the enzyme. IC_{50} The inhibition of FAX and Y fiber is equal to 33 and 48.8 mg/mL, respectively.

Based on the obtained results, the inhibitory property increases with the increase of the amount of total phenol in the fibers and the extracted fiber has different mechanisms in inhibiting the activity of this enzyme. For example, inhibitors such as acarbose inhibit the activity of the enzyme by forming a complex between the enzyme and the inhibitor. Dietary fibers reduce enzyme activity by making the environment viscous and reducing the rate of glucose release [27]. Based on Yan et al.'s (2019) study, which was conducted on the inhibitory effect of water-soluble dietary fibers extracted from wheat bran by different methods, all the obtained fibers had inhibitory properties. Inhibition of α-amylase enzyme by polysaccharide compounds and fibers is mostly related to fiber constituent units and functional groups linked to the structure [28]. Based on the study of Sun et al. (2016), the inhibitory property of phenolic compounds present in apple against α-amylase enzyme was investigated. Tannic acid, chlorogenic acid and caffeic acid present in apples inhibited α-amylase enzyme. The results of the interaction between the enzyme and phenolic compounds show a decrease in fluorescence intensity (the ability to form a complex between the active site of the enzyme and the phenolic compound) and all these results indicate the good inhibition of the compounds in apple against digestive enzymes [29]. Polyphenolic compounds found in tea (catechins, gallotannins, ellagitannins and other polyphenolic compounds) inhibit the activity of oral α-amylase enzyme [30, 31]. Tannins inhibit the activity of digestive enzymes, especially α-amylase, through the power of binding to protein through the creation of hydrophobic bonds, and are suitable for people with type 2 diabetes [32].

5-3- Rat intestinal α-glucosidase inhibition test

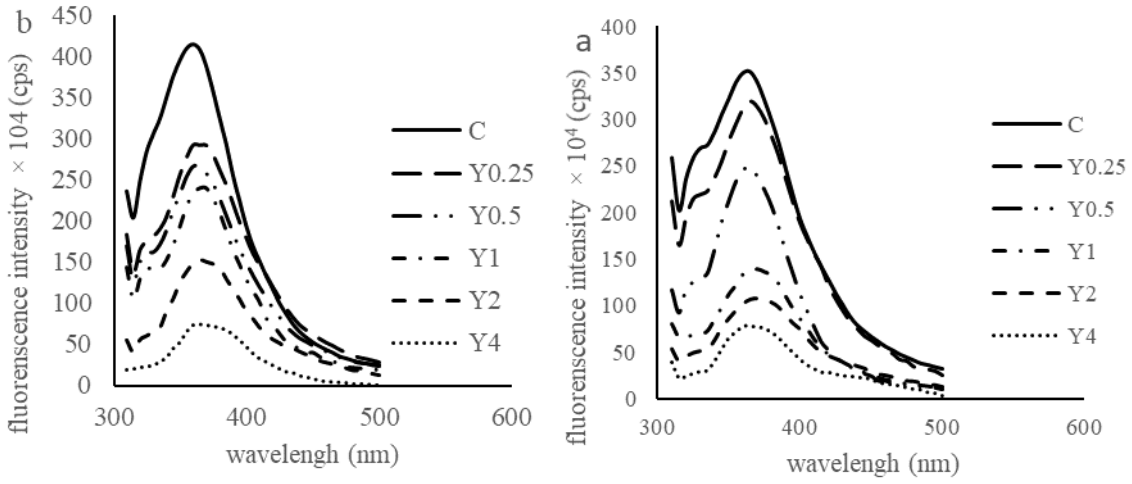
According to the results obtained from this test, the fiber obtained by method Y in the used concentration did not have an inhibitory effect, and the inhibition of FAX fiber in the used concentration (20 mg/mL) was 26.15%.

The α-glucosidase enzyme is one of the most important enzymes in the intestine, which is responsible for breaking down oligosaccharides and converting them into monosaccharides by separating their hydrogen compounds and breaking the (4-1)α bond. Phenolic compounds are one of the most important inhibitors of this enzyme. Phenols inhibit the enzyme by separating the released hydrogen ion from the active site of the α-glucosidase enzyme [27]. Based on this, the better performance of FAX extracted corn fiber can be attributed to having more total phenol than Y fiber. Polyphenolic compounds have the ability to form different bonds with proteins. The relationship between complex formation and enzyme inhibition is direct in that the hydroxyl compounds present in polyphenolic compounds form a link with the active site of the enzyme and cause inhibition of enzyme activity [33]. Phenolic compounds extracted from plant sources have inhibitory activity and (K_i) similar to compounds such as acarbose against α-glucosidase/maltase enzymes [34]. The phenolic compounds extracted from blueberry fruit inhibited the enzyme activity by occupying the active site of α-glucosidase enzyme by a non-competitive method [35].

3-6- intrinsic and extrinsic fluorescence

In the present study, the fluorescence intensity of α-glucosidase and α-amylase enzymes were

measured before and after combining with corn fiber gum in order to investigate the bonds and interactions between them (Figure 2). Amino acid tryptophan is the most important fluorescence emitting factor in proteins. As a result of protein bonding, tryptophan reacts with other amino acid compounds and the intensity of fluorescence decreases according to the amount and type of bonding created. Fluorescence emission spectra for α-glucosidase and α-amylase at $=290_{in}$ λ nm was obtained by adding extracted fibers. Both types of fiber compared to the control sample (sample without fiber) in all used concentrations caused a decrease in the intensity of the emitted fluorescence, which is caused by the involvement of the amino acid tryptophan. According to Sun et al.'s study (2016), phenolic compounds have the ability to form hydrogen bonds and form complexes with proteins through their hydroxyl groups and the remaining hydrophobic parts of amino acids in the active site of enzymes, thereby reducing the intensity of fluorescence in proteins. . Based on this, it can be said that with the increase in the concentration of phenolic compounds in the mixture, the fluorescence intensity will also decrease [29]. The interaction between fibers and proteins usually occurs through non-covalent bonds and changes in the tertiary structure of the protein. Based on the results of Figure (2), the intensity of fluorescence between two enzymes has been reduced by both types of extracted fiber compared to the control sample (no fiber). Therefore, both types of fiber have the ability to inhibit both types of enzymes by changing the third structure of the enzyme and by means of non-covalent bonds [36].



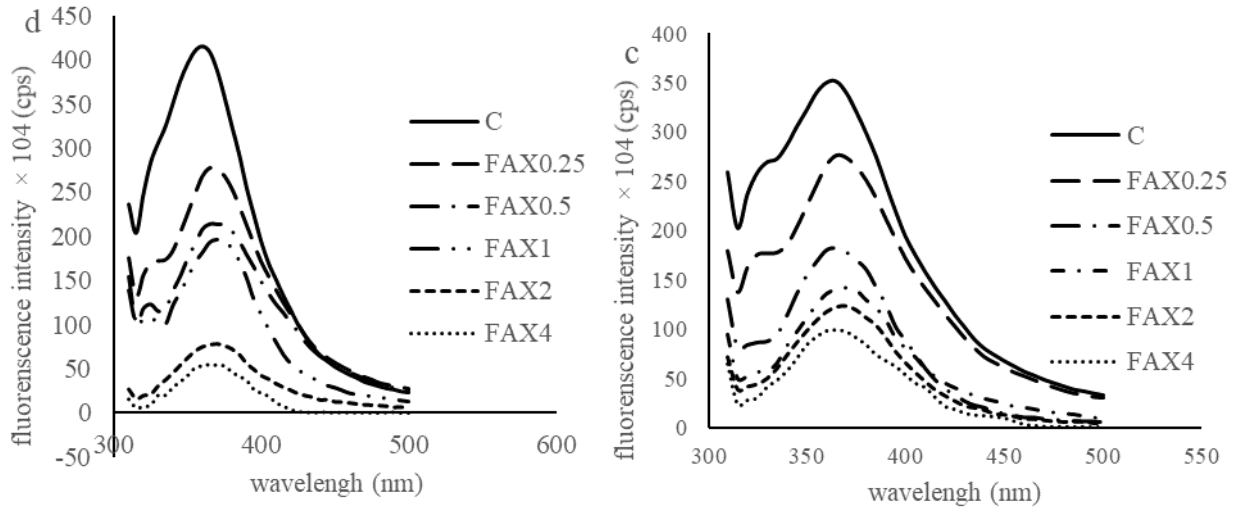
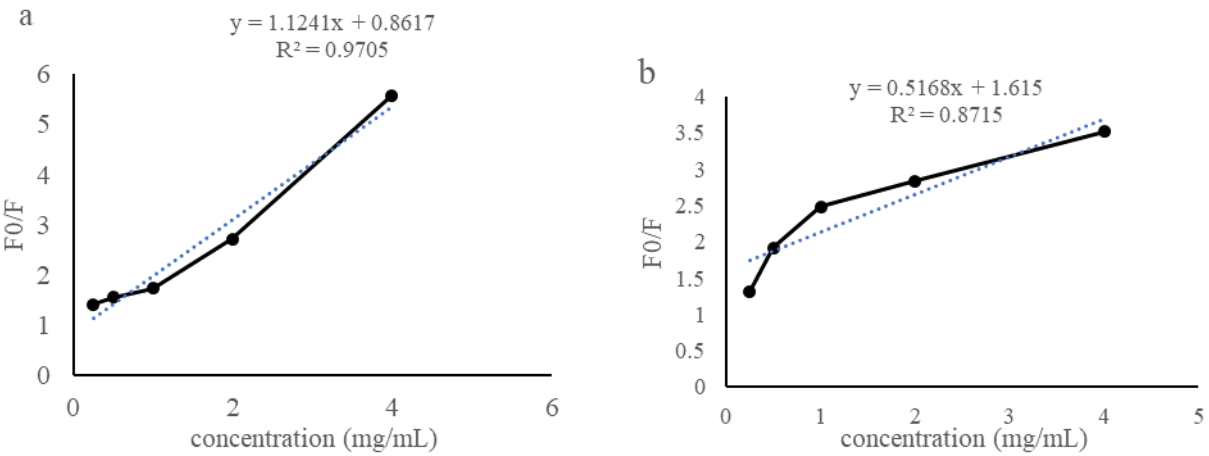


Fig 2 Intrinsic tryptophan fluorescence of α-amylase and α-glucosidase suspensions with different concentrations of FAX (c-d: 0, 0.25, 0.5, 1, 2, 4 mg/mL) and Y (a-b: 0, 0.25, 0.5, 1, 2, 4 mL). 4 mg/mL). (a): α-glucosidase-Y; (b) α-amylase-Y; (c): α-glucosidase -FAX; (d): α-amylase. FAX

Using the Stern-Volmer equation, it is possible to evaluate the collision damping effect. The diagrams in Figure 3 show the application of this equation in the fluorescence emission of the mixed system of α-glucosidase and α-amylase with both types of fiber extracted from corn bran. According to Figure 3, both types of fiber form complexes with both enzymes. The inhibitory effect of both types of fiber on α-amylase enzyme is more than α-glucosidase enzyme. Compared to Y fiber, FAX fiber has more interaction with both types of enzymes, which the result obtained from this section is completely consistent with the results of inhibition of fibers in the fluorescence section.



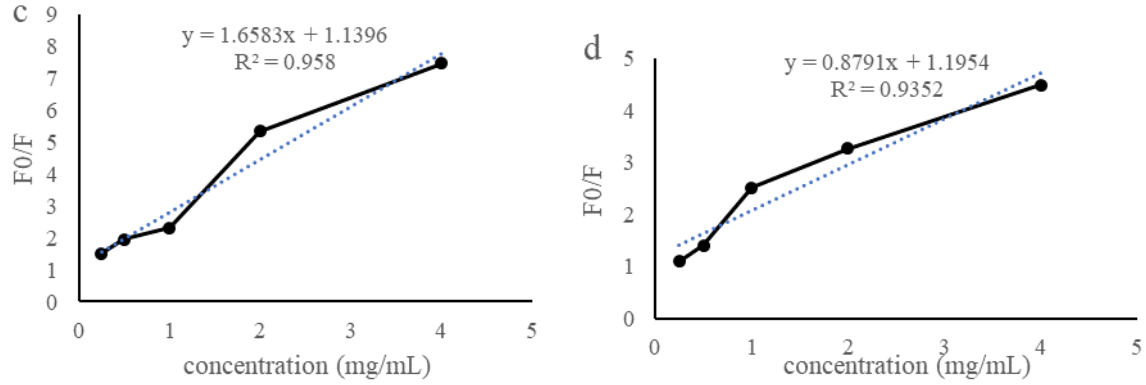


Fig 3 The quenching constant of the interaction between (a): α-amylase-Y; (b): α-glucosidase-Y; (c): α-amylase-FAX and (d): α-glucosidase-FAX.

4 - Conclusion

In this study, corn fiber was extracted from corn bran by two methods, with and without the presence of hydrogen peroxide, and its antioxidant and antidiabetic activity was investigated. Based on the obtained results, FAX fiber had more antioxidant and inhibitory activity than Y fiber against porcine α-amylase and rat α-glucosidase enzymes. Also, the fluorescence quenching results showed that the fibers inhibited the activity of both α-amylase and α-glucosidase enzymes by making changes in the tertiary structure of these enzymes through non-covalent interaction. In general, FAX extracted fiber gum can be useful in food formulations for the health of people due to its high phenolic compounds and anti-diabetic and antioxidant potential.

5- Gratitude

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6- Resources

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مقاله علمی_پژوهشی

بررسی برهم کنش صمغ فیبر ذرت با آنزیم α-آمیلاز و α-گلوکوزیداز و تاثیر آن بر فعالیت ممانعت کنندگی آنزیم‌ها

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چکیده

بیشترین ترکیب جانبی حاصل از فراوری ذرت در صنعت تولید نشاسته از ذرت، سبوس می‌باشد که فقط به صورت غذای دام از آن استفاده می‌شود. در این مطالعه خواص ضد اکسایشی و ضد دیابتی صمغ فیبر ذرت دارای فنل (FAX) و بدون فنل (Y) از سبوس ذرت مورد بررسی قرار گرفت. همچنین جهت بررسی سازوکار ممانعت کنندگی دو آنزیم α-آمیلاز و α-گلوکوزیداز، میزان شدت فلوئورسانس ذاتی و خارجی اندازه گیری شد. نتایج نشان داد بیشترین خاصیت مهارکنندگی رادیکال DPPH مربوط به فیبرFAX µmolTE/g ۰/۳۹۹ ± ۳۹/۷۴، و کمترین مربوط به فیبر استخراج شده Y µmolTE/g ۰/۲۵۷ ± ۳/۷۳ می‌باشد. همچنین نتایج فعالیت مهارکنندگی رادیکال کاتیونی ABTS مربوط به نمونه FAX برابر با µ molTE/g ۲/۹۹ ± ۱۳۷/۱۰ بوده و نمونه µ molTE/g Y ۱/۱۷± ۲۹/۶۸ بود. میزان مهار کنندگی فعالیت آنزیم α-آمیلاز خوکی نمونه FAX بیشتر از Y بوده و اختلاف معنی‌داری با یکدیگر داشتند (p < ۰/۰۵). نمونه فیبرFAX بیش‌ترین تاثیر را بر مهار فعالیت آنزیم گلوکوزیداز موشی داشت (۲۶/۱۵درصد) در حالیکه نمونه Yدر غلظت‌های استفاده شده فاقد خاصیت مهار آنزیم بود. همچنین کاهش شدت فلوئورسانس در اثر افزودن غلظت‌های مختلف هردو فیبر به آنزیم α-آمیلاز و α-گلوکوزیداز مشاهده شد ولی این شدت برای نمونه FAX بیشتر بود. فیبرهای تولیدی توانایی مهار کنندگی هر دو آنزیم را از طریق ایجاد تغییر در ساختمان سوم آنزیم به وسیله پیوندهای غیرکوالانسی را داشتند. بطور کلی نتایج نشان داد که فیبر با میزان فنل بالا از سبوس ذرت می‌تواند به عنوان یک منبع طبیعی دارای فعالیت ضد اکسایشی ومهار فعالیت آنزیم های α-آمیلاز و α-گلوکوزیداز محسوب شده و در تولید مواد غذایی فراسودمند مورد استفاده قرار گیرد.