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Utilization of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers powder in preparing healthy crackers with antioxidant, anti-inflammatory, and anti-diabetic properties *"in vitro"*

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ABSTRACT

This study provides an overview of Jerusalem artichokes (*Helianthus tuberosus*) and their potential uses in consumer foods as antioxidant, anti-inflammatory, and anti-diabetic agents. The sensory, physical properties, and color characteristics of crackers with 20, 30, and 40% substitution with Jerusalem artichokes (JA) tubers powder were evaluated, compared to the control crackers (50% whole wheat + 50% yellow corn). Chemical composition, minerals, phenolics, flavonoids, and carotenoids were determined in JA powder, control crackers, and 20% JA crackers. Antioxidant, anti-inflammatory, and anti-diabetic activities were determined. The addition of JA tubers powder increased the cracker's contents of fibers, ash, protein, iron, calcium, potassium, magnesium, and phosphorus when compared to the control crackers. Crackers with 20% JA tubers substitution had a significantly high level of phenolics, flavonoid, and carotenoids. Besides *in vitro* their antioxidant, anti-inflammatory, and anti-diabetic activities when compared to control crackers.

Key words: Anti-diabetic, Antioxidant, Anti-inflammatory, Chemical composition, Jerusalem artichoke.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder caused by insufficient or ineffective insulin. Chronic hyperglycemia contributes significantly to the development of organ damage in diabetes by producing reactive oxygen species (ROS). An organism's antioxidant defence mechanism is essential for mitigating the harmful effects of ROS (Robertson and Harmon, 2007). Thus, dietary antioxidant intake is crucial for preventing tissue and organ damage. In order to prevent pre-diabetes and manage type 2 DM, the American Diabetes Association has suggested medical nutrition therapy, which calls for consuming large amounts of soluble fiber (Franz et al., 2010). Despite having a long history of cultivation, the Jerusalem artichoke (*Helianthus tuberosus* L.), a perennial plant in the *Asteraceae* family, is still little known despite the fact that its tubers are consumed as food all over the world. Jerusalem artichokes (JA), which has been grown for generations, was initially used as food for humans and then as animal feed (Ben Chekroun et al., 1996). Because of its resistance to dry spells, frost, and poor soils, it is easy to grow. It may thrive in a variety of soil types, such as sandy soil, salt-affected soil, and marginal fields with almost no fertiliser (Razmkhah et al., 2017;

Fang et al., 2018; Kaszás et al., 2018). It is a tall (2.5–3.5 m) ornamental plant with somewhat smaller flowers that resemble sunflowers (Burdzenia, 2001). Due to its many applications, JA is attracting more attention (Abd Alla et al. 2014). It is added to sausages and pastries, consumed as a vegetable salad, and utilized as animal feed (Praznik et al., 2002; Panchev et al., 2011; Afoakwah et al., 2015; Afoakwah and Mahunu, 2022). The high levels of fructose and inulin in the tubers of JA are the primary causes of its acknowledged nutritional benefit. Inulin is the main dietary fiber in JA and a recognized prebiotic. It has been shown to lower blood glucose levels and maintain human mineral bioactivity (Afoakwah and Mahunu, 2022). Additionally, JA tubers are the source of fiber, also fructans, best known and the most appreciated components of Jerusalem artichoke tubers. The chemicals that make up fructans include inulin and oligofructose. A fructose oligosaccharide with two to ten monosaccharide residues is called oligofructose, while inulin has a fructan chain length that ranges from two to sixty units. According to some descriptions, a high-performance fructan has an average degree of polymerisation of 25.

The high-performance soluble type fructans (two to sixty monosaccharide residues) that JA stores as carbohydrates replace the insoluble fibers (Niness, 1999). The beneficial effects of fiber rich diet in the prevention of numerous illnesses led to a search for foods high in dietary fiber. Food enriched with soluble fiber derived from fruits, vegetables, and other unprocessed plant components has shown a notable increase (Dziugan et al., 2006). Additionally, JA generates a vast amount of green biomass that is a rich source of biomolecules, including proteins, polyacetylenic derivatives, sesquiterpene compounds, flavonoids, chlorophylls, phenolics, volatile essential oils (primarily β-bisabolene and 17 other identified volatile compounds), carotenoids, and some amino acids (AA), which have antibacterial, antitumor, antioxidant, and anti-inflammatory properties (Pan et al., 2009; Yuan et al., 2012; Chen et al. 2014; Long et al., 2016). In the study by Aslan et al. (2010), JA tubers have an anti-diabetic potential in streptozotocin - induced diabetic rats. According to Sedej et al. (2011), crackers are common snack food in the human diet. They are described as crisp, thin wafers or biscuits that are typically created with dough that hasn't been sweetened (Han et al., 2010). Crackers were examined in this study as a substitute food to consume the minerals and inulin that are present in large amounts in JA. Therefore, adding powdered Jerusalem artichoke tubers to crackers will result in fiber rich functional healthy snacks. Thus, this study aimed to evaluate chemical composition, total phenol, flavonoid and carotenoid contents and also in vitro antioxidant, anti-inflammatory and anti-diabetic activities were measured in JA tubers and JA crackers. Additionally, the sensory properties, physical measurements and color characteristics of produced crackers.

2. Materials and methods

Material

Jerusalem artichoke (*Helianthus tuberosus* L.) tubers were obtained in September 2023 from Hortculture research station Al-kanater El-khaireya, Agricultural Research Center, Dokki, Giza, Egypt. The other ingredients used were of common consumer brand and all chemicals used in the entire study were purchased from Al-Gomhorya Company for medicine and chemicals trading and medical devices, Mansoura, Egypt.

Preparation of Jerusalem artichoke tubers powder

Samples of JA tubers were carefully peeled, washed to remove impurities and then dried with blotting paper. After that, it was sliced and sun dried at 35: 37º C. Then, the dry material was ground until it could pass 60 mesh sieves, the flour was bagged up and stored at 4˚C until further analysis (Fig. 1).

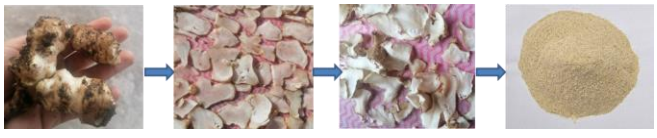

Fig. 1. Preparation of JA tubers powder

Lipid production and cultivation conditions. Crackers preparation

Flour preparation: the whole wheat flour was substituted with yellow corn flour in the fixed ratio of; Whole wheat flour: yellow corn flour = 50: 50. Substitution of flour blend with Jerusalem artichoke tuber powder: Flour blend (F) was partially substituted with 20%, 30%, and 40% of

JA powder. This made three different formulations for cracker preparation (F: JA = 80:20, 70:30 and 60:40).

Crackers preparation

Cracker bake trials were carried out in lab conditions. Equipment used in laboratories was used for ingredient weighing, processing, and baking. Samples of crackers were prepared using the common dough method. The dry ingredients were thoroughly combined, including a flour mixture of 50% whole wheat and 50% yellow corn, JA tubers powder, baking powder and salt. After that, water was gradually added and well combined to create a dough. To make sure the liquids were distributed evenly, the former dough was left to rest at room temperature for ten minutes. A dough cutter was used to cut the dough into round crackers after the dough had been manually sheeted into large sheets. The crackers were put in an oven tray that had been lightly dusted and oiled. The tray with the crackers was then baked for 20 to 30 minutes at 165 ºC. After baking, the crackers were allowed to cool for fifteen minutes at room temperature. Control crackers were made using a flour mix of 50% whole wheat and 50% yellow corn without JA tubers powder. Fig. 2 shows the final form of baked crackers.

Fig. 2. The baked crackers

Ethanolic extract of JA tubers and crackers powders.

Ethanolic extract of samples was prepared according to the method described by Elbadrawy and Mostafa, (2024). Briefly, 500 g of dried samples were macerated in 500 ml of ethanol overnight and filtered. The residue was resoaked in ethanol and filtered twice. The filtrate was collected and subjected to evaporation using a rotary evaporator to obtain the extract. It was then allowed to dry in a desiccator over anhydrous $CaCl_2$ until it reached a constant weight. The extract was stored under freezing till further analysis.

Sensory evaluation of crackers

A panel of ten skilled judges used a 5-point rating system to assess the crackers' sensory qualities. Judges were familiar with the rating technique, the names for each attribute, and the sensory qualities before they could start the exam. The judges randomly assessed the coded cracker samples based on their appearance, flavor, mouthfeel, color, hardness and overall acceptability. The Mansoura University Faculty of Specific Education's Research Ethics Committee granted permission for this research, (Code No. Nutrition 30; 9/2024).

Physical properties of crackers

According to Sai-Manohar and Haridas-Rao (1997), the physical characteristics were investigated inside the National Research Center in Giza, Egypt.

Determination of crackers' color

According to Sapers and Douglas (1987), an objective assessment of the crackers surface color was conducted at the National Research Centre in Giza, Egypt using a spectrocolorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode.

Chemical composition of JA powder and crackers

Moisture, ash and fiber contents of JA tuber powder, control crackers and JA crackers) were analyzed using the standard procedures of the Association of Official Analytical Chemists (AOAC, 2005). The percentage of crude lipid content was determined using the Conventional method according to Khilari and Sharma (2016). The protein content was determined using the Bradford assay according to the method described by Bradford 1976.

Minerals contents were estimated using the method of Bettinelli et al. (2000) using Inductivity Coupled Plasma (iCAP™ 7000 Plus Series ICP-OES, Thermo Scientific™).

Total phenolic, total flavonoid and carotenoid contents of JA tubers and crackers extracts

According to the methods of Singleton and Rossi (1965) and Sembiring et al. (2018), the total

phenolic and total flavonoid contents of the extracts were determined. The method of Carvalho et al. (2012) was used to determine the total amount of carotenoids.

Antioxidant activity of JA tubers and crackers extracts

Free radical scavenging activity of different extracts of leaves plant was measured by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) according to the method of González-Palma et al. (2016). In brief, three millilitres of various extracts in ethanol at varying concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml) were mixed with one millilitre of 0.1 mM DPPH solution in ethanol. After giving the mixture a good shake, it was allowed to stand for half an hour at room temperature. Then, a spectrophotometer (UV-VIS Milton Roy) was used to detect absorbance at 517 nm. The experiment was conducted in triplicate using ascorbic acid as the reference standard component. The Log dosage inhibition curve was used to determine the sample's IC 50 value. The following equation was used to determine the percentage DPPH scavenging effect: DPPH scavenging effect (%) = A0 - A 1 / A0 × 100.

A0: the absorbance of control reaction

A1: the absorbance in presence of test or standard sample.

Anti-inflammatory activity of JA tubers and crackers extracts

The anti-inflammatory activity was evaluated using the Ameena et al. (2023) method, which involved taking 50 µL of the sample and adding various concentrations to 450 µL of a 1% aqueous solution of bovine serum albumin. These concentrations ranged from 5µg/mL, 10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL, 50µg/mL, and 100 µg/ml. A tiny quantity of 1N hydrochloric acid was added to the solution to bring its pH down to 6.3. After 20 minutes of incubation at room temperature, these samples were heated in a water bath for 30 minutes at 55°C. A Biosystem 310 plus spectrophotometer was used to detect the absorbance at 670 nm after the samples had been. The reference medication used for comparison was diclofenac sodium. The experiment's control was dimethyl sulfoxide (DMSO).

The percentage of protein denaturation was determined using the following equation:

% Inhibition = (Absorbance of control - Absorbance of sample/Absorbance of control) x 100.

***In vitro* α-amylase inhibition of JA tubers and crackers extracts**

According to Wickramaratne et al. (2016), the 3,5-dinitrosalicylic acid (DNSA) method was used to carry out the α-amylase inhibition assay. To get concentrations ranging from 1.9 to 1000 µg/ml, the extract was first diluted in a minimum of 10% DMSO and then in a buffer (Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9). 200 µl of the extract and 200 µl of α-amylase solution (2 units/ml) were combined, and the mixture was incubated for 10 minutes at 30 °C. Each tube was then filled with 200 µl of the starch solution (1% in water (w/v)), and the tubes were incubated for three minutes. 200 µl of DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) were added to stop the reaction, and it was then heated for 10 minutes at 85–90 °C in a water bath. A UV-visible Biosystem 310 spectrophotometer was used to measure the absorbance at 540 nm after the mixture had been allowed to cool to room temperature and diluted with 5 millilitres of distilled water. The sample extract was substituted with 200 µl of buffer to create the Control 100%, which had 100% enzyme activity. In the absence of the enzyme solution, a blank reaction was made in a similar manner using the sample extract at each concentration. The following formula was used to determine the α-amylase inhibitory activity, which was represented as a percentage of inhibition: Plotting the extract concentration versus the percentage α-amylase inhibition allowed for the determination of the IC_{50} values.

% α amylase inhibition = 100 *($Abs_{100\%}$ control−Abs_{Sample})/ $Abs_{100\%}$ control

Statistical analysis

One-way analysis of variance (ANOVA) was used for statistical analysis, and the results obtained were displayed as mean± SD. According to Gomez and Gomez (1984) computer software was used to compare the means between groups using LSD at $p \leq 0.05$.

3. Results

Sensory evaluation of JA crackers.

The results represented in Table 1 show that crackers substituted with 20% JA tubers powder are the most preferred crackers. Fig. 3 shows the overall acceptability score values for three cracker samples of JA tubers powder compared to the control crackers.

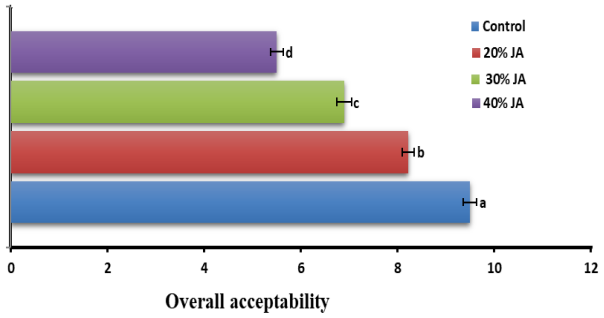

Fig. 3. Effect of JA tubers powder addition on crackers sensory evaluation+*+

Physical properties of JA crackers

The baking quality of the different tested crackers were represented in Table 2. The partial replacement of wheat flour with 20%, 30% and 40% JA tubers powder caused significant differences ($p \leq 0.05$) in specific volume, diameter and spread ratio compared with the control crackers. Significant differences in thickness values were noticed between the control crackers and crackers with JA tubers powder. In contrast, no significant differences were recorded between crackers with different levels of JA tubers powder at $p \leq 0.05$. After the control crackers score, crackers with 40% JA tubers powder recorded the highest spread ratio score (11.44±0.12 %) followed by 30% JA crackers (9.27±0.11 %), then the crackers sample with 20% JA tubers powder (7.47±0.15%). Meanwhile, 20% JA crackers recorded the highest specific volume (1.08±0.07 cm3/g) followed by 30% JA crackers (0.91±0.08 cm3/g), then the crackers sample with 40% JA tubers powder which recorded the lowest specific volume score (0.58±0.06 cm3/g).

Color characteristics of JA crackers

Color parameters (L*, a* and b*) represented in Table 3 were examined to find out how different concentrations of JA tubers powder affected crackers samples. The partial replacement of (whole wheat flour + yellow corn flour) with (20, 30 and 40) % JA tubers powder caused a significant decrease ($p \leq 0.05$) in lightness (L*), and a significant difference ($p \leq 0.05$) in yellow intensity (a*) and red intensity (b*) when compared with the control crackers. The various colors of whole wheat flour, yellow corn flour and JA powders employed in the crackers' manufacturing are assumed to be the cause of these findings.

Chemical composition of JA tubers powder, control crackers and 20% JA crackers

The chemical composition of JA tubers powder, control crackers, and 20% JA crackers is given in Table 4. Due to the high fiber, ash, and protein contents of JA tubers powder which recorded 9.03±0.45 %, 5.57±0.12 %, and 5.34±0.06 %, respectively. Data show that the addition of JA tubers powders significantly increased ($p \leq 0.05$) the cracker's contents of fibers (6.77±0.50%), ash (3.50±0.10%), and protein (20.16±0.07%) when compared to the control crackers which recorded 4.60±0.61, 3.07±0.15 and 14.74±0.06, respectively. On the other hand, results show that the addition of JA tubers powder significantly decreased ($p \leq 0.05$) the cracker's contents of total lipids (1.10±0.02 %) comparing with the control crackers (1.22 ±0.04 %).

Minerals contents of JA tubers, control crackers and 20% JA crackers

Results represented in Table 5 show that JA tubers are considered a rich source of elements such as iron, calcium, potassium, magnesium, and phosphorus, as it recorded 13.8, 297.0, 1912.0, 165.0, and 132.0 mg/100 g, respectively. Data show that the addition of JA tubers powders obviously increased the cracker's contents of the mentioned minerals when compared to the control crackers.

Total phenolic, flavonoid and total carotenoid content of JA tubers, control crackers and 20% JA crackers

Total phenols, flavonoids, and total carotenoids of JA tubers, control crackers, and 20% JA crackers are tabulated in Table 6. The results show that JA tubers had a high content of total phenols, flavonoids, and total carotenoids which recorded 29.23±0.87 mg GAE/gm, 89.07±2.31

mg QE/gm and 23.13±0.32 µg/gm, respectively. This high content obviously led to increasing the total phenols, flavonoids, and total carotenoids of 20% JA crackers (27.57±0.71 mg GAE/gm, 77.74±2.20 mg QE/gm, and 17.33±0.22 µg/gm) when compared to their scores in the control crackers which recorded 23.53±0.78 mg GAE/gm, 71.20±2.23 mg QE/gm, and 9.48±0.27 µg /gm, respectively. It is clear that crackers with 20% JA tubers powder have higher bioactive components, such as phenol, flavonoid, and carotenoid

Table 1. Effect of JA tubers powder addition on crackers sensory evaluation

Samples	Appearance (10)	Flavor (10)	Mouth feel (10)	Color (10)	Hardness (10)	Overall acceptability (10)
Control	9.4^{a} ±0.15	9.30^{a} ±0.13	9.76^{a} ±0.18	9.16^{a} ±0.12	9.18^{a} ±0.11	9.50^{a} ±0.14
20% JA	8.6^{b} ±0.11	8.42^{b} ±0.11	8.28^{b} ±0.13	8.64^{b} ±0.16	8.96^{ab} ±0.16	8.22^{b} ±0.12
30% JA	7.7^{c} ±0.12	6.98^{c} ±0.16	7.12^{c} ±0.14	7.16^{c} ±0.12	8.68^{b} ±0.17	6.90^{c} ±0.15
40% JA	6.2^{d} ±0.11	5.70^{d} ±0.12	6.58^{d} ±0.16	6.58^{d} ±0.13	8.74^{b} ±0.15	5.50^{d} ±0.13
LSD at 0.05	0.04	0.04	0.04	0.30	0.34	0.31

JA: Jerusalem artichoke, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$

Table 2. Effect of JA tubers powder addition on crackers physical properties

Samples	Physical parameters					
	Weight (g)	Volume (cm3)	Specific volume (cm3/g)	Thickness (cm)	Diameter (cm)	Spread ratio (%)
Control	4.7^{c} ±0.11	5.9^{a} ±0.18	1.25^{a} ±0.09	0.36^{b} ±0.06	4.9^{a} ±0.18	13.9^{a} ±0.11
20% JA	4.4^{d} ±0.12	4.7^{b} ±0.19	1.08^{b} ±0.07	0.52^{a} ±0.07	3.8^{d} ±0.16	7.4^{d} ±0.15
30% JA	6.4^{b} ±0.15	5.8^{a} ±0.18	0.91^{c} ±0.08	0.46^{ab} ±0.05	4.2^{c} ±0.13	9.2^{c} ±0.11
40% JA	6.7^{a} ±0.14	3.9^{c} ±0.21	0.58^{d} ±0.06	0.40^{ab} ±0.06	4.5^{b} ±0.12	11.4^{b} ±0.12
LSD at 0.05	0.25	0.39	0.16	0.14	0.34	0.04

JA: Jerusalem artichoke, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$.

Table 3. Effect of JA tubers powder addition on crackers measurement

Samples	Color parameters		
	L*	a*	b*
Control	61.66^{a} ±0.17	6.09^{c} ±0.6	29.65^{a} ±0.14
20% JA	59.99^{b} ±0.16	5.78^{d} ±0.07	21.01^{d} ±0.12
30% JA	57.09^{c} ±0.12	9.69^{b} ±0.08	23.02^{c} ±0.16
40% JA	52.25^{d} ±0.14	13.12^{a} ±0.09	25.03^{b} ±0.17
LSD at 0.05	0.34	0.16	0.04

JA: Jerusalem artichoke, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$.

Table 4. Proximate chemical composition of JA tubers powder, control crackers and 20% JA crackers

Parameters %	**JA tubers powder**	**Control crackers**	**20% JA crackers**	**LSD at 0.05**
Moisture	6.77^{c} ±0.25	8.00^{b} ±0.19	8.70^{a} ±0.20	0.47
Total lipids	0.28^{c} ±0.03	1.22^{a} ±0.04	1.10^{b} ±0.02	0.07
Ash	5.57^{a} ±0.12	3.07^{c} ±0.15	3.50^{b} ±0.10	0.33
Protein	5.34^{c} ±0.06	14.74^{b} ±0.06	20.16^{a} ±0.07	0.12
Fibers	9.03^{a} ±0.45	4.60^{c} ±0.61	6.77^{b} ±0.50	1.27

JA: Jerusalem artichoke, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$.

Table 5. Mineral contents of JA tubers, control crackers and 20% JA crackers

Elements	**Concentration (mg/100 g)**		
	JA tubers	**Control crackers**	**20% JA crackers**
Fe	13.8	1.5	3.0
Ca	297.0	151.0	216
K	1912.0	370.0	430
Mg	165.0	35.0	42
P	132.0	217.0	227
Na	130.0	109.0	77.8

JA: Jerusalem artichoke.

Table 6. Total phenolic, flavonoid and total carotenoid contents of JA tubers, control crackers and 20% JA crackers

Samples	**JA tubers**	**Control crackers**	**20% JA crackers**	**LSD at 0.05**
Total phenolic (mg GAE/gm)	29.23^{a} ±0.87	23.53^{b} ±0.78	27.57^{a} ±0.71	2.10
Flavonoid (mg QE/gm)	89.07^{a} ±2.31	71.20^{c} ±2.23	77.74^{b} ±2.20	6.07
Total carotenoid content (µg /gm)	23.13^{a} ±0.32	9.48^{c} ±0.27	17.33^{b} ±0.22	0.65

JA: Jerusalem artichoke, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$.

Antioxidant activity of JA tubers, control crackers and 20% JA crackers

Antioxidants play a vital role in defending the human body against generated free radicals. Table 7 and **Fig. 4** demonstrate how the DPPH scavenging % significantly rose in tandem with the extract concentration of JA tubers, control crackers and 20% JA crackers. Regarding JA tubers, the maximum DPPH scavenging activity % was 94.22±0.14, 92.82±0.18 and 91.28±0.22% at 1000, 500, and 250 µg/mL extract concentrations which were close to the standard percent to a large extent which recorded 98.01±0.20, 96.10±0.17, and 93.71±0.21% at the same concentrations, respectively. Moderate levels of DPPH scavenging activity were also seen at the lowest concentrations 3.9 and 1.95 µg/mL which reaching 46.20±0.24 and 37.95±0.20%, respectively. Because the antioxidant activity increases when the IC_{50} is low, the antioxidant's IC_{50} value was quite low at 4.46 µg/mL, which revealed the highly antioxidant activity of JA tubers close to the standard's IC_{50} (2.71 µg/mL).Based on the results, the antioxidant property of crackers with 20% JA tubers ranged between 89.69±0.23 and 57.50±0.16% at concentrations ranged from 1000 to 62.5 µg/mL which are significantly

($p \leq 0.05$) higher than the antioxidant property of control crackers which ranged between 69.57±0.18 and 43.61±0.21% at the same extract concentrations. Also, results demonstrated the highly antioxidant activity of JA crackers due to their antioxidant's IC_{50} value which was quite low at 37.31 µg/mL compared to its value in the control crackers (141.01 µg/mL). This shows that the addition of JA tubers significantly increases the antioxidant properties of crackers.

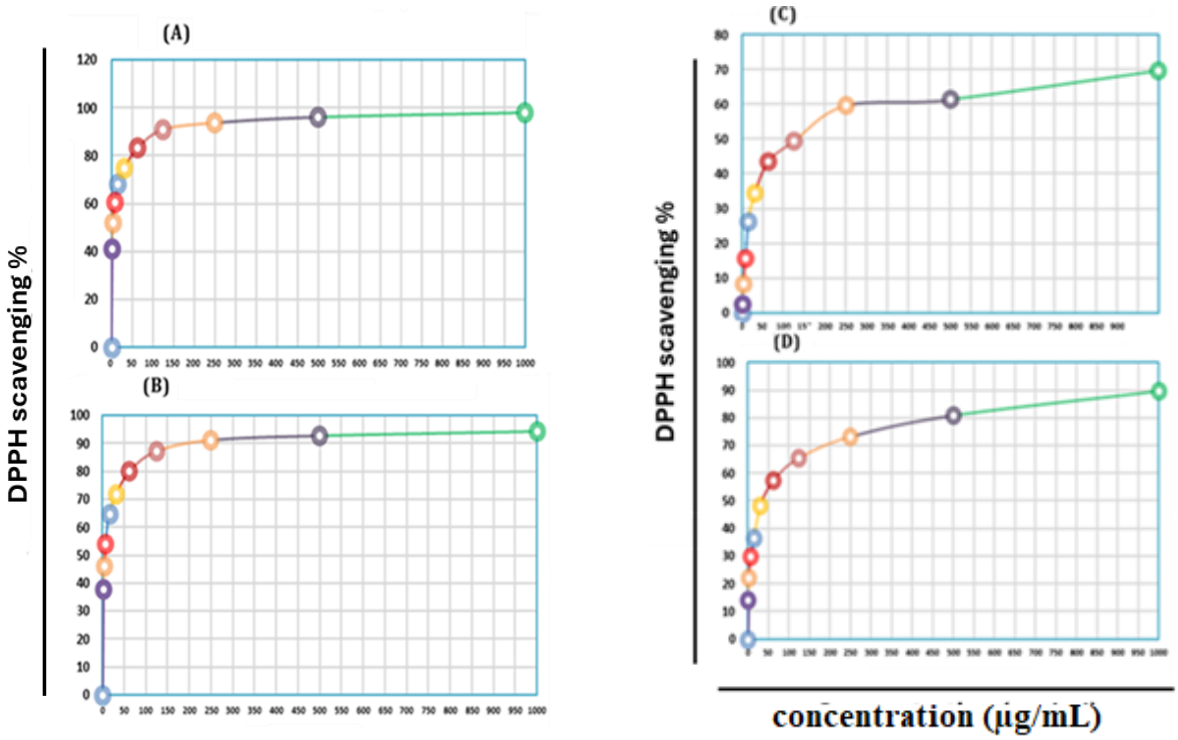

Fig. 4. Antioxidant activity of ascorbic acid (A), JA tubers (B), control crackers (C) and 20% JA crackers (D).

Table 7. Antioxidant activity of JA tubers, control crackers and 20% JA crackers20% JA crackers

Conc. (µg/ml)	DPPH scavenging%				LSD at 0.05
	Standard	JA tubers	Control crackers	20% JA crackers	
1000	98.01^{a} ±0.20	94.22^{b} ±0.14	69.57^{d} ±0.18	89.69^{c} ±0.23	0.36
500	96.10^{a} ±0.17	92.82^{b} ±0.18	61.33^{d} ±0.21	80.74^{c} ±0.22	0.39
250	93.71^{a} ±0.21	91.28^{b} ±0.22	59.57^{d} ±0.22	72.96^{c} ±0.26	0.41
125	90.90^{a} ±0.19	87.27^{b} ±0.22	49.29^{d} ±0.28	65.38^{c} ±0.26	0.21
62.5	83.15^{a} ±0.20	79.93^{b} ±0.22	43.61^{d} ±0.21	57.50^{c} ±0.16	0.35
31.25	74.92^{a} ±0.18	71.96^{b} ±0.14	34.45^{d} ±0.23	48.27^{c} ±0.11	0.37
15.62	68.41^{a} ±0.70	64.74^{b} ±0.53	26.49^{d} ±0.64	39.69^{c} ±0.55	1.14
7.81	60.64^{a} ±0.22	54.14^{b} ±0.35	15.55^{d} ±0.20	29.88^{c} ±0.35	0.66
3.9	52.17^{a} ±0.27	46.20^{b} ±0.24	8.34^{d} ±0.29	22.06^{c} ±0.28	0.53
1.95	41.09^{a} ±0.20	37.95^{b} ±0.20	2.37^{d} ±0.21	14.19^{c} ±0.16	0.09
IC_{50}	2.71 µg/mL	4.46 µg/mL	141.01 µg/mL	37.31 µg/mL	

JA: Jerusalem artichoke, Standard: Ascorbic acid, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$.

Anti-inflammatory activity of JA tubers, control crackers and 20% JA crackers (HRBC inhibition %)

The anti-inflammatory property of a food product is supported to control the inflammation in the body. Based on the result obtained in Table 8 and illustrated by Fig.5, all the JA tubers extract concentrations significantly increased the inhibition percent at $p \leq 0.05$. The maximal inhibition percentage reached 84.80±0.47% at 100 µg/mL which was close to the standard percent to a large extent which recorded 86.03±0.48% at the same concentration. The inhibition percentage decreased with the lowering of the extract concentration. Therefore, the JA extract indeed possessed anti-inflammatory properties. At 100 µg/mL concentration, the anti-inflammatory properties of crackers with 20% JA tubers powder substitution and control crackers were 82.05±0.38, and 62.14±0.42%, respectively. This proved that both crackers have high anti-inflammatory properties. This result shows that the incorporation of JA tubers powder significantly increases the functional properties of crackers.

Table 8. Anti-inflammatory activity of JA tubers, control crackers, and 20% JA crackers

Conc. (µg/ml)	HRBC Inhibition%				LSD at 0.05
	Standard	JA tubers	Control crackers	20% JA crackers	
100	86.03^{a} ±0.48	84.80^{b}±0.47	62.14^{d}±0.42	82.05^{c}±0.38	0.71
50	73.94^{a} ±0.48	72.37^{b}±0.34	49.31^{d}±0.35	71.33^{c}±0.47	0.59
40	64.65^{a} ±0.84	61.60^{b}±0.58	38.59^{d}±0.75	59.98^{c}±0.53	1.52
30	53.63^{a} ±0.28	52.25^{b}±0.24	23.00^{d}±0.38	48.91^{c}±0.30	0.67
20	44.29^{a} ±0.39	38.83^{b}±0.32	12.58^{d}±0.40	35.95^{c}±0.31	0.73
10	35.10^{a} ±0.72	27.67^{b}±0.67	8.94^{d}±0.77	25.07^{c}±0.73	0.91
5	25.14^{a} ±0.78	20.84^{b}±0.61	3.83^{d}±0.57	14.69^{c}±0.68	1.26
IC_{50}	20.03 µg/mL	23.1 µg/mL	71.81 µg/mL	26.88 µg/mL	

JA: Jerusalem artichoke, Standard: Diclofenac sodium, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$.

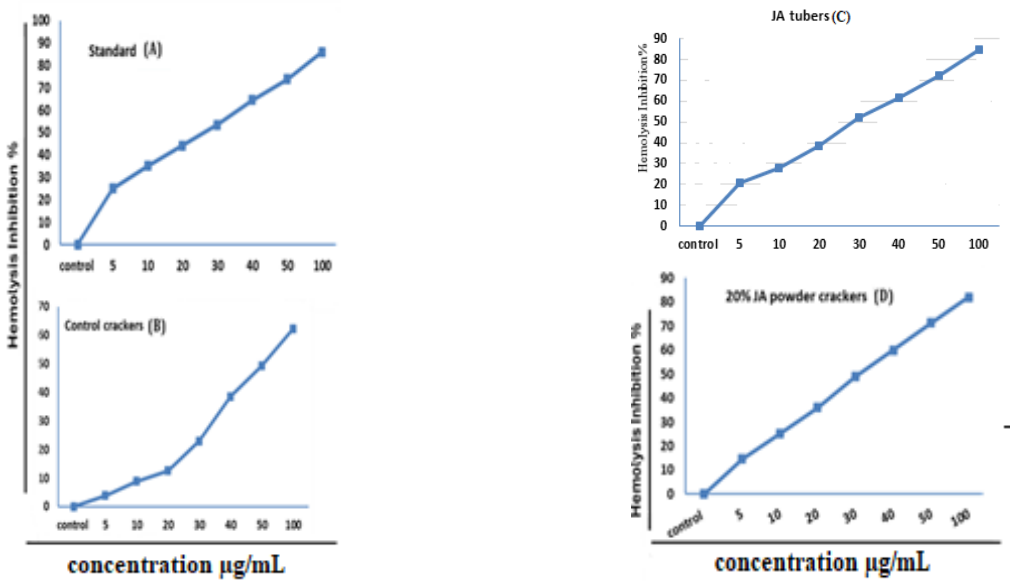

Fig. 5. Anti-inflammatory activity of standard (A), control crackers (B), JA tubers (C) and 20% JA crackers (D).

Anti-diabetic properties of JA tubers, control crackers and 20% JA crackers (α-amylase Inhibition%)

The anti-diabetic property of crackers was determined using an alpha-amylase inhibition assay percentage. The obtained results are summarized in Table 9 and **Fig 6.** As for JA tubers, the maximum amylase inhibition% was 90.35±0.14, 85.64±0.27, 79.23±0.22, 73.38±0.37, and 65.87±0.49% at 1000, 500, 250, 125, and 62.5 µg/mL extract concentrations which were close to that of the standard which recorded 95.81±0.20, 94.39±0.17, 90.98±0.26, 85.39±0.27, and 79.27±0.21 % at the same extract concentrations, respectively. High anti-diabetic properties were also seen at the low tuber extract concentrations of 7.8 and 3.9 µg/mL, reaching 40.55±0.56 and 34.10±0.46%, respectively. α-amylase inhibition increased with the increase in JA tubers supplementation. The control crackers showed an inhibitory activity ranging between 70.97±0.24 and 41.89±0.37 % with concentrations between 1000 and 62.5 µg/mL. While crackers with 20% JA tubers increased significantly ($p \leq 0.05$) α-amylase inhibitory activity as ranged between 84.24±0.17, and 58.30±0.28% at the same extract concentrations. This demonstrates that crackers that contain 20% JA tubers have anti-diabetic qualities and may be able to manage diabetes mellitus.

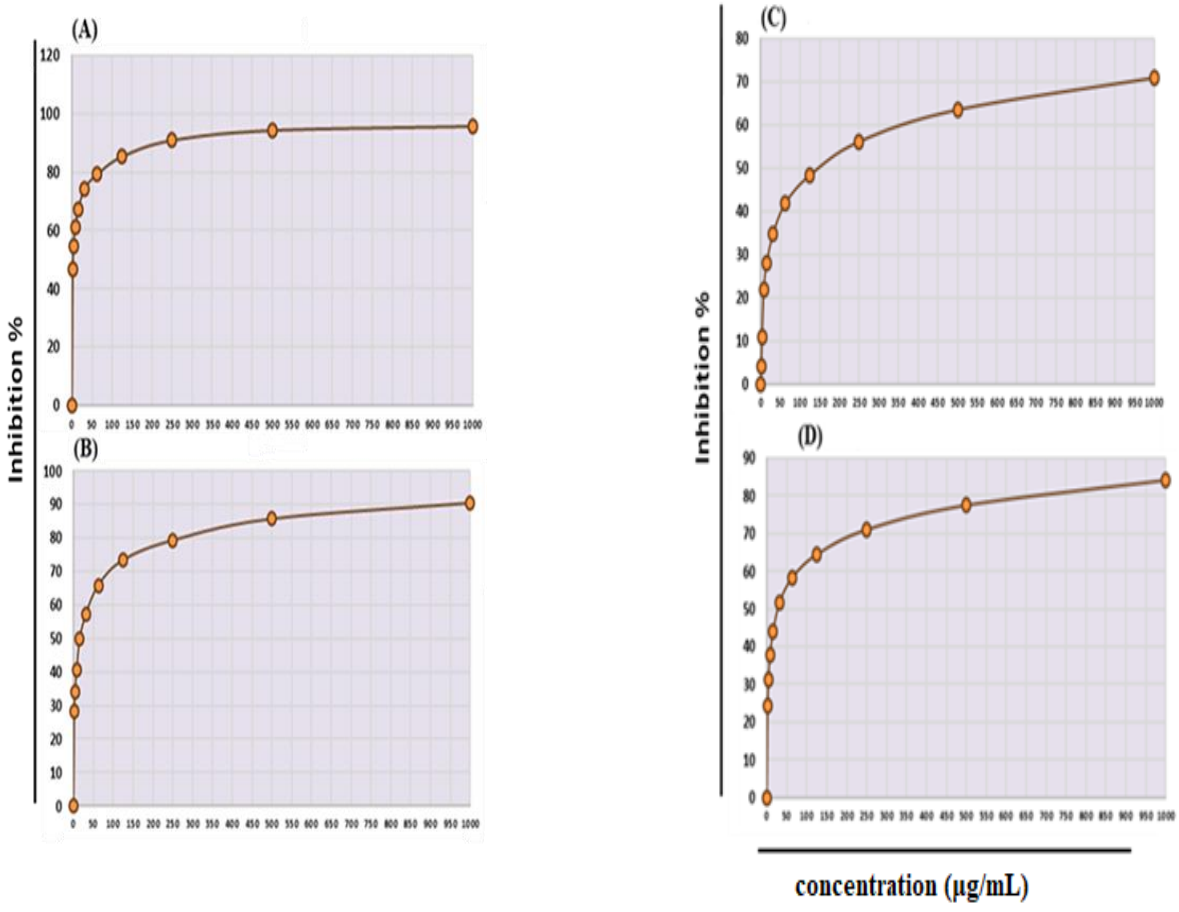

Fig. 6. Alpha amylase inhibition of standard (A), JA tubers (B), control crackers (C) and 20% JA crackers (D)

Table 9. Alpha amylase inhibition of JA tubers, control crackers and 20% JA crackers

Conc. (µg/ml)	α-amylase Inhibition%				LSD at 0.05
	Standard	JA tubers	Control crackers	20% JA crackers	
1000	95.81^{a} ±0.20	90.35^{b} ±0.14	70.97^{d} ±0.24	84.24^{c} ±0.17	0.42
500	94.39^{a} ±0.17	85.64^{b} ±0.27	63.56^{d} ±0.30	77.59^{c} ±0.19	0.37
250	90.98^{a} ±0.26	79.23^{b} ±0.22	56.19^{d} ±0.35	71.12^{c} ±0.23	0.32
125	85.39^{a} ±0.27	73.38^{b} ±0.37	48.40^{d} ±0.35	64.53^{c} ±0.29	0.70
62.5	79.27^{a} ±0.21	65.87^{b} ±0.49	41.89^{d} ±0.37	58.30^{c} ±0.28	0.51
31.25	74.41^{a} ±0.51	57.21^{b} ±0.34	34.68^{d} ±0.48	51.61^{c} ±0.42	0.99
15.62	67.22^{a} ±0.24	49.88^{b} ±0.35	28.15^{d} ±0.40	44.06^{c} ±0.30	0.61
7.81	61.20^{a} ±0.24	40.55^{b} ±0.56	21.74^{d} ±0.45	37.9^{c} ±0.28	0.48
3.9	54.50^{a} ±0.26	34.10^{b} ±0.46	10.97^{d} ±0.31	31.09^{c} ±0.27	0.44
1.95	46.70^{a} ±0.28	28.14^{b} ±0.23	4.16^{d} ±0.45	24.33^{c} ±0.44	0.38
IC_{50}	2.05 µg/mL	16.24µg/mL	136.4 µg/mL	27.7 µg/mL	

JA: Jerusalem artichoke, Standard: Acarbose, each value is the mean ± SD. Mean values in each column have different letters (a, b, c, d) are significantly different at $p<0.05$.

4. Discussion

There are several methods for adding Jerusalem artichoke inulin to a range of baked products. However, the inulin's prebiotic, fat-, and sugar-replacement properties are preserved, creating healthier substitutes for conventional baked goods. Inulin has extra health benefits and can be utilized as a fat and sugar substitute in baked goods like cakes, cookies, and breads. Additionally, inulin in baked goods might enhance moisture retention, extending the shelf life of cakes and breads (Franck, 2002). Results stated that crackers substituted with 20% JA tubers powder are the most preferred. These results agreed with Goranova et al. (2016) who reported that the sponge cakes that contained 20% JA powder scored similarly to the control samples in terms of color, odor, uniformity and size of cells, and sweetness. The sensory panel test did not find the 20% JA powder cakes' odor unpleasant, although it was more pronounced and specific to the control sample's odor. However, in research by Celik et al. (2013), it was found that cakes enriched with JA powder (5 and 10%) scored similarly for odor, but significantly lower on the flavor, crumb cell structure, chewiness, sweetness, and overall acceptability measures compared to the control samples. Furthermore, the crispiness and general acceptance scores of crackers using JA powder in the formulations were significantly higher ($p<0.05$). It can be concluded that the reduction in hardness value in these crackers makes them more palatable in terms of crispiness when comparing the changes in hardness values and crispiness scores linked to the addition of JA powder. All of the crackers' color, odor, and taste values were statistically ($p>0.05$) similar, despite the control and 30% JA powder samples having lower color and taste values (Ozgoren et al., 2019). Praznik et al. (2002) found that the organoleptic scores (appearance, crumb, crust, taste, and odor) of breads containing 10% and 12% JAP were comparable to those of the control sample. According to the findings of a customer assessment, glass noodles made by replacing 40% mung bean flour with JA flour had moderate overall liking ratings (Oupathumpanont and Chitravimol, 2016). Color is a crucial factor when assessing the sensory quality, consumer perception, and market value of food samples (Panghal et al., 2019).

The present study showed that crackers' luminosity scores decreased, while red and yellow intensity increased significantly by increasing the JA addition percent compared to the control crackers. The differences between crackers samples in color measurements may be due to the different colors of raw ingredients. Our results were in the same trend with the results of Ozgoren et al. (2019) who found that the inclusion of JA powder led to a substantial increase in a value and a significant

(p <0.05) decrease in the L and b values. Also, when JA powder was added to biscuits, the same color results were found in a study of Kārkliņa et al. (2012). The high inulin content of JA powder observed in this study is consistent with earlier research that found fructans in bread formulations favored the Maillard reaction, which causes non-enzymatic browning (Hager et al., 2011; Capriles and Areas, 2013). Because of the plant's tubers' high nutritional content, JA has been widely used as a food plant for many centuries. Vitamins, minerals, and the complex carbohydrate inulin found in the tubers can all help people stay healthy (Somda et al., 1999; Bach et al., 2013). Results showed that the addition of JA tuber powder significantly increased (p≤0.05) the cracker's contents of fibers, ash, and protein when compared to the control crackers. Results agreed with several previous research which found that JA's dry basis protein content ranged from 4.00 to 12.98%, its ash content ranged from 4.30 to 6.03%, its K concentration ranged from 21,000 to 32,850 ppm, and its calcium level ranged from 700 to 1850 ppm (Praznik et al., 2002; Yuan et al., 2008; Terzić et al., 2009; Cieślik et al., 2011).

On the other hand, Ozgoren et al. (2019), found that JA powder showed higher levels of soluble and insoluble dietary fiber, crude ash, Mg, Ca, K, P, total phenolic contents, and antioxidant activity than wheat flour, but lower levels of crude protein, crude oil, carbohydrate, and energy. However, the National Nutrient Database of the United States Department of Agriculture (USDA) stated that raw JA has 0.01% total lipid (fat), 10% total sugars, 0.5% minerals, and 78% moisture (Celik et al., 2013). The cultivar of JA, soil and climate conditions, harvesting time, and tuber storage conditions all affect how much soluble dietary fiber is present in JA. However, new research indicates that JA has a significant protein content, including important amino acids, in addition to its notable fructan content. Between 2 and 3 percent of fresh mass in JA tubers is protein (Aleknavičienė et al., 2009; Cieślik et al., 2011; Sawicka and Kalembasa, 2013; Danilčenko et al., 2013; Szewczyk et al., 2019). The fiber content in JA tubers ranges from 11.4–20.8 g 100 g-1 of DM (Žaldarienė, 2017). Compared to the roots of other known species, JA has a higher concentration of fiber and pectins (3.5 g per 100 g-1 of DM) (Sawicka, 2016). Dietary fiber's constituents benefit the human body in several ways, including increasing intestinal peristalsis, reducing the absorption of cholesterol and certain harmful substances in the digestive tract (a condition known as "slagging"), delaying the hydrolysis of carbohydrates, and lowering blood glucose levels (Okada et al., 2017).

In addition to having a high soluble fiber content, JA tubers also include a sizable amount of insoluble fiber. Fresh tuber mass has a raw fiber content of 0.9 to 1.9%, or 2.7–13% of DM (Danilčenko et al., 2017). The results showed that the addition of JA tubers powder obviously increased the cracker's contents of elements compared to the control crackers. Numerous vital processes in the human body depend on minerals. The development and upkeep of bones depend on calcium, phosphorus, and magnesium. Furthermore, P and Mg are crucial for energy metabolism, whereas Ca aids in blood coagulation. Along with calcium, phosphorus, and magnesium, the electrolyte K is essential for maintaining appropriate acid-base balance, osmotic pressure, and water balance as well as for neuronal transmission, muscular activity, and vascular dilatation and constriction. P and Mg function as cofactors in enzymatic processes or as parts of the enzyme systems. Furthermore, P is a part of cell membranes, nucleotides, and nucleic acids (Ervin et al., 2004).

The mineral content of JA tubers determines their utility as a raw material for food and medicinal production. Its tubers are distinguished by a high concentration of mineral components that produce alkali, primarily potassium (Scholz-Ahrens et al., 2007). According to Bergmann's dietary guidelines (1992) JA tubers are high in sodium, low in calcium and potassium, and sufficient in phosphorus and magnesium. According to the study's findings, adding

powdered JA tubers can raise the proportion of mineral requirements that are needed. Natural antioxidant substances are phenols and flavonoids which can scavenge free radicals, increase their dismutation into far less reactive molecules, chelate pro-oxidant metals, and decrease or increase the activity of certain enzymes (Heimler et al., 2017; Sharifi-Rad et al., 2018).

Our results showed that the addition of JA tuber powder to the cracker obviously increased the bioactive components, such as phenol, flavonoid, and carotenoid. The results agreed with the results of Ozgoren et al. (2019) who found that the total phenolic content and antioxidant activity values of the crackers were increased significantly ($p \leq 0.05$) by increasing the JA powder content in the formulation. The higher phenolic content of JA powder is linked to the higher antioxidant activity values and total phenolic content of crackers made with JA powder. Also, other research results stated that JA tubers contain high amounts of phenolic compounds, ranging from 1,477 to 1,802 mg kg-1 DM (Michalska-Ciechanowska et al., 2019). Mattila and Hellström (2007) assessed the content of polyphenols in tubers at 2,210 mg. kg-1DM which has strong antioxidant potential. Also, results were in harmony with Catană et al. (2018) who found that tuber powder has a high antioxidant potential and 18.51–44.03 mgGAE g-1 of polyphenols, making it beneficial for diets that prevent diseases caused by free radicals. Because of their anti-aging, regenerative, and antioxidant qualities, plant extracts high in antioxidants can be employed as active components in a variety of therapies. The results showed that the addition of JA tubers to the flour of crackers significantly increases the antioxidant properties of crackers. Our results agreed with the results of Afoakwah et al. (2015) who found that sausages enhanced with freeze-dried and oven-dried JA powder exhibited greater antioxidant activity than the control sample, which was in good agreement with these findings. In the same trend, Olagunju et al. (2018) stated that the cracker biscuits' antioxidant properties imply that the snacks may be useful radical scavengers that can both prevent major degenerative diseases linked to free radicals and act as a useful snack for dietary intervention. Also, Sat (2008) reported that children with kidney membranopathy or constipation can benefit from eating JA tubers, which are natural plant products that are antioxidants and immunostimulants. Inulin and fructose can be utilized to treat and prevent diabetes since they are ready products for the body's cells to consume. Because human intestinal enzymes can break down β-inulin between fructose monomers, it can be employed in functional foods and to treat type 2 DM, obesity, and blood sugar levels (Yang et al., 2015). When taken orally, inulin is metabolized in the mouth, stomach, and small intestine before being fermented by the gut flora in the large intestine. In this sense, using inulin does not affect blood sugar levels or the activation of insulin release (Aslan et al., 2010; Yang et al., 2015).

Results stated that α-amylase inhibition increased with an increase in supplementation with JA tubers. This demonstrates that crackers that contain 20% JA tubers have anti-diabetic qualities and may be able to manage diabetes mellitus. Accordingly, the high percentage of α-amylase inhibition might aid in delaying the absorption of carbs following foods. The crackers' strong digestive enzyme-inhibiting action may be due to the addition of JA tubers. These results agreed with previous research that stated that JA can treat type 2 diabetes by decreasing elevated blood glucose levels (Gupta and Verma, 2011; Horochowska et al., 2017; Catană et al., 2018). For people who are overweight or obese, tubers containing swellable ingredients are advised. This is caused by different types of fiber, primarily cellulose that come from the tubers. Inulin helps lower cholesterol, improve insulin resistance in diabetes, and strengthen the immune system. It is ideal for diabetics and individuals recovering from chemotherapy (Zhang et al., 2011; Yang et al., 2015). People with diabetes are more likely to tolerate fructose, thus JA is advised for them (Kronberga et al., 2013). There are several uses for JA's tubers components in human nutrition.

Its properties allow it to be incorporated into the diets of people with type 2 diabetes and obesity, and it was recently found that the extract of JA tubers acts as a cytotoxic agent against breast cancer cells (Samal et al., 2012; Horochowska et al., 2017).

Conclusion

Adding JA tubers powder increased the cracker's contents of fibers, ash, protein, iron, calcium, potassium, magnesium, and phosphorus compared to the control crackers. Crackers with 20% JA tubers substitution had a significantly high level of phenol, flavonoid, and carotenoid, besides their antioxidant, anti-inflammatory, and anti-diabetic activities "in vitro" compared to control crackers.

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