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CONTENTS

ORIGINAL PAPERS

INFLUENCE OF HIGH FLUORIDE IN DRINKING WATER ON SOME BIOLOGICAL AND HISTOLOGICAL MARKERS IN RATS

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ABSTRACT

Our study aimed to investigate the toxic effect of sodium fluoride (NaF) on some biological and histological markers in rats. Twelve male rats were divided into two groups (n=6), Control receved normal water and diet and NaF group received normal diet and water contaminated with NaF (400ppm) during 8 weeks. Various biochemical, hematological and oxidative stress parameters and histopathological analysis were estimated. Results showed that body weight gain was significantly decreased ($p<0.001$) in NaF exposed rats in comparison with the control group. Relative kidney weights of NaF exposed rats were significantly increased compared to control rats. Results showed an increase in serum urea ($p<0.01$), serum creatinine ($p<0.01$), serum albumin ($p<0.05$) levels and transaminases activities ($p<0.001$) in NaF groups compared to control. Results showed also, WBC and LYM were significantly increased ($p<0.01$ and $p<0.05$) respectively in sodium fluoride exposed rats compared to control. Results of oxidative stress showed that sodium fluoride increase Malondialdehyde (MDA) levels and decrease reduced glutathione (GSH) levels in liver and kidney tissues compared to control group. Histopathological analysis showed that NaF induced a high structural alteration in kidney and liver compared to control. **Conclusion:** High dose of fluoride in dirinking water may lead to a toxicity, oxidative stress and deep histological alteration in liver and kidney of rats.

KEYWORDS:
Fluoride, Toxicity, Kidney, Liver, Oxidative stress, Drinking water, Rats

INTRODUCTION

Human activities have resulted in increased contents of fluoride in water, fluoridated water is a natural source of fluoride pollution [1]. Oral care and food products all contribute to the total daily exposure to fluoride but the primary source of fluoride exposure, due to high consumption, is through drinking water, naturally occurring, or added [2]. Prolonged exposure to high fluoride results in damage to multiple soft tissues of the body inducing morphological changes in many organs, leading to an impairment of their function [3]. Kidneys, followed by the liver, accumulate more fluoride than any other organ system in the body [4]. Liver is the first and most easily invasive organ for poisons as vital digestion and detoxification organ [5], it is an important metabolic organ in the body, secreting bile and processing various nutrients into proteins. Most importantly, the liver functions in detoxifying tissue by transforming, neutralizing, and eliminating toxins through hepatocyte mediated enzymatic detoxification systems [6]. Kidney is the main tissue for the both elimination and retaining of fluoride [7], because renal excretion of fluoride is one of the most important mechanisms for the regulation of fluoride levels in the body [8]. Oxidative stress is an abnormal condition caused by an excess production of oxidants compared to the antioxidant [9], it has been considered as the main cause of several pathologies [10]. The objective of the current study was to assess the effect of fluoride in drinking water on liver and kidney functions, histological alterations and oxidative stress in rats.

MATERIALS AND METHODS

Animals care and experimental design. Twelve (12) healthy adult male Wistar rats, aged 10 weeks and weighted 153–205 g, were given from Algerian Pasteur Institute. Animals were kept in the controlled conditions with temperature 22.27±0.15 °C and humidity 72±1.62 % under standard conditions (12 h light/dark cycles) for 2 weeks to acclimatization. The rats were housed in plastic cage and were given free access to distilled water and commercialized food. The animals were divided into two (02) groups of six animals in each. Group 01 served as control group which received standard diet and water (Control). Group 02 received 400ppm of sodium fluoride in drinking water during 8 weeks

(NaF). All the experimental protocols involving animals were reviewed and approved by the department of cellular and molecular biology, El-Oued University Ethics Committee (approval number: 26 EC/DCMB/FNSL/EU2021).

Sacrifice and Samples preparation. At the end of study, the animals were fasted for 16 hours, and then anesthetized with chloroform by inhalation and sacrifice (by decapitation). When rats were sacrificed, blood glucose was measured during the sacrifice period. The blood was also transfused into EDTA tubes for lymphocyte and white blood cells accounts, then the blood obtained was centrifuged at 3000 rpm for 10 min, plasma was recovered and then rapidly frozen at -20 ° C for use in biochemical analysis (urea, creatinine, albumin, GOT, GPT). The tissues (kidney and liver) were removed, weighted, washed, and homogenized with Tris Buffer Saline (pH 7.4) and centrifuged at 9000 t/m +4 °C for 15 min, the supernatant was collected and used for the determinations of MDA and GSH levels.

Biochemical, Hematological and Oxidative stress biomarkers. Biochemical parameters were determined by methods using commercial reagent kits using auto-analyzer. The determination of hematological parameters performed using fully Auto Blood Cell Counter (ERMA). The levels of malondialdehyde in rats tissue homogenates was determined by thiobarbituric acid method as descripted in method of Yagi 1976 [11]. The amount of reduced glutathione in tissues was determined according to method of Weckbecker & Cory, 1988 [12].

Histological sections. After fixation, liver and kidney samples were dehydrated with a gradient series of ethanol, cleared by xylen and then embedded in paraffin. After that, the paraffin was cut into the sections of 4 µm on a rotary microtome and collected on slides. Then the slides were stained with hematoxylin and Eosin. Finally, the histological changes were observed using the optical microscope.

Statistical analysis. Mean and SEM calculated by the software (Minitab 17) using Student t test. Differences were considered statically significant at $p<0.05$.

RESULTS

Growth parameters . Body weight gain was very high significantly ($p<0.001$) decreased in NaF exposed rats in comparison with the control group. Relative kidney weights of NaF exposed rats significantly increased compared to control rats (Table 1).

Hemato-biochemical biomarkers. Results obtained in Table 2 showed an increase in serum urea ($p<0.01$), serum creatinine ($p<0.01$), serum albumin ($p<0.05$) levels and transaminases activities ($p<0.001$) in NaF groups compared to control. As shown in Table 2 also, WBC and LYM were significantly increased ($p<0.01$ and $p<0.05$) respectively in sodium fluoride exposed rats compared to control.

TABLE 1
Body weight gain & Relative organs Weight in control and experimental rats

	Control (n=6)	NaF (n=6)	*P-value*
Initial Body weight (g)	227.75 ± 1.38	198.8 ± 5.58	0.089
Body Weight gain (g/rat/day)	0.191 ± 0.091	-0.55 ± 0.042	0.000
Relative Liver Weight (%)	3.079±0.128	2.6468±0.0637	0.061
Relative Kidney Weight (%)	0.3099±0.0102	0.5465±0.0652	0.042

Comparison with the control group: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

TABLE 2
Hemato-biochemical biomarkers in control and experimental rats

	Control (n=6)	NaF (n=6)	*P-value*
Blood glucose (g/l)	2.17±0.239	1.728±0.17	0.325
Serum Urea (g/l)	0.62±0.023	0.76±0.031	0.009
Serum Creatinin (mg/l)	6.75 ±0.479	11.33 ±0.94	0.004
Serum ALT (UI/l)	45.5±3.52	83.5 ± 4.47	0.000
Serum AST (UI/l)	151± 8.41	226±5.48	0.000
Serum Albumin (g/l)	35.33± 0.55	37 ± 0.447	0.021
White Blood Cells (10^3/µl)	6.45 ±0.250	8.30±0.385	0.006
Lymphocytes (10^3/µl)	5.68 ± 0.140	7.43 ± 0.52	0.035

Comparison with the control group: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$,

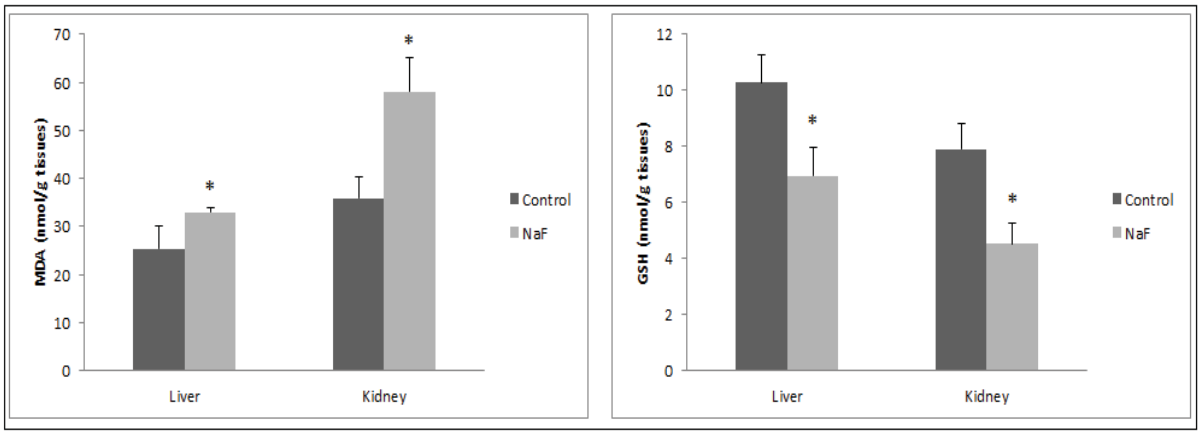

FIGURE 1
MDA and GSH levels in liver and kidney of control and experimental rats.

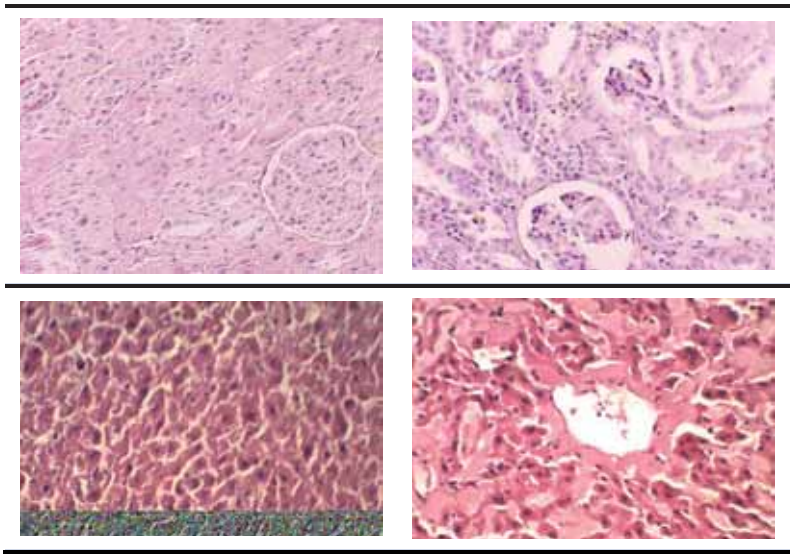

FIGURE 2
Kidney and liver histopathological changes. (a) Kidney of control group (b) Kidney of sodium fluoride exposed group (c)) Liver of control group (d) Liver of sodium fluoride exposed group .

Oxidative stress markers. Results presented in Table 2 and Figure 1 showed that Sodium fluoride significantly increase ($p<0.05$) MDA levels and decrease ($p<0.05$) GSH levels in liver and kidney tissues compared to control group.

Hepatic and renal histopathological study. Histopathological analysis of kidney and liver were shown in (Figure 2), Kidney tissue of NaF exposed group shows necrosis, tubule dilatation and degeneration, glomerular atrophy and inflammation (Figure 2b). In the other hand, Liver architecture was normal and clear in rat tissues of control group (Figure 2.d). Microscopic examination of liver in the sodium fluoride treated group showed lobules liver structure was not clear, liver cells were abnormally arranged and swollen with big sinusoid space between them, and presence of vacuoles, (Figure 2e).

DISCUSSION

In the present in-vivo study, hepatic function biomarkers (ALT, AST) activities were increased in fluoride exposed group, these results are in accordance with the findings of Raina et al., [13] who found the same results like us. Plasma enzymes activities rise when the membranes of only very few cells are damaged. Liver cells contain more AST than ALT, but ALT is confined to the cytoplasm in which its concentration is higher than that of AST. In inflammatory or infective conditions: the cytoplasmic membrane sustains the main damage, leakage of cytoplasmic contents causes a relatively greater increase in plasma ALT than AST. In infiltrative disorders, in which there is damage to both mitochondrial and cytoplasmic membranes, there is a proportionally greater increase in AST activity than ALT [14]. Our hematological findings confirm the inflammation statue which revealed that both LYM and WBC were increased in this rats group, These results

in the line of that obtained by Ameeramja et al., [15], who found that exposure to fluoride can associated with elevation of WBC level. Our results present liver oxidative stress statue**,** where elevated MDA has been considered as a reliable biomarker for oxidative stress in animals exposed to environmental contaminants [16], hence the liver dysfunction caused by the fluoride induced inflammatory in addition of that of oxidative stress because AST activity is greater than that of ALT. Reduction in relative liver weight (RLW) observed in that group, this observation is conflicting with our previous study [17] when we observed hepatomegaly, but the results of present study is in accordance with the study of. Adelakun *et al*., [18] which present also reduction of RLW after fluoride exposure. Sodium fluoride can cause liver damage by inducing cell apoptosis, RLW reduction may be refer to apoptosis of hepatocytes [19]. Liver function alterations caused by sodium fluoride exposure are confirmed by histopathological examination, which showed necrosis, edema and degeneration in the hepatocytes. These data are in line with the previous studies [20]. Relative kidney weight significantly increased in fluoride exposed rats in comparison with control rats. Fluoride is freely filtered through the glomerulus and undergoes a variable degree of proximal tubular re-absorption [21]. The long-term accumulation of Fluoride in the kidney can lead to tissue structure and functional damage [22]. Therefore, histological sections revealed various tissue damages in NaF exposed rats: necrosis, tubule dilatation and degeneration, glomerular atrophy and inflammation. According to results of Tian et al., [23] which revealed that Fluor caused mitochondrial damage in the kidney, they speculate that fluoride toxic effects can cause excessive production of ROS and interfere with the kidney antioxidant defense system, resulting in oxidative stress and renal tissue damage. Our results present elevation of MDA level and reduction of that of GSH indication oxidative stress statue in kidney of NaF exposed rats.

CONCLUSION

In conclusion, findings of the present study revealed that sodium fluoride-induced hepato-nephrotoxicities through oxidative stress and apoptosis in rats, which could be due to its antioxidant and anti-inflammatory activities.

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Declaration of interest. All authors have none to declare

Authors' Contribution. S.D. substantially contributed to the conception and design, analysis of the laboratory parameters, drafting the manuscript and analysis and the interpretation of the data A.D. was responsible for the designing of the study, revising and drafting the manuscript.

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RESPONSE OF SOME SUGAR BEET VARIETIES TO DIFFERENT IRRIGATION INTERVALS

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ABSTRACT

A field experiment was conducted at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate in 2015/16 and 2016/17 seasons to investigate the behavior of five sugar beet varieties under different irrigation intervals; ie. Irrigation every 18 days (I_1), every 24 days (I_2) and every 30 (I_3) represented eight, six and four irrigation, respectively to determine sugar beet quality attributes as well as estimates some water relationships. A strip plot design with three replicates was used. The vertical plots were occupied with the three irrigation intervals and the horizontal plots were devoted to the five sugar beet varieties.

1 - The results obtained indicated that, sugar beet varieties were differed significantly in all the studied traits except for root/top ratio, sodium content and sugar lost to molasses% in 1st season, as well as potassium content in both seasons. Betamax variety ranked the first for most traits comparing with the other tested varieties

2 - The interaction effect between sugar beet varieties and irrigation water regimes were significant for all studied traits. 3 - Irrigating beet plants treatments with four (I2) and six (I3) irrigations led to saving about 870.55 m3 (30.45 %) and 331.63 m3 (11.60 %) water/fed across the two seasons, respectively, compared with eight irrigations. the highest mean values for the productivity of irrigation water kg/m3 (PIW).

3 - irrigated sugar beet plants six times led to significant increments in root diameter, fresh and foliage weight/plant, root/top ratio, sucrose, corrected sugar percentages as well as, yields of root and sugar/fed, while sugar lost to molasses% decreased when had irrigated with four irrigations in both seasons. Meantime, alpha amino-N and sodium contents were increased in the case of irrigating beet plants with eight irrigations in both seasons.

4 - Water regime 24 days intervals (six irrigations) during the two growing seasons can be recommended to get the highest root and sugar yields/fed as well as the best quality characteristics of sugar beet under conditions

KEYWORDS:
Irrigation, intervals, sugar beet, varieties

INTRODUCTION

In Egypt, water is the most critical factor in crop production with rainfall is low and with an erratic distribution. Therefore, almost agricultural production depends mainly upon irrigation, Optimum irrigation is one of the most vital factors in sugar beet production, as it can increase yield and reduce water costs and fertilizer in an area of water scarcity, aiming at saving water and maximizing its productivity [1]. In this concern [2] mentioned that root length was significantly increased with a longer irrigation period every 7 weeks compared with irrigation every 3 weeks. [3] revealed that increasing the drought period resulted in a significant increase in root length, gross sugar, white sugar percentages while , the root, top yields/fed , root diameter and yield/fed were decreased , meanwhile white and gross sugar yield/fed and sugar purity % were not affected. They added that water deficit showed relatively the smallest leaf growth. [4] concluded that irrigation requirement of sugar beet is fairly low, not more than 4 to 5 irrigations amounting to 37.5-60 cm would be required to obtain the roots and quality satisfactorily. [5] found that excess or deficit irrigation affects crop growth negatively. They showed that irrigations should be scheduled in such a way as to supply the optimum amount of water throughout the growing period depending upon soil type and climatic conditions. [6] indicated that deficit irrigation practices in sugar beet significantly decreased the root fresh weight/plant, root and sugar yields/fed. They added that by increasing the amount of irrigation water, root and white sugar yields increased while the sugar content decreased.

All sugar beet cultivars sown under Egyptian conditions are imported from global breeding sources. Therefore, evaluation of these varieties is required locally is very important to select the best ones. In this connection, [7] revealed that the evaluated sugar beet varieties exhibited significant differences in all yield criteria due to the difference in their gene make-up, which plays an important role in plant structure and morphology. [8] indicated that SD-

PAK09/07 variety attained the highest sugar yield (9.35 tons/ha) with highest sugar contents (12.60 %) and root yield (74.2 tons/ha) followed by California and Magnolia with sugar yield of 7.08 and 6.99 tons/ha, respectively. They reported insignificant difference among varieties in root yield and root size. [9] found that, the tested sugar beet varieties showed different performance with respect to sugar %, fresh root and gross sugar yield. They recorded maximum fresh root beet yield produced by Monte Rose, DS-9004 and R-Hist. Similar trends were recorded for gross sugar yield.

Consequently, this study aimed to investigate the behavior of five sugar beet varieties under different water regimes, and the effect of irrigation intervals on yield quantity and quality features of sugar beet in north delta conditions.

MATERIALS AND METHODS

A field trial was conducted at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate in 2015/16 and 2016/17 seasons to evaluate the performance of five sugar beet varieties concerning their response to three irrigation intervals; eight irrigation (I_1) Irrigation every 18 days , six irrigation (I_2) Irrigation every 24 days and four irrigation (I_3) Irrigation every 30 days with relation to yield, quality attributes of sugar beet crop (*Beta vulgaris var. saccharifera,* L.), as well as some water relationships. Five multi-germ sugar beet varieties namely; Golori, Toro, Almaz, Betamax and Demapoly. A strip plot design with three replicates was used. The vertical plots were occupied with the three irrigation intervals and the horizontal plots were devoted to the five studied varieties. The area of each plot was 21 m^2 (1/200 fed) including 6 ridges of 7 m in length and 0.5 m in width. All agricultural practices were done as recommended by Sugar Crops Research Institute except irrigation was done according to the study protocol.

The soil samples from various depth for the experimental site were analyzed before planting, which physical analysis is shown in Table 1.

The recorded data: The following studied traits of sugar beet were determined at harvesting:

I. Growth characters: 1. Root length (cm).2. Root diameter (cm).3. Root fresh weight/plant (g).4. Top fresh weight/plant (g).

II. Productivity traits: At harvest, all plants in each plot were taken in kg and converted to determine the following characteristics: 1. Root and top yields/fed (ton), 2. Corrected sugar yield/fed (ton) was calculated according to the following method of **[10]**:

Corrected sugar yield/fed (ton) = (root yield/fed (ton) x corrected sugar % / 100

TABLE 1
Physical properties of the experimental site during 2015-2016 and 2016-2017 seasons.

Soil depth (cm)	Particle size distribution			Soil texture	F.C %	PWP %	B_d g/cm^3	AW %
	Sand %	Silt%	Clay%					
	2015/2016 season							
0-15	12.33	33.02	54.65	Clay	44.20	22.01	1.11	22.10
15-30	19.51	34.60	45.89	Clay	42.30	21.15	1.15	21.15
30-45	20.00	39.10	40.90	Clay	40.10	20.05	1.13	20.05
45-60	20.10	40.20	39.70	Clay	36.75	18.38	1.17	18.37
Mean	18.00	37.00	45.00	Clay	40.84	20.42	1.14	20.42
	2016 /2017 season							
0-15	20.95	34.00	53.50	Clay	49.60	24.80	1.05	24.80
15-30	20.80	33.65	45.40	Clay	37.14	18.57	1.09	18.39
30-45	20.70	43.60	35.30	Silt clay	35.90	17.95	1.21	17.95
45-60	20.50	40.70	35.50	Silt clay	34.21	17.11	1.27	17.10
Mean	18.70	38.15	43.15	Clay	39.21	19.61	1.16	19.60

III. Root quality characteristics and chemical constituents were taken in five garded plants from each plot: 1. Sucrose percentage (Pol%) was estimated in fresh samples of sugar beet roots, using "Saccharometer" according to the method described in **[11]**.

2. Impurities%: K, Na and a-amino N contents were estimated as meq/100 g beet according to the procedure of sugar company by Automated Analyzer as described in **[12]**.

3. Sugar lost to molasses percentage (SLM %) was calculated using the following formula according to **[10]** as follows:

SLM% = 0.29 + (Na + K) 0.343 + 0.094 (α-amino N).

4. Corrected sugar% was calculated according to equation of **[12]**as follow:

Corrected sugar% = (Pol% - 0.29) - 0.343 (K + Na) - a-amino N (0.0939).

Where: K, Na and a-amino N were determined as meq/100 g beet.

5. Quality index (QZ %) was calculated according to the following equation:

Q Z % = (Corrected sugar% x100) / Pol %.

IV. Estimate of some water relationships as following equations: 1. Amount of irrigation applied water (AW, cm and m^3/fed),The amount of applied irrigation water was measured by using cut-throat flume (30 and 90 cm) for each irrigation treatment, then seasonal applied water was recorded through the whole growing season and calculated as cm^3/fed and according to **[13]**

2. Productivity of irrigation water (PIW, kg/m^3): Productivity of irrigation water was calculated according to **[14]**.

PIW = $\frac{Y}{AW}$ (kg/m^3) Where: PIW = productivity of irrigation water (kg/m^3) Y = root yield (kg/fed) AW = applied water (m^3/fed).

Statistical analysis: The collected data were statistically analyzed according to Gomez and Gomez (1984) and Snedecor and. Cohran.1989 by means of "MSTAT-c" computer software package.

RESULTS AND DISCUSSION

Effect of irrigation intervals on root length, root diameter and root fresh weights during 2015/2016 and 2016/2017 seasons. Results in Table 2 cleared that root length decrease by increasing number of irrigations during the two experimental seasons. Regarding root diameter of sugar beet increased significantly by the increasing intervals between irrigation in both seasons. Irrigating sugar beet plants with four irrigations was enough to produce the tallest roots compared to the other two irrigation treatments (6 and 8 irrigations) in both seasons. Meantime, increasing the applied irrigation treatments up to eight irrigations led to positive and appreciable effects on root diameter during the two seasonal. These findings may be due to sugar beet allocates more photo-assimilates to root growth under adequate irrigation conditions, which can lead to greater root development and as a result, an increase in the root diameter and the consequent increase in the root/top ratio (Table 2). These results coincide with those found by **[4]**.

Where, I_1, I_2 and I_3 refer to irrigation every 18 days (eight irrigation), irrigation every 24 days (six irrigation) and irrigation every 30 days (four irrigation)**, respectively.**

For the varietals difference, results in the same Table showed that the evaluated sugar beet varieties varied significantly in root length and diameter, .

Betamax variety showed a significant superiority over than the other four varieties in root length and diameter in both seasons. The variance among the tested sugar beet varieties can be attributed to their gene make-up. These results are in agreement with those obtained by **[9,15] .**

Concerning to the interaction, data in Table 2 indicated that the interactions between irrigations treatment and varieties affected significantly on root length, and diameter, in the first season only. It was cleared that most of these varieties could grow successfully under the various irrigation intervals but, Betamax variety exhibits the highest values in root length in the case of irrigating it with four irrigations. Likewise, irrigating Betamax variety with six irrigations achieved the highest values in root diameter and fresh weight/plant in 1st season. There was no significant interaction caused by other cases i.e. the increase given by the irrigation treatments occurred unaffected by the status of the varieties.

The results in Table 3 manifested that irrigation intervals had significant effects on top fresh weight/plant, top fresh weight/ plant (kg). Irrigating beet plants every 24 days interval (6 irrigations) caused an increase of both top fresh weight/plant, top fresh weight/fed over those irrigated with the other two irrigations (4 and 8 irrigations) in both seasons. The increment in root yield amounted to (4.42 and 3.67 tons/fed) comparing with that irrigated with 8 irrigations. These results are in harmony with those obtained by **[3]**.

As for varieties, there were significant between them regarding **Root fresh weight (g)** and **Top fresh weight / plant (kg)** Table 3 presented that the Betamax variety exhibited significant high values for these traits across studied environments. Mean while, no interaction effects between irrigation treatments and varieties were detected for the mention traits

TABLE 2
Root length (cm), and root diameter (cm) of five sugar beet varieties as affected by irrigations intervals treatments in 2015/2016 and 2016/2017 seasons

	Root length (cm)								Root diameter (cm)							
	2015/2016				2016/2017				2015/2016				2016/2017			
	I_1	I_2	13	Mean	I_1	I_2	13	Mean	I_1	I_2	13	Mean	I_1	I_2	13	Mean
Golori	27.0	30.3	31.7	**29.7**	27.0	31.3	32.2	**30.2**	9.3	8.1	6.3	**7.9**	10.7	7.7	6.9	**8.4**
Toro	29.0	32.7	36.0	**32.6**	29.7	36.3	35.6	**33.9**	11.2	8.4	7.2	**8.9**	11.3	9.0	7.8	**9.4**
Almaz	28.0	31.3	34.3	**31.2**	27.3	34.7	32.4	**31.5**	9.7	8.2	6.7	**8.2**	10.9	8.1	7.3	**8.8**
Betamax	30.3	35.0	37.3	**34.2**	30.7	39.8	37.3	**35.9**	11.5	8.6	7.9	**9.3**	11.6	9.8	8.1	**9.8**
Dimapoly	26.3	30.0	29.7	**28.7**	26.3	29.7	31.3	**29.1**	8.7	7.9	5.8	**7.5**	10.1	7.5	6.3	**8.0**
Mean	**28.1**	**31.9**	**33.8**		**28.2**	**34.4**	**34.5**		**10.1**	**8.2**	**6.8**		**10.9**	**8.4**	**7.3**	
LSD at 0.05 % **Irrigation (I)**	**1.6**				**2.3**				**1.4**				**0.9**			
Varieties (V)	**1.2**				**1.5**				**0.4**				**0.4**			
I x V	**2.1**				**N.S**				**0.7**				**N.S**			

4. Root/top ratio and root yield/fed. Results in table 4 showed that both traits i.e. root /top ratio and root yield/fed were significantly variated from irrigation treatment to another. It's cleared that the when give six irrigation (I_2) lead to increase in both traits in the aforementioned traits in both seasons. The increase in root /top ratio reached 26.47 and 51.41 in the first season and 49.00 and 73.08 in the second season comparing with I3 and I1, respectively for root /top ratio. As for root yield/ fed the highest values were detected by I3 (27.33 ton) and I2 33.39 at first and second season, respectively It was found that varieties had a significant effect on foliage weight, root/top ratio and root yield/fed in both seasons. Betamax variety surpassed significantly the other varieties in top fresh weight; root/top ratio and root yield/fed. Otherwise, Demapoly variety recorded the lowest values of the three traits in both seasons. The differences among the tested varieties might be principally due to the genetic variation. These finding are in harmony with those obtained by [15,16,17] Moreover, data in Table 4 revealed that the interactions between watering number and varieties insignificantly affected top fresh weight/plant, root/top ratio in both seasons while, root yield/fed significantly increased in 1st season. Meantime, Betamax variety gave the highest values in root yield/fed recording (34.20 ton/fed), just in case of it irrigating every 24 days (six irrigation), in exchange for 18.11 ton and 30.44 when it is watering with 4 and 8 irrigation respectively, in the first season.

5. Sucrose%, and alpha amino-N. Results in Table 5 affirmed that sucrose%, and alpha amino-N was increased significantly as the applied irrigations number in both seasons. Irrigating sugar beet with six irrigations attained the highest values of sucrose%. Otherwise increasing irrigation intervals up to 8 irrigations led to a significantly increase in the content of alpha amino-N in roots compared to the other two irrigation treatments. While irrigating beet plants at 30 days intervals (four irrigations) reduced the values of alpha amino-N, these were true in both seasons. **[6].**

TABLE 3
Root fresh weight (g) and Top fresh weight/plant (kg) of five sugar beet varieties as affected by irrigations intervals in 2015/2016 and 2016/2017 seasons

		Root fresh weight (g)								Top fresh weight / plant (kg)							
		Season 1				Season 2				Season 1				Season 2			
		I_2	I_1	13	Mean	I_2	I_1	13	Mean	I_2	I_1	13	Mean	I_2	I_1	13	Mean
Golori		0.79	0.95	0.58	0.77	0.85	1.32	0.60	0.92	0.42	0.59	0.34	0.45	0.58	0.55	0.35	0.49
Toro		0.81	1.21	0.59	0.87	0.88	1.38	0.63	0.96	0.52	0.7	0.41	0.54	0.66	0.63	0.5	0.6
Almaz		0.81	1.06	0.59	0.82	0.86	1.34	0.61	0.93	0.45	0.63	0.35	0.48	0.63	0.73	0.39	0.59
Betamax		0.84	1.28	0.62	0.91	0.89	1.41	0.65	0.98	0.56	0.75	0.43	0.58	0.78	1.02	0.57	0.72
Dimapoly		0.78	0.93	0.58	0.76	0.84	1.26	0.6	0.9	0.37	0.53	0.34	0.42	0.52	0.49	0.34	0.45
Mean		0.81	1.82	0.59		0.86	1.34	0.62		0.46	0.64	0.37		0.63	0.69	0.39	
LSD at 0.05 %	Irrigation (I)	0.08				0.71				0.04				0.02			
	Varieties (V)	0.49				0.06				0.06				0.05			
	I x V	N.S				N.S				NS				NS			

TABLE 4
Root /top ratio and root yield/fed of five sugar beet varieties as affected by irrigations intervals in 2015/2016 and 2016/2017 seasons.

	Root /top ratio								Root yield/fed							
	Season 1				Season 2				Season 1				Season 2			
	I_1	I_2	1_3	Mean	I_1	I_2	1_3	Mean	I_1	I_2	1_3	Mean	I_1	I_2	1_3	Mean
Golori	1.15	2.01	1.68	1.61	1.11	2.18	1.33	1.54	16.44	31.64	26.05	24.71	18.34	31.32	28.27	25.98
Toro	1.41	2.28	1.73	1.81	1.55	2.34	1.73	1.87	16.68	32.89	29.73	26.43	19.40	33.48	28.51	27.13
Almaz	1.33	2.19	1.70	1.74	1.23	2.19	1.57	1.66	16.89	31.15	27.35	25.13	18.17	32.57	28.35	26.36
Betamax	1.55	2.30	1.76	1.87	1.70	2.57	1.79	2.02	18.11	34.20	30.44	27.58	20.05	33.55	30.68	28.09
Dimapoly	1.14	1.98	1.64	1.59	0.93	1.97	1.14	1.35	16.26	28.90	23.11	22.76	18.06	31.02	27.31	25.46

Mean		**1.42**	**2.15**	**1.70**		**1.30**	**2.25**	**1.51**		**16.88**	**24.60**	**27.33**		**18.80**	**32.39**	**28.62**
LSD at 0.05%	**Irrigation (I)**		**0.29**				**0.19**				**1.32**				**0.43**	
	Varieties (V)		**NS**				**0.22**				**0.50**				**0.72**	
	I x V		**NS**				**NS**				**0.87**				**NS**	

TABLE 5
Sucrose % and alpha amino nitrogen content of five sugar beet varieties as affected by irrigations intervals in 2015/2016 and 2016/2017 seasons

		Sucrose %								**Alph amino nitrogen content**							
		Season 1				**Season 2**				**Season 1**				**Season 2**			
		I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean
Golori		17.93	19.77	17.82	18.51	16.94	21.61	18.69	19.08	1.03	1.46	1.23	1.24	0.95	1.14	1.15	1.08
Toro		17.73	20.92	18.67	19.11	17.49	22.27	19.40	19.72	0.96	0.98	1.11	1.02	1.25	0.93	1.04	1.07
Almaz		17.58	20.35	18.26	18.73	17.18	21.99	19.08	19.42	1.00	1.07	1.36	1.14	1.03	1.24	1.17	1.15
Betamax		17.83	21.67	18.66	19.39	18.38	22.63	19.99	20.33	1.12	0.83	1.23	1.06	1.09	1.22	1.26	1.19
Dimapoly		17.27	19.25	17.66	18.06	16.59	21.39	18.46	18.81	1.29	1.35	1.29	1.31	0.93	1.38	1.48	1.26
Mean		17.67	20.39	18.21		17.32	21.98	19.12		1.08	1.14	1.24		1.75	1.18	1.22	
LSD at 0.05 %	**Irrigation (I)**		1.36				0.41				0.29				0.12		
	Varieties (V)		0.34				0.44				0.14				0.12		
	I x V		**0.59**				**0.76**				**0.24**				**N.S**		

In the same Table 5, it could be noticed that significant differences among sugar beet cultivars were found in sucrose% and alpha amino-N content in root in both seasons. The highest sucrose % (19.11%) was obtained by Toro variety in 1st season while in the second one Betamax variety recorded the highest percentage of sucrose (20.33%). On the other hand, both Glori and Demapoly varieties were given the highest alpha-amino content in the first season while, in the second one, Betamax and Demopoly varieties attained the higher values of this trait, compared to the other three varieties which recorded the lowest ones. Otherwise, the results indicated a negative response in sodium content due to evaluated sugar beet varieties. This view agrees with those concluded by **[18]**

Concerning the interaction, data in Table 5 showed that the interaction between the number of

irrigations and varieties influenced significantly sucrose% and sodium content in both seasons, and alpha amino-N in the first season. Betamax variety gave the highest sucrose values (21.67 and 22.63 %) in both seasons respectively. As well as, the lowest sodium in both seasons and alpha amino-N contents in the first season, just in case of irrigating with six watering, compared to the other varieties.

6. Sodium content, and Potassium content, sugar lost to molasses and corrected sugar percentages. The results in Tables 6 and 7 revealed that **Sodium** and potassium content, sugar lost to molasses and corrected sugar percentages were significantly affected by the applied irrigations number. Supplying beet plants with four irrigations during season reduced the values of potassium content and the lowest quantities of sugar lost to molasses% in both seasons, compared with other irrigation treatments which gave the highest values. Likewise, irrigating beet plants with four irrigations attained a broached increase in the values of corrected sugar% amounted by (18.34% and 19.61%) in both seasons respectively, compared with the other two irrigation treatments which recorded the lowest percentage in both seasons. These observations coincide with those found by **[3,6].**

In respect to the differences among sugar beet cultivars, data in the same Tables 6 and 7) affirmed that Sugar beet variety Betamax recorded the highest values of corrected sugar%. Meanwhile, Almaz variety recorded the lowest value of sugar lost to molasses %. On the other side Demapoly variety recorded the maximum value of sugar lost to molasses and consequently the lowest amount of the corrected sugar%. The variations among the tested sugar beet varieties in these traits might be due to their gene make-up. These results agree with those obtained by **[15]**.

TABLE 6
Sodium content and Potassium content of five sugar beet varieties as affected by irrigations intervals in 2015/2016 and 2016/2017seasons

		Sodium content								**Potassium content**							
		Season 1				**Season 2**				**Season 1**				**Season 2**			
		I_1	I_2	I_3	Mean	**I_1**	I_2	I_3	Mean	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean
Golori		2.04	2.07	2.09	**2.06**	1.84	1.91	2.22	**1.99**	6.10	6.18	6.12	**6.13**	5.92	5.70	6.39	**6.00**
Toro		1.91	2.20	2.10	**2.07**	1.54	1.64	2.49	**1.89**	5.81	5.76	6.22	**5.93**	5.94	6.47	5.77	**6.06**
Almaz		1.72	2.14	2.44	**2.10**	1.56	1.83	2.39	**1.93**	5.83	6.52	6.21	**6.19**	5.97	5.80	6.09	**5.95**
Betamax		2.01	1.70	2.29	**2.06**	1.72	1.73	2.11	**1.96**	6.07	6.51	6.25	**6.28**	5.95	5.85	6.02	**5.94**
Dimapoly		2.01	2.09	2.46	**2.19**	2.09	2.13	2.43	**2.22**	6.12	6.22	6.50	**6.28**	6.15	6.48	6.22	**6.28**
Mean		**1.94**	**2.04**	**2.31**		**1.75**	**1.85**	**2.39**		**5.99**	**8.24**	**6.26**		**6.15**	**6.48**	**6.22**	
LSD at 0.05 %	**Irrigation (I)**	**0.40**				**0.21**				**0.40**				**0.80**			
	Varieties (V)	**N.S**				**0.16**				**NS**				**NS**			
	I x V	**0.28**				**0.27**				**NS**				**NS**			

TABLE 7
Sugar lost to molasses % and Corrected sugar % of five sugar beet varieties as affected by irrigations intervals in 2015/2016 and 2016/2017seasons

		Sugar lost to molasses %								Corrected sugar %							
		Season 1				Season 2				Season 1				Season 2			
		I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean
Golori		6.10	6.18	6.12	**2.37**	5.92	5.70	6.39	**2.41**	2.36	2.34	2.42	**15.95**	2.48	2.24	2.51	**17.07**
Toro		5.81	5.76	6.22	**2.27**	5.94	6.47	5.77	**2.38**	2.34	2.31	2.17	**16.50**	2.28	2.45	2.42	**17.67**
Almaz		5.83	6.52	6.21	**2.44**	5.97	5.80	6.09	**2.34**	2.27	2.41	2.65	**16.28**	2.37	2.27	2.38	**17.43**
Betamax		6.07	6.51	6.25	**2.49**	5.95	5.85	6.02	**2.33**	2.47	2.42	2.59	**16.88**	2.34	2.29	2.37	**18.37**
Dimapoly		6.12	6.22	6.50	**2.44**	6.15	6.48	6.22	**2.48**	2.32	2.57	2.42	**16.62**	2.35	2.67	2.43	**16.67**
Mean		**2.35**	**2.41**	**2.45**		**2.36**	**2.38**	**2.42**		**14.53**	**18.34**	**15.86**		**15.95**	**19.61**	**16.76**	
LSD at 0.05 %	**Irrigation (I)**	**0.40**				**0.80**				**0.37**				**0.26**			
	Varieties (V)	**NS**				**NS**				**NS**				**0.17**			
	IxV	**NS**				**NS**				**0.32**				**NS**			

TABLE 8
Quality% and corrected sugar yield/fed (ton) of five sugar beet varieties as affected by irrigations intervals in 2015/2016 and 2016/2017seasons

	Quality%								Corrected sugar yield/fed (ton)							
	Season 1				Season 2				Season 1				Season 2			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
Golori	72.20	87.77	81.00	**80.32**	76.75	89.63	81.03	**82.47**	2.11	4.48	3.07	**3.22**	2.11	4.48	3.07	**3.69**
Toro	75.18	89.61	82.55	**82.45**	79.02	88.98	81.99	**83.33**	2.34	6.15	3.99	**4.16**	2.34	6.15	3.99	**4.03**
Almaz	75.75	86.98	80.51	**81.08**	77.94	89.85	81.67	**83.15**	2.33	5.10	3.41	**3.62**	2.33	5.10	3.41	**3.85**
Betamax	74.93	88.10	81.30	**81.44**	78.44	90.00	83.30	**83.91**	2.53	6.57	3.96	**4.35**	2.53	6.57	3.96	**4.37**
Dimapoly	71.51	87.42	80.24	**79.72**	75.73	87.52	80.49	**81.25**	2.05	4.24	3.30	**3.20**	2.05	4.24	3.30	**3.44**
Mean	**73.91**	**87.98**	**81.12**		**77.58**	**89.20**	**81.70**		**2.28**	**5.51**	**3.55**		**2.74**	**4.51**	**3.73**	

6.00	**Irrigation (I)**	**2.28**	**1.53**	**0.562**	**0.16**
LSD at 0.05 %	**Varieties (V)**	**1.03**	**1.09**	**0.113**	**0.18**
	IxV	**1.78**	**NS**	**0.195**	**0.31**

With respect to the interactions between irrigation intervals and varieties, data in Table 9 clear that the interaction affected significantly sugar lost to molasses in the first season and corrected sugar% in both seasons, while potassium content failed to reach the significance level. It was found that Toro variety had the lowest value of sugar lost to molasses. while Almaz variety had the highest value in the 1st season. Also, the Betamax variety gave the highest corrected sugar% (19.09 and 20.36%) when it is watering by six irrigation during the two growing seasonal respectively. These results may be due to genetic variation.

7. Quality % and corrected sugar yield/fed. Data in Table 8 revealed that quality and corrected sugar yields/fed of sugar beet were markedly affected by the applied irrigations number in both seasons.

Increasing the number of irrigations to six irrigations during the season led to a significant increase in corrected sugar yield/fed and quality%, it was enough to attain the highest values of corrected sugar yield/fed and improved juice quality% than those irrigated by the other irrigations ones (4 and 8 irrigations) in both seasons. These increases may be due to the amount of rainfall in the 1st season was increased than the second one (Table 8). As well, these results may be due to higher values of sucrose and lower, sodium accumulation and improved potassium uptake in roots% (Table 6) respectively. The reduction in quality and sugar yield/fed caused by irrigating beet plants at 18 days intervals (8 irrigations), may be attributed to the decrease in sucrose% and increase impurity. These results are in harmony with those of [2,5,18]

As for the differences among sugar beet varieties Betamax variety significantly surpassed those other varieties on their effect of quality%, was it recorded the highest value of quality%, as well as it gave (4.35 and 4.37-ton sugar/fed.) in both seasons respectively, compared to the other four tested varieties which, recorded the lowest yields in both seasons. These results related to the advantage which, obtained from corrected sugar % and root yield/fed for this variety. These results are in agreement with those confirmed by **[7,18]**. In view of the interaction data, in Table 8 cleared that the interaction between the number of irrigations and varieties influenced significantly the quality index in the 1st season and corrected sugar yield/fed in both seasons. Toro variety gave the highest quality index values (89.61 %) in case of watering every 24 days intervals in 1st season, while the highest values of corrected sugar yield were recorded by Betamax variety (6.57 and 5.86 tons) in both seasons.

Under North Delta conditions, it can be recommended to irrigating sugar beet Betamax variety, every 24 days interval (six irrigations) to get the highest sugar beet root and sugar yields, however for getting high sugar quality.

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In vitro ANTIFUSARIAL ACTIVITY OF THE EXTRACTS OF *Punica Granatum* L OBTAINED BY SOXHLET METHOD; AGAINST *Fusarium Oxysoprum f.sp Albedenis*; IN THE SOUTHWEST OF ALGERIA

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ABSTRACT

The biological activities of medicinal plants have been recognized for centuries. Among them, *Punica granatum* L is very commonly used for its medicinal virtues and has many therapeutic properties and targets various diseases such as cardiovascular disorders, diabetes, etc. It also has several properties due to its richness in phytochemicals at high concentrations, such as polyphenols against vegetable and palms diseases.

Fusarium oxysporum f.sp. albednis is one of the most dangerous fungi that causes Bayoud disease, causing significant losses of date palms in Algeria.

The aim of the present study is focused on the evaluation of the *in vitro* antifungal activity of a *Punica granatum* L fruit extracts obtained by the Soxhlet method, considered as a hot method using heating with the following solvents: distilled water, methanol, ethyl acetate, and chloroform.

The evaluation of antifusarial activity of *Punica granatum* L. was carried out by using the dilution and the direct contact method to determine the minimum inhibitory concentration (MIC).

The results of the extraction yield show that distilled water and methanol are the two best solvents which give a good yield compared to chloroform and ethyl acetate; 52% for methanol, but the low yield is 3.96% for chloroform and 3.33% for ethyl acetate.

The antifungal activity results of these extracts on the solid and liquid medium, the PDA and the PDB, respectively, show that they have good inhibitory activity against all the tested fungi.

According to the results obtained in the solid medium, the extracts tested have an inhibitory activity against *Foa*, with different MIC values ranging from 0.6 mg/ml to 5.5 mg/ml.

The result of growing on liquid medium, shows that with each increase in the concentration of the extracts in the culture medium, there is a decrease in the fungal biomass, and there is a fungicide activity for all extracts used.

KEYWORDS:
Punica granatum L, *Fusarium oxysporum f.sp. albedinis*, antifusarial activity, date palm

INTRODUCTION

Medicinal plants have played an essential role in the development of human medicinal culture. These creatures have always virtually been at the forefront of disease fighting in all cultures and civilizations. Many modern medicines are derived from traditional healing methods, which are regarded as rich resources **[1].**

Essential oils and extracts from aromatic plants have long been used for a wide variety of medicinal and domestic purposes. The antimicrobial properties of medicinal plants like *Punica granatum* L against food-related microorganisms, as well as their applications in the food system, have been studied and reviewed **[2]**.

Plants have been used as a primary means of disease treatment from ancient times and until nowadays, a significant number of species have been reported to possess various pharmacological properties **[3-4]**. Indeed, for thousands of years, medicinal plants have been used to treat health disorders, to add flavor and conserve food, as well as prevent disease epidemics **[5]**. The *Punica* genus is a well-known source of secondary metabolites which is widely spread in the Northern Hemisphere and is used in folk medicine as well **[6].**

Punica granatum L., or locally named "El Roman" (pomegranates), belongs to the Punicaceae family. It is widely cultivated throughout Central Asia, the Himalayas, the Middle East, and the American Southwest, and is believed to originate from Iran and Afghanistan and the Mediterranean area, including Algeria, which lies on the arid slopes and hills**.** It is one of the important endemic plants of Algeria, growing in most regions throughout the country, especially in arid and semi-arid regions due to its ability to adapt to adverse ecological conditions **[7].**

It is a shrub or a small tree that measures between five and eight meters tall **[8]**.

According to the holy Quran, pomegranates (Pg) grow in the gardens of paradise, and the Quran has recited the Pg twice as an example of God's good creations. The fruit of the Pg has extensively been used as a traditional remedy against acidosis, dysentery, microbial infections, diarrhea, helminth infections, hemorrhages and respiratory pathologies. Pg seeds have also been shown to contain the estrogenic compounds, estrone and estradiol **[9-10]**. Furthermore, the dried pericarp and the juice of the fruit are considered beneficial for the treatment of colic, colitis, menorrhagia, oxyuriasis, headache, diuretic, acne, piles, allergic dermatitis, and treatment of oral diseases. Recent studies have shown new scientific investigations into the traditional uses of Pg **[11]**.

In order to find other alternatives to fight the date palm disease, or Bayoud, which is caused by a most dangerous fungus called "*Fusarium oxysporum f.sp*. *albednis"*causing significant losses of date palms in Algeria, we have studied and focused in this present study, the antifusarial activity of *Punica granatum* L peels extracts obtained by the Soxhlet method, using the following solvents: water, methanol, ethyl acetate and chloroform; against ten (10) strains of *Fusarium oxysporum* f. sp. *albedinis* (FOA) causing palme date fusariosis.

MATERIALS AND METHODS

Plant Material. The selected samples of fruits of "*Punica granatum* L." were collected from the Bechar oasis, southwest of Algeria; and from an area in Beni Ounif known as "El ksar,"north of Bechar, during January and February 2021. This collected vegetal material was dried for 15 days in the dark and ground by an electric grinder machine to obtain a fine powder and less at ambient laboratory temperature (20 °C–28 °C).

Preparation of plant extracts. Soxhlet extraction method was used to extract plant components and the extraction process was performed using 30g of plant material and 150 ml of different solvents included distilled water, methanol, ethyl acetate and chloroform. The extraction process was done for 6 hours at boiling temperature of the solvent. The crude extract of each solvent was evaporated using a rotary evaporator. The weight of all extracts were measured after solvent evaporation and kept in the fridge at 4°C for biological effect purpose. **[12-13-14]**.

Determination of extraction yield. The extraction yield is expressed, for each solvent, by the ratio between the mass of the extracted material and the mass of the plant material used.

Where:

M: mass in grams of the resulting dry extract;
M_0: mass in grams of initial plant material.

***In vitro* Antifungal Activity. Fungal Strains.** The antifungal activity was evaluated by both direct contact and dilution methods on ten selected strains of fungi, *Fusarium oxysporum* f. sp. *Albedinis* FOA (1), FOA (2), FOA (3), FOA (4), FOA (5), FOA (6), FOA (7),FOA (8),FOA (9) FOA (10), that were isolated from date palms from the Beni-ounif area infected by the fusariosis disease. Fungal spores were prepared by growing mold on potato dextrose agar (PDA) at 25 °C for 7 days, and spores were suspended in sterile 1% tween-80. Spore count was performed by using a hemocytometer and adjusted to obtain10^5–10^6spores/ml with potato dextrose broth (PDB) **[15].**

Fungal DNA Isolation, Purification and Quantification. The total DNA of dried mycelium of each strain was extracted and purified as described by **Kumar et *al*., (2012) [16]**. In the Universal method: a single protocol universally used for plants, algae, blood, bacteria, and fungi will be more demanding than those suited specifically to a particular biological material. The extraction buffer used in this case included 100 mM Tris-HCl, pH 8.0, 25 mM EDTA, 1.5 M NaCl, 2% CTAB, β-mercaptoethanol, and PVP (in case of the plant sample). The method utilizes the classical protocol for homogenization by liquid nitrogen, incubation in a water bath at 65°C, and deproteinization was performed by chloroform–isoamyl alcohol, followed by ethanol precipitation and washing. As an alternative to the above, other universal methods termed as the "nuclei method" and "protoplast method" may be successfully used to prepare high quality, megabase-sized DNA.

The RNA was eliminated by adding proteinase K (RNase;10 mg/ml). The DNA was dissolved in 200µl TE buffer (10 mM Tris-HCl, pH 6.0, 1 mM EDTA, pH 8.0), quantified and diluted to an approximate concentration of 5 ng/µl for PCR reactions **[17].**

PCR Amplification of Fungal DNA. The primers used were preselected among several primers because they permitted to reveal polymorphism on small DNA samples used for preliminary trials. The PCR techniques were optimized according to primers. Reaction was achieved by 1 cycle consist of 4 min of denaturation at95 °C followed by 30 cycles consisting of 1 min at 94 °C,1 min at 36 °C and 1 min 30 s at 72 °C. One cycle for 15 min at 72 °C was conducted at the end. PCR reactions were performed in a total volume of 25µl, containing 25 ng genomic DNA, 10X Taq buffer, 10 mM dNTP, 25 mM MgCl2, 1-unitTaq DNA polymerase (Promega) and 10 mM primer.For FOA-specific amplification, PCR primers included two primer pairs; FOA1 (CAGTTTATTAGAAATGCCGCC) coupled with BIO3

(GGCGATCTTGATTGTATTGTGGTG) and FOA28 (ATCCCCGTAAAGCCCTGAAGC) coupled with TL3 (GGTC GTCCGCAGAGTATAC-CGGC) [18].

FOA-specific PCR reactions were performed according to Fernandez et *al*. (1998) [18]. As follows: 1 cycle for 4 min at 95 °C followed by 30 cycles for 30 sat 92°C,30 sat 60° Cand30sat 72 °C for the FOA1-BIO3 primer pair; and 30 cycles for30 sat 92 °C, 30 sat 62°C and 45s at 72°C for the FOA28 TL3 primer pair. Thereafter, a cycle of 15 min at 72°C was conducted. PCR reactions were performed in a total volume of 20 µl, containing 10–100 ng genomic DNA,10 X Taq buffer,0.2 mM of dNTP, 1.5 mM MgCl2, 1unit Taq DNA polymerase (Promega) and 1µM primer, as previously described by Fernandez et *al*. (1998) [18]. The PCR reactions were incubated in a TC 3000 Thermocycler (Progene, Techne England). All amplification products were separated in stained agarose gels (1.8% w/v; 15 × 10 cm, W × L) with ethidium bromide in TAE buffer electrophoresed at 100 V for 1 h 30 min **[19]**. The DNA weight marker used was λ (lambda) digested by enzymes Hind III and EcoR1. At the end of electrophoresis, the gels were visualized by UV illumination and photographed using a Bioprint System 3000WL X-PRESS computer assisted machine (software BIO-1D).

Dilution method (MIC). The minimum inhibitory concentration (MIC), defined as the lowest concentration of the treatment that inhibits visible fungal growth, was determined with a microdilution method. Antifungal tests were performed according to the method reported by Hassikou et *al*. (2002) **[20],** with the antifungal activity evaluated via the dilution and the direct contact method. The direct contact method on solid medium was performed in Petri dishes containing PDA, a culture media and extract, at different concentrations (0.2, 0.4, 0.6, 0.8, 1 mg/ml) which were inoculated with fungus suspension obtained from pure culture, and incubated at 25 °C for 7 days. The diameter of the fungus colony was obtained by calculating the average of two perpendicular diameters compared with the witness petri dishes. However, the direct contact method on liquid medium or the minimum fungicide concentration (MFC) is defined as the lowest concentration that kills 99.9% of the final cell concentration. To determine it, the method described by **Canton et *al*. (2003) [21]** was used. Concentrations strictly above the MIC will be used. It was performed in sterile vials where the fungal inoculum was taken and added to 50 ml of PDBac medium, and then precise volumes of the extract were taken according to the given concentrations. As a witness, sterile vials are prepared using PDBac culture medium and fungal inoculum only. At the end, incubation is done at 25°C for 14 days. After determining the MIC, aliquots of the wells with concentrations corresponding to the MIC as well as with higher concentrations were used to inoculate PDA plates for the determination of the minimum fungicidal concentration (MFC). It was defined as the lowest treatment concentration required killing a pathogen and corresponded to an invisible growth of the subculture. Three replicates of each assay were assessed.

RESULTS

Extraction yield. The extraction yield of *Punica granatum* L with different solvents is shown in Table 1.

Antifungal Activity. Analysis of the Population of *F. o.* f. sp. *Albedinis* by the Specific PCR Technique. The amplification of genomic DNA by a specific primer couple to Foa, TL3-FOA28, revealed a specific band which has a size of 400 bp (Figure 1). However, this band has also been amplified in the case of six strains of *Fusarium oxysporum*, of which one was isolated from the Beniounif palm grove. For amplification; a specific band of size 200 bp using BIO3-FOA1 coupled primer revealed a specific band. The assembly of the two types of bands revealed by two primer couples (TL3-FOA28 and BIO3-FOA1) only gave 30% of reliability. The dendrogram based on recombined results obtained by two primer couples permitted to distinguish two big distinct groups (data not shown): the groups 1 and 2 are composed of a mixture of FOA strains from different origins and *Fusarium oxysporum* strains **[22]**.

Dilution Method (MIC). The inhibition growth zones measured by the dilution method (MIC) are presented in Tables 2, 3, and 4. The antifungal activity of studied extracts against ten strains of *Fusarium oxysporum f. sp*. *Albedinis* showed that *Punica granatum* L extracts have great antifungal activity against all the investigated strains. These results revealed that the fungal growth was inhibited completely. Nevertheless, S3 was the most sensitive strain with a minimal inhibition concentration value of 0.6, 1.8, and 3 mg/ml form ethanol, ethyl acetate, and chloroform extracts, respectively (Figures 2,3,4).

The results of the antifungal parameters (MIC and MFC) of our extracts of *Punica granatum* L by the direct contact method on liquid medium are summarized in Table 5.

DISCUSSION

The results of the extraction yield show methanol is the best solvent which gives a good yield compared to chloroform and ethyl acetate, 52% for methanol, but the low yield is between 0.5% to 3,96 % for

TABLE 1
Extraction yield of *Punica granatum* L with different solvents.

	Soxhlet method		
Solvents	MOH	CHLF	EA
Extraction yield (%)	52	3.96	3.33

MOH: Methanol; CHLF: Chloroform; EA: Ethyl acetate.

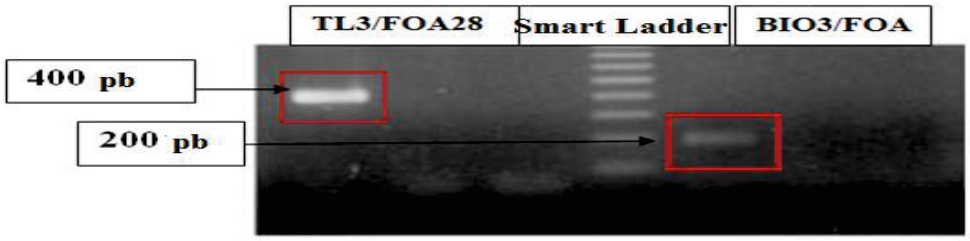
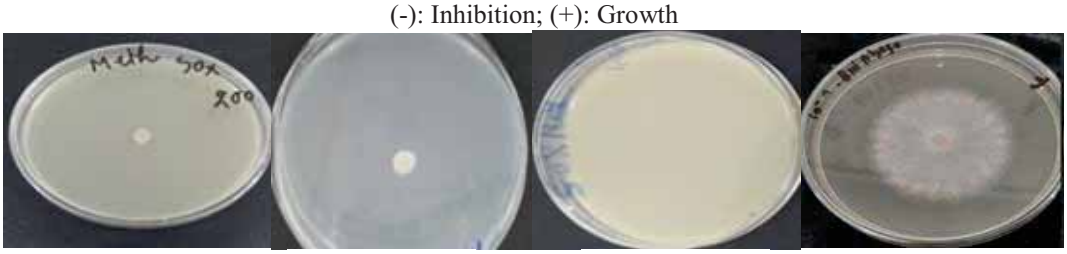


FIGURE 1
Amplification of genomic DNA of FOA by PCR method (Original source, 2022).

TABLE 2
MIC of *Punica granatum*L methanol extracts (mg/ml) on fungal strains.

Strains	Witness	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8
S1	+	+	+	+	+	+	**MIC**	-	-	-
S2	+	+	+	+	+	+	+	+	**MIC**	-
S3	+	+	+	**MIC**	-	-	-	-	-	-
S4	+	+	+	+	+	**MIC**	-	-	-	-
S5	+	+	+	+	+	+	**MIC**	-	-	-
S6	+	+	+	+	**MIC**	-	-	-	-	-
S7	+	+	+	+	+	+	+	**MIC**	-	-
S8	+	+	+	+	+	**MIC**	-	-	-	-
S9	+	+	+	+	+	+	+	**MIC**	-	-
S10	+	+	+	+	+	+	+	+	**MIC**	-

(-): Inhibition; (+): Growth



Witness **0.3 mg/ml** **0.6 mg/ml** **1 mg/ml**

FIGURE 2
Effect of *Punica granatum* L methanol extracts with various concentrations against S3 of FOA (Original Source, 2022).

TABLE 3
MIC of *Punica granatum* L for ethyl acetate extracts (mg/ml) on fungal strains.

Strains	Witness	1.2	1.4	1.6	1.8	2	2.2	2.4	2.6	2.8	3	3.2	3.4
S1	+	+	+	+	+	+	**+**	**MIC**	-	-	-	-	-
S2	+	+	+	+	+	+	**MIC**	-	-	-	-	-	-
S3	+	+	+	**+**	**MIC**	-	-	-	-	-	-	-	-
S4	+	+	+	+	+	**+**	+	**MIC**	-	-	-	-	-
S5	+	+	+	+	+	+	+	+	**MIC**	-	-	-	-
S6	+	+	+	+	+	+	**MIC**	-	-	-	-	-	-
S7	+	+	+	+	+	+	+	+	**MIC**	-	-	-	-
S8	+	+	+	+	+	+	+	+	+	**MIC**	-	-	-
S9	+	+	+	+	+	+	+	+	+	+	+	**MIC**	-
S10	+	+	+	+	+	+	+	+	+	**+**	**MIC**	-	-

(-): Inhibition (+): Growth

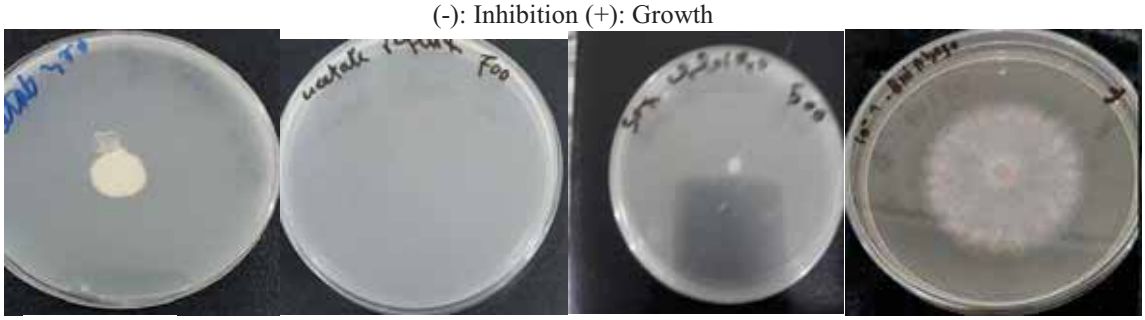


FIGURE 3
Effect of *Punica granatum* L ethyl acetate extracts with various concentrations against S3 of FOA (Original Source, 2022).

TABLE 4
MIC of *Punica granatum* L for chloroform extracts (mg/ml) on fungal strains.

Strains	Witness	1.2	1.4	1.6	1.8	2	2.5	3	3.5	4	4.5	5	5.5	6
S1	+	+	+	+	+	+	+	+	+	+	**MIC**	-	-	-
S2	+	+	+	+	+	+	+	+	+	+	+	**MIC**	-	-
S3	+	+	+	+	+	+	+	**MIC**	-	-	-	-	-	-
S4	+	+	+	+	+	+	+	+	**MIC**	-	-	-	-	-
S5	+	+	+	+	+	+	+	+	+	**MIC**	-	-	-	-
S6	+	+	+	+	+	+	+	+	+	+	**MIC**	-	-	-
S7	+	+	+	+	+	+	+	+	+	+	+	+	**MIC**	-
S8	+	+	+	+	+	+	+	+	+	+	**MIC**	-	-	-
S9	+	+	+	+	+	+	+	+	+	**MIC**	-	-	-	-
S10	+	+	+	+	+	+	+	+	+	+	+	+	**MIC**	-

FIGURE 4
Effect of *Punica granatum* L ethyl acetate extracts with various concentrations against S3 of FOA (Original Source, 2022).

chloroform and between 1,15 % to 3.33% for ethyl acetate.

Our returns of the extraction yield of *Punica granatum* L extracts (Table 1) are low compared to those obtained by **Manisha Bapodara et** *al,* **(2011);** Ferial A. Ismail **et** *al*, (2014); Wapwera Augustine **et** *al*, (2021) [23-24-25]. This difference is related to several factors, such as the geographical area of collection, climate, stage of development and the season.

According to these *in vitro* results, (Tables 2, 3, and 4) and the inhibition rates of each extract of *Punica granatum* L, it is generally noted that the methanolic extract has a stronger inhibitory activity than the ethyl acetate and the chloroform extracts.

However, it has a great and good inhibitory antifungal activity against all the investigated fungi. The concentration inhibition is between 0.6 and5 mg/ml. The diameters of inhibition growth zone ranged from 15 to 50 mm with the highest inhibition zone values observed against the strain of *Fusarium oxysporum* f. sp. *Albedinis*(3) FOA(3) (50 mm). However, the studied fungi did not show the same sensitivity to different solvents.

The difference in the degree of this effect is possible due to:

- The nature of the secondary metabolites extracted and/or their quantities, the major compounds

TABLE 5
Activity for each extracts of *Punica granatum* L on fungal strains.

	Methanol extracts				Ethyl acetate extracts				Chloroform extracts			
Strains	MIC	MFC	MFC/ MIC	**Activity**	MIC	MFC	MFC/ MIC	**Activity**	MIC	MFC	MFC/ MIC	**Activity**
S1	1.2	1.4	1.16	Fungi-cide	2.4	2.6	1.08	Fungicide	4.5	5	1.11	Fungi-cide
S2	1.6	1.8	1.125	Fungi-cide	2.2	2.4	1.09	Fungicide	5	5.5	1.1	Fungi-cide
S3	0.6	0.8	1.33	Fungi-cide	1.8	2	1.11	Fungicide	3	3.5	1.16	Fungi-cide
S4	1	1.2	1.2	Fungi-cide	2.4	2.6	1.08	Fungicide	3.5	4	1.14	Fungi-cide
S5	1.2	1.4	1.16	Fungi-cide	2.6	2.8	1.07	Fungicide	4	4.5	1.125	Fungi-cide
S6	0.8	1	1.25	Fungi-cide	2.2	2.4	1.09	Fungicide	4.5	5	1.11	Fungi-cide
S7	1.4	1.6	1.14	Fungi-cide	2.6	2.8	1.07	Fungicide	5.5	6	1.09	Fungi-cide
S8	1	1.2	1.2	Fungi-cide	2.8	3	1.07	Fungicide	4.5	5	1.11	Fungi-cide
S9	1.4	1.6	1.14	Fungi-cide	3.2	3.5	1.09	Fungicide	4	4.5	1.125	Fungi-cide
S10	1.6	1.8	1.125	Fungi-cide	3	3.2	1.06	Fungicide	5.5	6	1.09	Fungi-cide

in the fruits of *Punica granatum* L are polar substances such as flavonoids and tannins [26-27-28-29]. It is possible that the antifungal effect is linked to these secondary metabolites (this difference depends on the nature of the solvent used and their polarities; water and methanol are more polar than ethyl acetate and chloroform).

-The method of extraction used, the temperature, and the time of extraction. According to Roy **et** *al* (2007) [30], and Walid F **et** *al*., (2022) [31], temperature can affect the amount of polyphenols and their biological effects. They reported that a temperature at 100°C for 10 to 30 min affects the content of phenolic compounds and their antioxidant activities. However, heating at 50°C for 10 to 30 min preserves 80-100% of the polyphenols and their biological effects.

In the literature, pomegranate juice and wine have become increasingly popular because of the attribution of important biological actions to this plant [32], including cardiovascular protection [33]. In traditional Ayurvedic medicine, all parts of PG are used for the treatment of various disorders. PG flowers (PGF) have been prescribed in Unani and Ayurvedic medicine for the treatment of diabetes [34]. Recently, it has been reported that the aqueous methanolic (50% v/v) extract from PGF showed hypoglycemic activity in a diabetic animal model [35].

Punica granatum L (pomegranate) is a deciduous shrub. Nowadays, besides its use as a fruit, its medicinal properties have attracted the interest of researchers of many countries. Pomegranate fruit has medicinal properties such as anti-inflammatory and antibacterial activities. Pomegranate seed oil has inhibitory effect on skin and breast cancers. It has phytoestrogenic compounds and the fruit is rich in phenolic compounds with strong antioxidant activity. Ellagic acid is one of the main components of pomegranate with phenolic structure and antioxidant activity [36-37-38-39-40].

CONCLUSION

From the above study, one can conclude that the extract of pomegranate fruits, which contains secondary methabolites, is useful for the treatment of several infections and vegetables inflammatory disorders due to fungi of *Fusarium oxposum* fsp *albedenis*. The *Punica granatum* L extracts have a good antifungal activity. The strong antifungal activity against an array of filamentous fungi strains is an indication of the broad spectrum of the antifungal potential related to this type of extract. This could make it a promising element of natural compounds for the development of safer antimicrobial agents.

These results suggest the possibility of using this raw material in pharmaceuticals as a fertilizer powder. Further studies and investigations are needed.

ABBREVIATIONS

CHLF: Chloroform
DNA: Deoxyribonucleic acid
DW: Distilled water.
EA: Ethyl acetate;

EDTA: Ethylene diamine tetra-acetic acido
Et OH: Ethanol
FOA: *Fusarium oxposum* f.sp *albedenis*
MFC: Minimum fungicidal concentration
MIC: Minimum inhibitory concentration
Me OH: Methanol
PCR: Polymeras chain reaction.
PDA: Potato dextros agar.
PDB: Potato dextrose broth.
PG: Pomegranate
PGF: Pomegranate flowers.
RNA: Ribonucleic acid

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EFFECT OF COOKING AND STORAGE VARIABLES ON ANTIOXIDANT COMPONENTS AND ACTIVITIES OF COMMON VEGETABLES: A COMPARATIVE ASSESSMENT

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ABSTRACT

Vegetables, important source of nutrients and phytochemicals are processed and stored in different ways prior to consumption influencing their nutritional quality. Antioxidants have been recognized for their protective role in disease prevention in huma diets. The effect of domestic cooking procedures (pressure, microwave or conventional) and storage [refrigeration (7 days), freezing (40 days)] on antioxidant components and activity of carrot (*Daucus carota,* subsp. sativus), cabbage (*Brassica oleracea* var. capitata), pumpkin (*Cucurbita maxima)* and cauliflower (*Brassica oleracea* var. botrytis) were studied. Thermal treatments of vegetables resulted in reduction of total phenols, tannins and ascorbic acid, while carotenoids and β-carotene were retained. Refrigeration and freezing caused further losses of these components. Raw and cooked vegetables showed high antioxidant activity measured through total antioxidant activity, reducing power and free radical scavenging assay, which varied depending upon vegetable and could be correlated with their antioxidant components.

KEYWORDS:
Domestic cooking, phenolics, carotenoids, storage, antioxidant activity

INTRODUCTION

Vegetables are important sources of nutrients and phytochemicals in diets, as they contribute large amounts of vitamins and minerals. Through their antioxidant function, they are capable of preventing or delaying onset of chronic diseases [1]. Antioxidants are constituents of foods that decrease adverse effects of reactive oxygen and nitrogen species, or both on normal physiological function in humans providing protection against cellular damage and inflammatory conditions. The major dietary antioxidants contributed by vegetables are vitamin C, vitamin E and carotenoids, flavonoids, phenolic acids and phenolic diterpenes [2,3].

Vegetables may be eaten raw or cooked, the latter a more common way of using large quantities of vegetables. Industrially, vegetables are minimally processed, frozen or canned for further processing at home, while at domestic level, there are several methods employed to cook them such as conventional cooking, steaming, microwave cooking, frying, or baking. Cooking softens the vegetable, removes bitterness and improves palatability. Cooking alters the compositional profile of vegetables. Both cooking and storage can affect the antioxidant components and activity. Many studies have shown that blanching/cooking can affect the antioxidant components of vegetables. However, studies report both beneficial and adverse effects. Thermal treatments may destroy phenolics or increase their bioavailability by releasing them from matrices and increasing their solubility [4-7].

As most common vegetables are consumed after cooking, it is important to know effect of cooking on antioxidant components and activity. Khachik et al. [8] reported that carotenoid content in green vegetables are affected when cooked. Bunea et al. [9] found that blanching improves carotenoids in vegetables as they are better extracted due to thermal treatment. A decrease in total phenolics of different vegetables on cooking has been reported [10,11]. Heat treatments lead to the destruction of the heat-labile vitamin, ascorbic acid, which imparts antioxidant activity to vegetables. The extent of positive, or negative, influence would depend on the type of vegetable, the cultivar, growing conditions, cooking conditions and storage variables. The present study was undertaken to determine the effect of cooking method and storage on antioxidant properties of four common vegetables.

MATERIALS AND METHODS

The study involved selection of vegetables, subjecting them to three different domestic cooking treatments and analyzing their antioxidant components and antioxidant activity. The effect of refrigerated and frozen storage on processed vegetables was also determined. The respective raw samples served as controls.

TABLE 1
Processing conditions and moisture content of samples

Sample	Raw weight (g)	Weight after cooking (g)	Time taken for cooking (min)	Water used for cooking (ml)	Moisture content (%)
Carrot					
Raw	-	-	-	-	88.4
Pressure cooking	150	140.9	15	23	90.5
Microwave cooking	150	138.1	7	60	89.5
Conventional cooking	150	146.1	14	65	91.7
Cabbage					
Raw	-	-	-	-	92.8
Pressure cooking	150	158.6	15	45	95.3
Microwave cooking	150	142.5	7	58	93.1
Conventional cooking	150	161.4	15	107	95.7
Pumpkin					
Raw	-	-	-	-	94.0
Pressure cooking	150	164.6	15	29	96.0
Microwave cooking	150	160.7	5	48	95.2
Conventional cooking	150	173.4	12	20	95.6
Cauliflower					
Raw	-	-	-	-	89.7
Pressure cooking	150	175.6	15	50	93.7
Microwave cooking	150	160.5	7	60	91.3
Conventional cooking	150	172.6	15.3	152	92.2

Materials . The vegetables used for the study were carrots (*Daucus carota,* subsp. sativus), cabbage (*Brassica oleracea* var. capitata), pumpkin (*Cucurbita maxima)* and cauliflower (*Brassica oleracea* var. botrytis). All vegetables were procured fresh from the local market, washed, cleaned, and cut. The chemicals used for the study were procured from E-Merck, Mumbai and Qualigens Fine Chemicals, Mumbai. The chemical, DPPH (1,1-diphenyl-2-picryl hydrazyl) was procured from Sigma-Aldrich Co., USA).

Methods. Processing of samples. The samples were subjected to pressure cooking (PC), microwave cooking (MWC) and boiling considered as conventional cooking (CC). Weight of the sample taken, water used for cooking and cooking time for each vegetable was recorded (Table 1). For pressure cooking, samples were placed in a container with water inside the pressure cooker and cooked for about 10 min. For microwave cooking, samples were placed in a glass container with water, covered lightly and cooked. For conventional cooking, vegetables were boiled with water in a vessel covered with a lid. The cooking time for each vegetable was standardized individually and criterion used was a desired soft texture when pressed between thumb and finger. Moisture content of all samples was determined, which was used for equalizing analyzed constituents and activity for each sample.[For estimating moisture, weighed sample was placed in a hot air oven maintained at 110ºC±10ºC for 3 h, cooled to room temperature in a desiccator and weight loss determined [12]. Raw and cooked samples were analyzed for effect of cooking on the antioxidant components and antioxidant activity on first day.

Storage Study. The cooked and raw samples were packed in polyethylene bags and stored (i) under refrigeration (5°C) for 7 days and (ii) in freezer (-18°C) for 40 days and the analysis for antioxidant properties repeated.

Analysis. Preparation of sample extract. To conduct various analyses, a 30 g portion of cooked sample was homogenized in 60 ml of water. The mixture was passed through a filter paper and centrifuged at 3000 rpm for 10 min. The supernatant was again filtered using Whatman no. 1 filter paper and the filtrate used for analyses.

Antioxidant components . i). Total phenolic compounds were measured using Folin-Ciocalteau reagent. A 0.2 ml of sample was mixed with 1.0 ml of Folin–Ciocalteau reagent and 0.8 ml of Na_2CO_3. The volume was made up to 10 ml using water:methanol (4:6), allowed to stand for 30 min and absorbance read at 740nm using spectrophotometer [13]. Tannic acid was used as standard and the results expressed as tannic acid equivalents /100 g sample.

ii). Tannins were determined colorimetrically based on measurement of blue colour formed by reduction of phosphortungstomolybdic acid by tannin like compounds in alkaline solution. A 0 to 10 ml

aliquots of the standard tannic acid solution were pipetted into 100 ml volumetric flasks containing 75 ml of water. A 5.0 ml Folin-Denis reagent and 10 ml Na_2CO_3 solution were added into each volumetric flask and made up to 100 ml with water. For the test sample, an aliquot of the filtrate containing not more than 0.1 mg of tannic acid was used and treated similarly. The colour was measured after 30 min at 760nm against a blank adjusted to 0 absorbency. Results were expressed as mg tannic acid equivalent/100g sample [14].

iii). Total and β-carotene were estimated by extracting in acetone and transfer to petroleum ether phase. Total carotene was read colorimetrically using petroleum ether for baseline correction. The β-carotene was separated by column chromatography and read colorimetrically [15].

iv). Ascorbic acid was estimated with 2, 6-dichlorophenol indophenol visual titration based on the reduction of dye colour from blue to pale pink by ascorbic acid [15].

Antioxidant activity. i). Total antioxidant activity was determined by the phosphomolybdenum method which is based on reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of green phosphate/ Mo (V) complex at acidic pH [16]. An aliquot of 0.1 ml sample was combined with 1.0 ml of reagent solution. Tubes were capped and incubated at 95^0C for 90 min. After cooling to room temperature, absorbance was measured at 695nm against a blank. The total antioxidant activity was expressed as equivalents of ascorbic acid (mmoles/g of sample).

ii). Free radical scavenging activity (FRSA) was determined with DPPH, a commercial oxidizing radical which is reduced by antioxidants. The disappearance of the DPPH radical absorption at a characteristic wavelength is monitored by decrease in optical density [17]. Different concentrations of the extract were placed in test tubes and volumes made up to 1.0 ml with methanol. A 4.0 ml of 0.1 mM methanolic solution of DPPH was added and the volume was made up to 5.0 ml. Tubes were shaken vigorously and allowed to stand for 20 min at room temperature. The control was prepared as above without any sample and methyl alcohol was used for base line correction. Changes in absorbance of sample were measured at 517nm. Free radical scavenging activity was expressed as inhibition percent.

iii). Reducing Power was determined with reduction of Fe^{3+}/ ferricyanide complex to the ferrous form by antioxidants. The Fe^{3+} formed is monitored by measuring the formation of Perl's Prussian blue at 700nm [18]. Different amounts of extracts in 1.0 ml of distilled water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mix was incubated at 50°C for 20 min, 2.5 ml of 10% TCA was added and centrifuged at 3000 rpm for 10 min. An upper layer of the solution (2.5 ml) was mixed with equal amount of distilled water and 0.5 ml of ferric chloride and absorbance measured. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical Analysis. Mean and standard deviations were computed from raw data. Student's T-test was used to test the statistically significant differences between the cooking methods and storage. To determine effect of cooking, the cooked samples were compared with the raw sample. And for effect of storage, the stored samples were compared with the respective first day counterpart, raw vs raw and cooked vs cooked. Probability level was fixed at 5%.

RESULTS AND DISCUSSION

The results of each parameter analyzed are discussed under two categories, namely (i) effect of cooking and (ii) effect of storage. For the effect of cooking, the cooked samples are compared with raw sample (both fresh and stored) and for effect of storage, the comparison is made between the initial (1^{st}) day respective controls (raw vs raw and cooked vs cooked).

Effect of cooking and storage on phenolic compounds and tannins. Effect of cooking and storage on total phenols and tannin content of vegetables is compiled in Table 2. Phenolic compounds are secondary plant metabolites which account for majority of the antioxidant activity in fruits and vegetables. Redox property of polyphenols along with the ability to donate hydrogen and singlet oxygen quenching makes them potent antioxidants. Processing conditions can modify the phenolic content in foods to varying extent depending upon the type of treatment. As given in Table 2, cooking reduced the total phenols in PC and CC cabbage samples, however, MWC sample showed higher content than the raw counterpart, though the difference was not significant. The CC sample recorded a significant decrease of 59% in comparison to raw. On storage in fridge, (4°C), while no change was seen in the fresh sample, all cooked samples showed significant decrease after a week. On frozen storage, processed samples were similar to uncooked sample as the latter itself had a decreased phenolic content. Carrot showed least total phenols content among all analyzed vegetables, however, a higher marginally significant content of total phenols could be estimated in all cooked carrot samples, the increase ranging from 48-124% over the uncooked counterpart. This could be because of release of phenolic constituents

TABLE 2
Effect of cooking and storage on total phenols and tannin content of vegetables (mg/100g)

Storage Days	Raw		Pressure cooked		Microwave cooked		Conventionally cooked	
	1	2	3	4	5	6	7	8
Total Phenols								
Cabbage								
1	21.00±0.84	a	14.80±1.10	b	22.73±2.81	a	12.41±0.00	b
8	20.62±0.53 ^{ns}	a	11.93±1.15 ^{ns}	bc	15.32±0.57 ^{ns}	b	9.25±0.40**	c
40	9.41±1.18**	a	10.71±0.22*	a	15.49±1.94^{ns}	a	7.47±0.17***	a
Carrot								
1	5.65 ±0.70	b	9.47±0.69	a	8.29±0.55	a	12.53±1.90	a
8	5.24±0.06*	a	4.68±0.01*	a	4.35±0.39*	a	3.85±0.01*	b
40	6.40±1.13^{ns}	a	6.25±0.02*	a	8.79±0.97^{ns}	a	5.59±0.82*	b
Cauliflower								
1	24.02±0.22	a	19.06±0.58	b	15.03±0.14	c	16.51±1.44	bc
8	21.41±0.69*	a	10.27±0.45**	c	14.14±1.88^{ns}	b	10.80±0.28*	c
40	21.87±0.30*	a	18.54±1.66^{ns}	a	22.24±0.01***	a	17.14±1.91^{ns}	a
Pumpkin								
1	28.20±0.49	a	23.43±0.28	b	24.21±0.14	b	16.08±1.11	c
8	23.50±1.06*	a	12.01±0.54**	c	18.41±0.42**	b	14.37±1.73^{ns}	bc
40	11.65±0.92**	b	11.32±0.97**	b	16.04±0.28***	a	15.34±0.21^{ns}	a
Tannins								
Cabbage								
1	29.45±0.20	b	18.95±0.16	c	33.94±0.55	a	14.60±0.70	d
8	27.75±0.84^{ns}	a	12.44±0.33**	c	16.62±0.30***	b	9.95±0.41*	d
40	11.78±0.03***	b	11.35±0.23***	b	15.48±0.24***	a	6.04±0.03**	c
Carrot								
1	14.40±0.01	a	13.67±0.01	b	7.90±0.04	c	5.40±0.02	d
8	12.40±0.03***	a	6.25±0.03*	b	7.90±0.03 ^{ns}	b	5.40±0.06 ^{ns}	c
40	8.80±0.4**	a	7.81±0.78*	a	9.28±0.28^{ns}	a	4.36±0.26*	b
Cauliflower								
1	39.23±1.47	a	23.75±0.27	b	25.64±1.15	b	14.97±0.66	c
8	20.50±0.64**	a	13.68±0.15***	b	13.14±1.00**	b	11.36±0.38^{ns}	c
40	23.74±0.20**	b	16.76±1.33*	c	27.42±0.23^{ns}	a	21.49±0.15**	b
Pumpkin								

1	27.70±0.14	a	21.16±0.23	c	26.18±0.44	b	20.40±0.26	c
8	21.55±0.41**	a	15.27±0.21**	b	21.54±0.95*	a	14.94±0.70**	b
40	20.80±0.71**	ab	12.91±0.21***	c	21.39±1.44*	a	17.57±1.39^{ns}	b

Statistical analysis: (i) Effect of storage:- Comparison of stored sample with the respective first day control in column 1, 3, 5 and 7 on application of 'T' test; *: P≤0.05,**: P≤0.01, ***: P≤0.001, ns: no significant difference.
(ii) Effect of cooking:- Different alphabets in column 2, 4, 6 and 8 indicate significant differences among differently cooked samples on application of ANOVA.

from the food matrix, as noted in many earlier studies. Cooking disrupts the matrix facilitating a higher estimation of phenolics [6,19]. Refrigerated storage of cooked samples showed a decrease in total phenols of all (significant for PC and CC samples), while frozen storage did not differ from raw control.

Cauliflower, *Brassica oleracea* is low in fat, high in dietary fiber, folate, water and vitamin C. As a member of the brassica family, cauliflower shares with broccoli and cabbage several phytochemicals which are beneficial to human health. Particularly, they have been found to be anti-mutagenic. In present study, the phenolic compounds in cauliflower exhibited significant cooking losses (range, 15.03 – 19.06 mg/100g) in comparison to raw samples (24.02 mg/100g). Storage of cooked samples in fridge showed reduction of phenolic content in comparison to raw, while there was no change in frozen storage. Ahmad & Ali [2] in their study on effect of heat treatments on phytochemical content of cauliflower also showed that water blanching and water cooking recorded highest loss of phenolic compounds. The losses were comparatively lesser in microwave cooking. The predominant phenolics identified in raw cauliflower as reported in their study were protocatechuic acid, quercetin, pyrogallol, vanillic acid, coumaric acid, and kaempferol. A study by Ali [20] on effect of cooking on bioactive components of cauliflower also reported that boiling process had a negative effect on phytochemical content.

Pumpkin, *Cucurbita maxima* is a gourd-like squash of the genus *Cucurbita* that are typically orange or yellow and have many creases running from the stem to the bottom. They have a thick shell on the outside, with seeds and pulp on the inside. They are a good source of β- carotene. It was observed that cooking reduced total phenol content in all samples of pumpkin (Table 2). The loss in MWC and PC pumpkin was around 15 and 17% of the raw counterpart respectively, while CC lost 43% of phenolics. This shows that conventional cooking had maximum negative effect on the phenolic content of pumpkin.

When effect of storage on phenolic content of cabbage was analyzed, all samples showed a decrease on refrigeration, the results being significant only for CC sample (74.5% of initial day). On frozen storage, raw sample exhibited a significant decrease (44.8% of initial value), PC, a marginal decrease, (72.4% of initial content) and CC, also a highly significant decrease (60.2% of initial). No change was observed in MWC sample. The decrease in phenolic content in cooked samples could be due to the breakdown of phenolics during cooking. In all the three cooked samples and in the raw cabbage, storage reduced the phenolics content with varying level of significance. For carrot, the effect of storage showed a marginal decline of total phenols in all samples on refrigeration and in PC and CC on freezing (66 and 44.6% of their respective controls). Raw and MWC samples exhibited an insignificant increase in comparison with their respective controls. In cauliflower, the changes seen in phenolic content on storage were as follows – the control sample showed a marginal reduction and cooked samples showed varying results. PC samples showed significantly lower values in refrigerated samples (53.9% of 1st day value) and there was no change in frozen sample after 40 days. MWC stored well in fridge with no loss and showed a significant increase on freezing (22.24 mg/100g). CC sample followed the pattern seen in PC variation. For pumpkin, all samples stored at 4^{o}C also had lower phenols in comparison to their respective controls. Storage for 40 days further decreased the phenolics in cooked and raw samples. Storage effect showed significant reduction in raw/PC/MWC samples in comparison to respective controls while the decrease in CC was non-significant. Thus, both cooking and storage reduced total phenols in pumpkin. A reduction in phenolic compounds on cooking is attributed to a breakdown of heat-labile phenolics or disruption of polyphenoloxidases causing a reduction in the phenolic acids [21,22].

The overall results can be summarized as follows – thermal treatment and refrigerated storage decreased phenolic compounds in cabbage, cauliflower and pumpkin irrespective of cooking methods. Carrot followed a different pattern showing an increase. For frozen storage, results were variable and the effect was marginal. Among cooking methods, losses were more in conventional cooking (or boiling) and lesser in microwave cooking. The results are similar to the observations of many authors who state that boiling causes maximum loss of phenolic contents in vegetables to varying extent [2,5,20].

The effect of cooking on tannins (compiled in Table 2) was as follows, on initial day, the tannin content of cooked cabbage enhanced significantly in MWC variation (by 15% over raw control) and deceased in PC and CC variations by 36 and 50% respectively. On storage, tannin consistently decreased

TABLE 3
Effect of cooking and storage on total carotenoids and β-carotene content of vegetables (mg/100g)

Storage Days	Raw		Pressure cooked		Microwave cooked		Conventionally cooked	
	1	2	3	4	5	6	7	8
Total Carotenoids								
Cabbage								
1	0.174±0.001	a	0.068±0.001	c	0.067±0.001	c	0.074±0.001	b
8	0.016±0.001***	a	0.006±0.001***	c	0.004±0.001***	d	0.01±0.001***	b
40	0.034±0.014***	a	0.021±0.003**	b	0.021±0.001***	b	0.02±0.001***	c
Carrot								
1	10.58 ±0.438	a	12.00±0.417	a	11.06±0.247	a	10.86±0.254	a
8	5.78±0.156**	a	5.86±0.106**	a	6.01±0.184**	a	6.29±0.226**	a
40	3.45±0.162**	a	3.53±0.077**	a	2.76±0.254***	a	3.05±0.078***	a
Cauliflower								
1	0.532±0.016	d	1.395±0.021	a	0.905±0.021	c	1.053±0.094	bc
8	0.099±0.016**	a	0.052±0.006***	a	0.032±0.006***	c	0.041±0.004**	b
40	0.025±0.005***	a	0.043±0.006***	a	0.037±0.005***	a	0.049±0.005**	a
Pumpkin								
1	0.993±0.002	c	1.00±0.059	b	0.90±0.004	d	1.299±0.003	a
8	0.102±0.009***	c	0.810±0.113*	a	0.414±0.009***	b	0.434±0.008***	b
40	0.142±0.004***	d	0.291±0.034**	c	0.498±0.098*	a	0.312±0.037***	b
β-carotene								
Carrot								
1	10.34 ±0.304	d	16.22±0.156	a	10.62±0.233	c	11.29±0.261	b
8	7.24±0.071**	a	7.66±0.156***	a	7.57±0.41*	a	7.82±0.24**	a
40	6.16±0.22**	a	4.78±0.28***	b	3.28±0.25**	c	5.76±0.24**	a
Pumpkin								
1	1.074±0.019	c	1.75±0.113	a	0.961±0.022	d	1.38±0.028	b
8	0.124±0.018***	c	0.869±0.021**	a	0.468±0.043**	b	0.458±0.033**	b
40	0.232±0.001***	c	0.275±0.065**	c	0.429±0.071**	a	0.303±0.051**	b

Footnote: as under Table 2.

in all samples, both raw and cooked. Carrot showed a decline in tannin content in all cooked samples on 1st, and 8th day, though on frozen storage, differences were lesser. The MWC sample showed an increase and PC and CC samples showed a decrease. A similar trend was also seen in cauliflower with a decline in tannin content of all cooked samples on 1st and 8th day and on 40th day, the MWC cauliflower showed a significant increase while others showed a decrease. In pumpkin, PC sample had decreased tannin on 1, 8th and 40th day; MWC only on 1st day, and CC on 1st and 8th day.

TABLE 4
Effect of cooking and storage on vitamin C content of vegetables (mg/100g)

Storage Days	Raw		Pressure cooked		Microwave cooked		Conventionally cooked	
	1	2	3	4	5	6	7	8
Cabbage								
1	41.04±1.16	a	18.19±0.572	c	26.95±0.403	b	15.36±0.679	d
8	22.35±0.636**	a	2.69±0.148***	b	2.73±0.056***	b	2.66±0.127***	b
40	2.01±0.07***	d	13.76±0.106**	a	6.13±0.445***	b	4.91±0.282**	c
Carrot								
1	7.60±0.424	a	5.03±0.381	b	7.49±0.551	a	7.38±0.219	a
8	7.19±0.84^{ns}	a	3.66±0.289 ^{ns}	b	4.99±0.162*	a	4.44±0.77*	a
40	5.82±0.226*	a	3.69±0.063*	b	4.86±0.396*	a	3.87±0.141**	b
Cauliflower								
1	75.00±2.83	a	21.65±2.62	d	42.70±2.69	c	37.75±2.76	bc
8	43.75±2.89**	a	12.15±1.91^{ns}	c	22.80±0.42**	c	19.10±0.99 *	b
40	14.83±1.63**	a	11.17±0.88*	a	12.71±0.381**	a	12.61±0.368**	a
Pumpkin								
1	6.80±0.707	a	3.57±0.431	b	5.73±0.353	a	3.72±0.24	b
8	6.33±0.24^{ns}	a	3.61±0.219 ^{ns}	b	5.30±0.261 ^{ns}	a	3.52±0.057 ^{ns}	b
40	5.49±0.233 ^{ns}	a	3.51±0.332 ^{ns}	b	5.36±0.311 ^{ns}	a	3.59±0.254 ^{ns}	b

Footnote: as under Table 2.

When effect of storage was analyzed, the tannin content showed a decline in all raw and PC samples both on 8th and 40th day. In MWC/CC sample, cabbage and pumpkin (8th and 40th day), and cauliflower (8th day) decreased. Carrot retained tannins on 8th day in MWC and CC sample and varied on frozen storage. Tannins generally decreased on cooking and storage, effect was lesser in MWC sample and more in PC and CC samples. As stated earlier, it has been observed that in microwave cooking loss of antioxidant components and activity is lesser in comparison to other cooking methods [2,4].

Effect of cooking and storage on total carotenoids, β-carotene and ascorbic acid. Carotenoids are natural pigments that are responsible for the bright colours of plants, flowers, fruits, and vegetables. Among all vegetables analyzed, cabbage had least carotenoids, which reduced further on cooking in all samples (Table 3). On storage also, all cooked ones had lesser carotenoids in comparison to raw. Carrots were richest in total carotenoids (range, 10.58-12.00 mg/100g), which were retained well in all cooked samples on storage too. In cauliflower, higher carotenoid could be estimated in cooked samples in comparison to raw, though on storage, a reverse trend was observed. Pumpkin is also very rich in carotenoid pigments, and both on cooking and storage, a higher amount could be estimated in comparison to respective raw controls. Podsedek [23] in her review on natural antioxidants and antioxidant capacity of brassica vegetables states that carrots were found to be richest sources of carotenoids, and white cabbage was reported to have a very low amount, which is similar to our findings.

When effect of storage was analyzed, all samples, both raw and stored showed a reduction in total carotenoids both on refrigerated and frozen storage, indicating fragility of carotenoid pigments to storage. The extent of reduction varied depending on the vegetable. Retention of carotenoids was better in refrigerated carrot (48.8-57.9%) compared to frozen carrot (25.00-32.6%). Cabbage had higher retention on freezing. In cauliflower, storage losses were maximum and pumpkin had higher carotenoids in PC and CC refrigerated samples.

β-carotene could be estimated only in carrots and pumpkin and followed similar trend as carotenoids (Table 3). The effect of cooking was marginal,

with slight changes, though on storage, there was a considerable reduction in β-carotene content of both vegetables. Among storage conditions, refrigerated samples retained more of β-carotene.

Vitamin C (ascorbic acid) is an essential nutrient for humans. It is a water-soluble vitamin, involved in many metabolic functions, such as biosynthesis of collagen. It is said to reduce levels of C-reactive protein (CRP), a marker of inflammation and possibly a predictor of heart disease [24]. Vitamin C functions as an enzyme cofactor, a radical scavenger, and as a donor/acceptor in electron transport at the plasma membrane. It is a very powerful antioxidant capable of scavenging the superoxide and hydroxyl radicals, as well as regenerating α-tocopherol [25].

Though vegetables are generally recognized as good sources of vitamin C, the process of cooking can partially destroy it as it is highly susceptible to high temperature. The extent of destruction is directly related to the time and temperature of cooking. As evident from Table 4, all cooked samples had lower level of vitamin C than the raw counterpart. On storing, there was a further loss of vitamin C. Among cooking methods, the loss in microwave cooking was lesser which can be associated with a lesser amount of time taken for cooking in microwave compared to other cooking methods. Among the vegetables analyzed, cabbage and cauliflower were better sources of vitamin C than carrot and pumpkin. Varying losses of vitamin C on blanching, cooking and storage of vegetables has been reported by many workers, the extent of losses depending upon the method of cooking and storage conditions [23].

Effect of cooking and storage on antioxidant activity of vegetables. Antioxidant activity of vegetable samples measured through total antioxidant assay is presented in Table 5. For cabbage, PC sample initially showed decreased activity, which retained better under refrigeration, and was lesser in frozen storage. MWC sample showed consistent higher value for all and CC was lower in comparison to raw. Carrot had highest total antioxidant activity among all vegetables (35588 mmols/g) on cooking and storage. Total antioxidant activity decreased in all samples and reduction was significantly more in PC/CC samples. In case of cauliflower, all cooked samples

TABLE 5
Effect of cooking and storage on total antioxidant activity of vegetables (mmoles/g)

Storage Days	Raw		Pressure cooked		Microwave cooked		Conventionally cooked	
	1	2	3	4	5	6	7	8
Cabbage								
1	17422±125	b	9987±54	c	21167±28	a	6665±138	d
8	7843±111***	c	8555±54**	b	9733±138***	a	5855±818*	d
40	10745±166***	a	7886±0***	b	11336±691**	a	5532±54**	c
Carrot								
1	35588±333	a	21806±880	c	26800±288	b	23920±936	bc
8	14814±97***	b	11588±217**	c	16630±315***	a	10196±76**	c
40	30363±69**	a	13915±13**	c	25212±397*	b	14031±107**	c
Cauliflower								
1	12745±28	a	7370±186	c	11202±68	b	6971±94	c
8	10696±236**	a	5683±78**	c	6925±68 ***	b	6990±13^{ns}	b
40	7559±69***	a	7229±93^{ns}	a	7070±136***	a	6484±432^{ns}	a
Pumpkin								
1	19725±2939	a	13452±433	b	13568±437	b	10361±680	bc
8	6912±125*	a	5399±2.5**	bc	6765±41.0**	a	6430±13.0*	b
40	15069±153^{ns}	a	6651±41**	c	9496±218**	b	8112±381^{ns}	b

Footnote: as under Table 2.

TABLE 6
Effect of cooking and storage on reducing power of vegetables
(Absorbance measured at 700 nm, 1.2 mg)

Storage Days	Raw		Pressure cooked		Microwave cooked		Conventionally cooked	
	1	2	3	4	5	6	7	8
Cabbage								
1	0.785±0.024	c	0.875±0.016	b	1.178±0.101	a	0.582±0.024	d
8	1.052±0.008**	a	0.638±0.045*	b	1.102±0.020^{ns}	a	0.423±0.005*	c
40	0.539±0.042*	b	0.617±0.030**	b	1.075±0.002^{ns}	a	0.387±0.001**	c
Carrot								
1	0.507±0.014	b	0.647±0.01	a	0.614±0.014	a	0.491±0.01	bc
8	0.621±0.002**	a	0.143±0.002***	d	0.359±0.011***	b	0.259±0.001***	c
40	0.223±0.004**	c	0.242±0.011***	bc	0.352±0.005***	a	0.252±0.002***	b
Cauliflower								
1	1.374±0.087	b	1.270±0.062	b	1.670±0.014	a	1.172±0.026	b
8	1.457±0.027*	a	0.864±0.027**	c	1.480±0.009***	a	1.082±0.041^{ns}	b
40	0.854±0.005***	a	0.806±0.009***	b	1.198±0.098***	a	0.898±0.070**	a
Pumpkin								
1	0.743±0.011	a	0.522±0.007	b	0.680±0.083	a	0.550±0.007	b
8	0.439±0.043^{ns}	a	0.279±0.001*	b	0.378±0.045^{ns}	a	0.348±0.006**	a
40	0.393±0.006*	a	0.210±0.002**	b	0.354±0.011*	a	0.242±0.006***	b

Footnote: as under Table 2.

had significantly lower antioxidant activity initially and under refrigerated storage. Freezing did not influence total antioxidant activity and it was similar to raw control (6484-7559 mmols/g). A reduction in total antioxidant activity of cauliflower was also reported by Ahmad & Ali [2] on blanching and cooking by different methods in comparison to raw sample.

For pumpkin, on day 1, the cooked samples had lesser total antioxidant activity than raw, though difference was significant only for CC. Stored samples also showed a reduction in total antioxidant activity of pumpkin. When effect of storage was considered in relation to their initial value, most of the vegetable showed lower activity in both storage methods with few exceptions (cauliflower, refrigerated CC and frozen PC/CC; pumpkin, raw and CC). The overall results show that total antioxidant activity reduced on cooking and storage. Among storage methods, frozen storage was better.

The antioxidant activity of vegetables measured through reducing power is presented in Table 6. Detailed figures for all the concentrations used for determining reducing power along with the related data is compiled in Supplementary file for reference. The effect of cooking on cabbage showed that on pressure and microwave cooking, the reducing power increased, whereas CC sample showed a decrease on first day as well as on storage in comparison to raw control. In PC and MWC samples results varied on storage, with a significant decrease in PC refrigerated sample, and an increase in MWC frozen sample. In carrot, on day 1 trend was similar to cabbage, on refrigerated storage, a significant decrease and on frozen storage an increase was seen in all. In cauliflower, PC and MWC were consistently lower on cooking and on storage too, MWC followed a different trend with higher activity. In pumpkin, all cooked samples had a lower range of reducing power in comparison to respective raw control, though differences were non-significant for MWC samples.

On storage, raw refrigerated cabbage, showed significantly higher reducing power, PC and CC samples were lower and MWC remained same. In carrot, all cooked samples showed decreased reducing power on storage, while raw, refrigerated sample had higher reducing power. Cauliflower followed a trend similar to cabbage with MWC sample showing

higher reducing power than raw control. In pumpkin all stored samples, both on 8th and 40th day, had less antioxidant activity when measured through reducing power. The overall results show that storage was detrimental to antioxidant activity and among cooking methods, microwave cooking retained better activity.

The antioxidant activity measured through FRSA showed that on cooking cabbage samples exhibited increased FRSA, though on storage for 7 days, PC and CC sample showed a decrease while MWC was more in comparison to raw (Table 7). [Detailed figures for all the vegetables showing FRSA are given in Supplementary file along with data for reference.] On frozen storage, cooked samples had higher activity than the raw control. Carrot samples also showed an increased FRSA, in all cooked samples initially and on storage. Cauliflower was higher in cooked sample initially, though on storage it decreased and on frozen storage, there was a significant decrease in all, both in raw and cooked samples. For pumpkin, on cooking, all samples showed higher FRSA, though on storage, all samples decreased in antioxidant activity (with exception of MWC sample on 8th day).

On storage, in cabbage, under refrigeration, there was a significant decrease in all cooked samples. On freezing, raw, PC and CC samples showed a reduction, though to lesser extent, while MWC sample showed an increase. In carrot, there was an increase in raw and PC, while others decreased. In cauliflower, on refrigeration, cooked sample showed a decrease, and in raw sample, there was an increase. However, under frozen storage, all samples showed a drastic reduction in FRSA. In pumpkin, a decrease in FRSA of all samples was observed during storage to varying extent. Overall, it can be said that, while cooking increased the FRSA in all vegetables, storage showed a decreased the activity.

TABLE 7
Effect of cooking and storage on free radical scavenging activity of vegetables (percent, concentration, 0.8 mg)

Storage Days	Raw		Pressure cooked		Microwave cooked		Conventionally cooked	
	1	2	3	4	5	6	7	8
Cabbage								
1	78.6±8.76	b	141.57±0.275	a	158.50±0.282	a	85.06±0.516	b
8	75.55±0.008^{ns}	b	29.63±0.48***	c	103.64±4.143**	a	28.48±2.41***	c
40	43.57±0.742*	c	88.26±2.50**	b	172.8±2.55*	a	50.45±2.17**	c
Carrot								
1	24.23±0.22	c	51.36±0.57	b	65.84±1.40	a	41.77±2.09	b
8	32.84±0.8**	b	55.38±8.14 ^{ns}	b a	60.23±0.36*	a	37.45±2.52 ^{ns}	b
40	33.9±3.95 ^{ns}	b	53.12±2.26 ^{ns}	a	58.89±5.51 ^{ns}	a	32.95±2.23 ^{ns}	b
Cauliflower								
1	43.31±0.565	b	68.20±2.573	a	62.89±0.014	a	45.49±4.157	b
8	56.77±3.429*	a	34.11±1.590**	b	53.53±0.395***	a	31.91±2.941^{ns}	b
40	4.51±0.155***	b	3.11±0.098***	c	8.96±0.700***	a	8.23±014**	a
Pumpkin								
1	44.15±2.651	b	48.98±2.453	b	64.65±4.115	a	54.06±0.657	a
8	40.01±0.296 ^{ns}	b	33.53±0.579*	b	58.84±3.691 ^{ns}	a	16.42±4.454**	c
40	23.40±1.767*	a	8.48±0.692**	b	15.58±5.918*	a	17.14±0.968***	a

Footnote: as under Table 2.

TABLE 8
Correlation analysis between antioxidant components and antioxidant activity of vegetables (R value)

Antioxidant assay	Antioxidant components				
	Total phenols	Tannins	Carotenoids	β-carotene	Vitamin C
Cabbage					
Total antioxidant activity	0.752	0.801	0.608	-	0.653
Reducing power	0.802	0.734	0.078	-	0.364
DPPH Assay	0.556	0.488	0.192	-	0.327
Carrot					
Total antioxidant activity	0.432	0.492	0.392	0.260	0.657
Reducing power	0.412	0.695	0.764	0.668	0.785
DPPH Assay	0.207	-0.264	0.049	0.003	-0.25
Cauliflower					
Total antioxidant activity	0.493	0.722	0.271	-	0.889
Reducing power	0.076	0.276	0.442	-	0.662
DPPH Assay	-0.175	0.026	0.654	-	0.538
Pumpkin					
Total antioxidant activity	0.559	0.753	0.408	0.531	0.436
Reducing power	0.819	0.869	0.622	0.601	0.573
DPPH Assay	0.646	0.661	0.588	0.590	0.344

It has been reported that varying levels of antioxidant activities observed in vegetables on cooking and storage are related to their lipophilic or hydrophilic nature of components [23]. Differences are also seen depending on the genotype of vegetables. Hence, multiple assays are generally recommended and are said to be better indicators to assess the antioxidant activity of any food. Our earlier studies indicate that storage had a detrimental effect of antioxidant components and activity in legumes and amaranth green leaves mixes used as food model. Storage of mix in dehydrated form at ambient temperature was more detrimental than frozen storage in wet form [26,27]. In another study, cookies formulated with 4% of carrot pomace and stored retained 75% of total carotenoids and 69% of β- carotene [28]. In contrast, *Citrus aurentium*, a fruit processed into preserved products such as pickles and preserve exhibited increased antioxidant activity on storage [29].

Correlation analysis between antioxidant components and antioxidant activity of vegetables . Since the vegetables analyzed exhibited varying levels of antioxidant activity with different assays, an attempt was made to correlate the antioxidant activity with the analyzed antioxidant components and results are presented in Table 8. The R values indicate that in case of cabbage, all three assays showed positive correlation with total phenols, tannins and vitamin C. Carotenoids correlated well only with total antioxidant activity. The antioxidant components of carrots correlated well with total antioxidant activity and reducing power while DPPH showed a very poor correlation. For cauliflower a better correlation was observed between total phenols and tannins with total antioxidant assay, while carotenoids and vitamin C showed a positive correlation with all assays. In case of pumpkin a high correlation could be seen among all assays with all the components analyzed.

CONCLUSION

The overall observations of the study can be summarized as follows – In all vegetables, there was a reduction in total phenols and tannins on cooking to varying degrees. Among cooking methods, micro-

wave cooking was better than others, and conventional boiling was least favorable. On storage, also significant losses were seen in all, the effect being more on refrigeration than freezing. Carotenoids and β-carotene behaved differently on cooking, wherein many samples showed an enhanced content in cooked sample, however, on storage, there was a significant decline. Ascorbic acid declined both on cooking and storage in all. The antioxidant activity differed according to vegetable. The total antioxidant activity decreased for all vegetables on cooking and storage. It correlated well with the antioxidant components of vegetables. The antioxidant activity measured using reducing power showed different results among vegetables, PC and MWC cabbage and carrot showed an increase while pumpkin showed a decrease in all. Reducing power also decreased in all vegetables on storage, though it correlated well with the antioxidant components. FRSA increased on cooking, though storage decreased it. Better correlation was observed between FRSA and antioxidant components of cabbage and pumpkin. In conclusion, thermal treatments of cabbage, carrot, cauliflower and pumpkin resulted in reduction of total phenols, tannins and ascorbic acid, while carotenoids and β-carotene were retained well. Refrigeration and freezing caused further losses of these components. Both raw and cooked vegetables showed high antioxidant activity which could be correlated well with their antioxidant components.

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AUTHOR INDEX

SUBJECT INDEX

AFS– GUIDE FOR AUTHORS

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