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THE INFLUENCE OF DIFFERENT LIGHT MODES ON CUCUMBER (*CUCUMIS SATIVUS L.*) SEEDLING PERFORMANCE

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ABSTRACT

In this experiment, the effect of light modes was evaluated on morphological parameters, chlorophyll contents and biochemical contents of cucumber seedlings *(Cucumis sativus L.)* grown under controlled and protected condition. The study was laid out using orthogonal design involving a combination of; light intensity, the ratio of LEDs and photoperiod (Light and Dark) The treatments consisted of three light intensity regimes; 80, 100, and 150 $\mu mol.m^{-2}.s^{-1}$ PPFD, provided by light-emitting diodes (LEDs) composed of LEDs values ranging from Red and Blue LEDs (R:B) 30:70, 50:50, and 70:30 with three different photoperiods of 10/14h, 12/12h, and 14/10 (light/dark). White florescent light was used as a control. From the results obtained, plant length, hypocotyls length, stem diameter, leaf area and soluble sugar content were highest when exposed to light mode of LM9, while the lowest values for the above parameters were obtained under LM1. Higher nitrate and soluble protein contents were obtained with LM3. The results showed that growing cucumber under high light intensity (150 $\mu mol.m^{-2}.s^{-1}$ PPFD) in the combination of Red and Blue LEDs (70:30) under the regime of 12/12 (light/dark) produced a significant increase in growth as well as a higher photosynthetic capacity in the plants.

KEYWORDS:
LED light qualities, Intensities light, Photoperiod, Chlorophyll contents, Biochemical content, Cucumber seedling.

INTRODUCTION

Photosynthetic lighting suitable for indoor cultivation in controlled environments has boosted crop productivity in densely populated areas, space missions, and bio-regenerative life support facilities [1]. The spectral properties of electrical light sources must meet the physiological requirements of plants for photosynthesis and photomorphogenic development [2]. However, the distribution and variations in the range of conventional light sources used (fluorescent tubes, sodium lamps, and metal halide) are fixed and may not be ideal for different crop species' light requirements. LEDs have emerged and have great potential for horticultural lighting due to energy efficiency, longevity, and flexibility of their application [3]. Moreover, LEDs are becoming more suitable in research and the commercial agriculture under controlled conditions due to their low radiation, heat and wide spectral adaptation [4].

Brown, Schuerger [5], and Tennessen, Singsaas [6] have opined that, when growing conditions were supplied with LEDs and modified to emit light photons at certain wavelengths, plant light requirement research can determine the various functions of light properties in terms of spectral irradiance. It is worth noting that, LEDs provides the ideal spectral distribution range that promotes plant growth with optimum life span and light energy efficiency [7]. During changes from the conventional to LEDs light sources, numerous inventions were experienced, such as combining fluorescent and LED lights [8], replacing fluorescent light tubes with LEDs [9] and retrofit LEDs for fluorescent fixtures devoid of ballast [10]. Many scientists have tried to explain the impact of light on growth, development, morphology and photosynthesis in different plants [11-14]. Combining LEDs light was observed to strongly effect the physiological and developmental processes of plants [15-19]. Many scientists [18, 20-24], have reported varying reports on crops grown under different intensity of illumination that gave insight on the growth and development of plants, as well as their photoperiodic requirements [18, 25].

In the present study, we had 3 factors with 3 levels L9 (3^3) are shown in Table 1. We need 27 treatments to test all possible combinations. Therefore, the Orthogonal Experimental design method was used to make specific test arrangements. We used only 9 treatments + control, these nine treatments represent all 27 treatments and that reduced the workload to one-third. Thus, the intents of this

study are conducted to determine the best-expected treatment for the effect of combined intensities, qualities, and the photoperiod of LEDs on the growth, pigments, and biochemical contents of cucumber (*Cucumis sativus var. Building no. 4*), and compare them with white fluorescent light.

MATERIAL AND METHODS

Growth conditions and Plant materials. All experiments were conducted in LED light chambers at Fujian Agriculture and Forestry University, Fuzhou. The experimental system included 10 chambers; each had a dimension of 60 x 60 x 60 cm. The details of growth conditions and LEDs light are shown in Table 1 and Figure 1. The manufacturer of the tested LED lamps is Kedao Technology Corporation (Huizhou, China) with the type of UH-BLDT0510. Cucumber (*Cucumis sativus var. Built No. 4*) "黄瓜建着 4 号" seeds were sown in 32-cell plug trays (W 2.6 cm × L 2.6 cm ×H 4.3cm/cell) that was fill with commercial growing substrate (N_1:P_1:K_1 ≥ 3%, Organic matter≥ 45% pH 5.5-6.5). Ten days after planting, the germinated seedlings were transferred to pots (W 10 cm × L 10 cm ×H 8.5 cm), and were left there for 20 days. In total, 20 seedlings were sown in each growth box. Irrigation was provided for the seedlings daily or as required. One week after sowing, seedlings started to receive fertilization based on water-soluble fertilizers (compound fertilizers "N20: P20: K20+TE", Ruierkang Co., Russia, and Stimufol Amino (compound fertilizers "N 25%, P 16%, K 12%, Amino acids 2%, Boron 0.044%, Fe 0.17%, Molybdenum 0.001%, Zink 0.03%, Copper 0.085, Cobalt 0.01%, Mg 0.02%, Manga 0.085% and EDTA" Shoura Co., Egypt.) two times per week through irrigation.

Multiple- factor experiment design. Table 1 shown the multiple-factor experimental regular fractional design, L9 (3^3) was used, i.e. 3 levels were chosen for each of the 3 improvement criteria and 9 tests from all possible combinations. When considering the technical feasibility of the advanced LED lighting unit, the parameters for improving the lighting system in the factory were chosen at the following levels:

A. The intensities of LEDs light averaged over the whole time of plant growing period, PPFD (A1-A3): 80, 100, 150 $\mu mol.m^{-2}s^{-1}$;

B. The ratio of PPDD values from Red and Blue LEDs (B1-B3): (R:B) =30:70, 50:50, and70:30.

C. The light period during Day/ Night (C1-C3): 10/14h, 12/12h, and 14/10.

Measurements and calculations. Measurement of Growth and biomass parameters. Growth parameters were estimated 30 days after planting. Measurement of plant height was taken from the base of the rhizome to the top of the plant using a ruler (cm). Stem diameter was measured using digital calipers (mm) and the fresh and dry mass was weighed using an electronic balance (0.0001 g). Total leaf area (cm^2) (summation of leaf areas) was estimated as described by Pandey and Singh [26]. Fresh shoots and roots were put in Petri dishes without cover and transferred to a drying oven at 75 °C for at least 48 h to obtain the dry weight.

TABLE 1
Parameters of the LED light properties used in the study

Treatments	Photon flux density ($\mu mol.m^{-2}.s^{-1}$)	Light spectral ratios Red: Blue	Photoperiod Light/Dark	Peak wave length λp (nm)	layout of the L9 (3^3) matrix		
					A	B	C
LM1	80±2	R30:B70	10h /14h	660: 460	1	1	1
LM2	80±2	R50:B50	12h /12h	660: 460	1	2	2
LM3	80±2	R70:B30	14h /10h	660: 460	1	3	3
LM4	100±2	R30:B70	12h /12h	660: 460	2	1	2
LM5	100±2	R50:B50	14h /10h	660: 460	2	2	3
LM6	100±2	R70:B30	10h /14h	660: 460	2	3	1
LM7	150±2	R30:B70	14h /10h	660: 460	3	1	3
LM8	150±2	R50:B50	10h /14h	660: 460	3	2	1
LM9	150±2	R70:B30	12h /12h	660: 460	3	3	2
CK	115±2	---	12h /12h	544	------	------	------

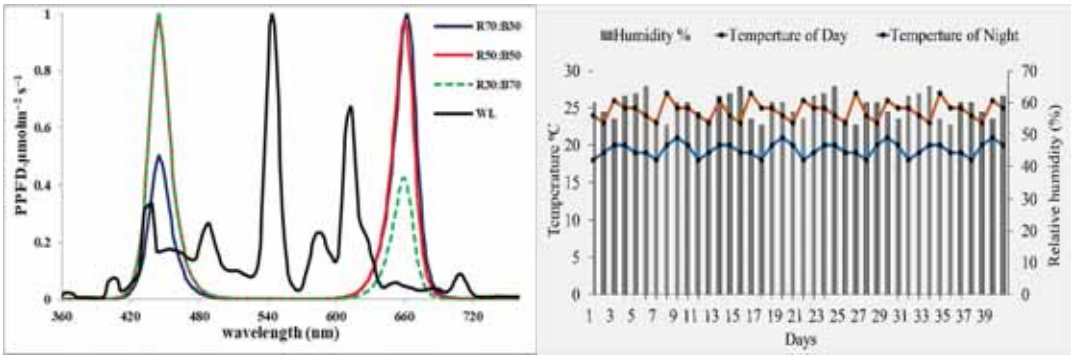

FIGURE 1
Spectrum distribution of the treatments light and control in the experiment.

Measurement of Chlorophyll Content. The chlorophyll contents were determined from fresh leaves of the cucumber plants in each of the treatments after 45 days of transplanting. Tissues of fresh leaves (0.2 g) were removed, ground well then used in 5 ml of 95% ethanol and filtered, the filtrate was made up to 25 ml by adding 95% ethanol. Absorbance of the filtered solution at 665 nm (OD665), 649nm (OD649) and 470 nm (OD470) was measured using a spectrophotometer (UV-5100B, Unico. Shanghai, China) while the chlorophyll content was determined using the equations below [27]:

Chl-a (mg/g) = $(13.95OD_{665}-6.88OD_{649})V/200$ W.

Chl-b (mg/g) = $(24.96OD_{649}-7.32OD_{663})V/200W$.

C (mg/g) = $(1000OD_{470}-2.05$Chl a-114.80Chl b$)$ V/ (245×200 W).

Where (Chl-a) = chlorophyll-a, (Chl-b) = chlorophyll-b, (C) = carotenoid, mg/g; (V) = volume (25 ml.) and (W) = sample weight (mg).

Measurement of Biochemical Contents. To measure the biochemical contents, fresh leaves were chopped into small pieces and fresh samples weighed (0.5g, 0.2g, and 0.5g) for nitrate, protein, and sugar respectively. they were used to determine the content of soluble nitrate [28]. The soluble protein content was evaluated using coomassie brilliant blue G250 method [29]. Also, the content of soluble sugar was evaluated using the anthrone colorimetric method [30]. The absorbance of the solution extracted was estimated at 410nm (OD410), 630nm (OD630) and 595 nm (OD595) using a UV-5100B spectrophotometer (Unico, Shanghai, China). The biochemical contents were expressed using the following equations:

Soluble nitrate content ($mg.kg^{-1}$ FW) =(C×VT)/ (W×VS).

Soluble protein content ($mg.g^{-1}$) =(C×VT)/ (VS×W×1000).

Soluble sugar content (%) =(C/VS×VT)/ (W×106)×100.

Where C = nitrite (µg/ml)/ sugar (%)/ protein ($mg.g^{-1}$) value from the standard curve, VT = total volume of samples extracted (ml), VS = taken sample solution (ml), and W = fresh leaf weight (g).

Statistical analysis. The Orthogonal Experimental design method was used to determine the number of experiments to be conducted. All the data were subjected to one-way analysis of variance (ANOVA). Duncan's multiple range tests [31] was used to test the significant difference between the means at 0.05 significance level using SPSS software (Version 16 SPSS Inc. Chicago, Illinois).

RESULTS

Growth parameters. Tables 2 showed the effect of mode of LED light on plant morphology and growth characteristics of cucumber seedlings. We can see from these Tables that light ratios with LEDs had significant effects on morphological appearances of cucumber seedlings (Table 2). The plant height, stem diameter, total leaf area, hypocotyl length, fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight were significantly higher under LM9 treatment than other treatments which were (12.4cm), (0.84 mm), ($192cm^2$), (8.43cm), (12.1g.), (1.79 g.), (2.1g.), and (0.143mg.) respectively, while the lowest value was for all of them were observed under LM1. Water content of plants irradiated with LM1 (92.4%) was significantly larger than those under the other mode of LEDs light which were values do not differ statistically with LM2 and LM3.

Chlorophyll and carotenoid contents. The contents of chlorophyll and carotenoid in leaves of cucumber seedlings under different mode of LEDs light were shown in Table 3 and they appeared to have significant differences. Compared with WL treatment, the content of Chl-a, Chl-b and carotenoid in leaves of cucumber seedlings (Table 3) with LM9 was higher than those with the other modes of LEDs light which were values do not differ statistically with LM7 and LM8, while that of LM1 mode showed the lowest Chl-a, Chl-b and carotenoid.

TABLE 2
Effect of modes LED light on plant morphology and growth characteristics of cucumber seedlings

Treat-ments	Plant height (cm)	Stem di-ameter (mm)	Total leaf area (cm^2)	Hypo-cotyl length (cm)	Fresh weight		Dry weight		Water content %
					Shoot (g.)	Root (g.)	Shoot (g.)	Root (mg.)	
LM1	6.3 ±0.55c	0.3±0.03 c	88 ±2.08c	2.76 ±0.12c	5.3±0.32 c	0.72±0. 01c	0.73 ±0.09c	0.06±0.0 1c	92.4 ±0.93a
LM2	6.6 ±0.20c	0.4±0.06 c	101 ±4.93bc	3.63 ±0.17c	6±0.32c	0.83±0. 02c	0.83±0.09 c	0.07±0.0 1c	89.8 ±0.47a
LM3	7±0.47c	0.46±0.0 6c	109 ±1.86bc	4±0.45c	6.3±0.26 c	0.85± 0.04c	0.88±0.03 c	0.07± 0.02c	88.5±0.61 a
LM4	7 .3 ±0.44bc	0.53±0.0 2b	116 ±3.84b	4.66 ±0.09c	6.7±0.18 c	0.9±0.0 3bc	0.96±0.03 c	0.09 ±0.01bc	85.8 ±1.18b
LM5	8.5 ±0.29bc	0.55±0.0 2b	126 ±1.53b	5±0.36bc	8.5±0.57 bc	0.97±0. 02bc	1.3±0.10b c	0.095±0. 01bc	84.5 ±1.62b
LM6	9.3±0.3 7bc	0.57±0.0 1b	133 ±0.88b	5.6 ±0.61bc	8.8±0.12 bc	1.05 ±0.24bc	1.33±0.09 bc	0.099±0. 01bc	83±1.52b
LM7	10±0.3 0ab	0.73±0.0 3a	155 ±2.60a	6.7 ±0.35ab	10.9±0.3 0a	1.48±0. 07ab	1.7±0.07a	0.126±0. 02a	81.6 ±2.42c
LM8	12±0.5 8a	0.81±0.0 3a	171 ±1.53a	7.7±0.47 a	11.4±0.5 8a	1.71±0. 07a	1.9±0.001 a	0.129 ±0.03a	81 ±2.04c
LM9	12.4 ±0.75a	0.84±0.0 1a	192 ±1.20a	8.43 ±0.35a	12.1±0.5 2a	1.79±0. 10a	2.1±0.20a	0.143 ±0.01a	80.7 ±1.19c
WL	6.7 ±0.15c	0.55±0.0 2b	122 ±1.45b	3.09 ±0.36c	6.8±0.22 c	0.9±0.0 2c	0.9±0.06c	0.088 ±0.03bc	85±1.83b

Values are means of three replicates, Different letters in the same column indicate significant differences according to Duncan's multiple range test at $P \leq 0.05$

TABLE 3
Effect of modes LED light on chlorophyll-a, chlorophyll-b and carotenoid content in cucumber seedlings

Treatment	Chlorophyll a ($mg \cdot g^{-1}$)	Chlorophyll b ($mg \cdot g^{-1}$)	Carotenoid ($mg \cdot g^{-1}$)
LM1	0.63 ±0.04c	1 ±0.03c	4.1 ±0.29c
LM2	0.73 ±0.16c	1.17 ±0.15c	4.14 ±0.88c
LM3	0.77 ±0.33c	1.4 ±0.12c	4.41 ±0.11c
LM4	0.88±0.29bc	1.85 ±0.55bc	5.9 ±1. 63b
LM5	0.94±0.05bc	1.95 ±0.06bc	6.2 ± 0.40b
LM6	0.98±0.20bc	2 ±0.15bc	6.88 ±0.72b
LM7	1.33 ±0.07a	2.55 ±0.03a	8.91 ±0.82a
LM8	1.44±0.32a	3 ±0.70a	9.51 ±1.34a
LM9	1.55±0.11a	3.11 ±0.13a	10.3 ±1.68a
WL	0.77 ±0.07c	1.6±0.20c	4.99±1.13c

Values are means of four replicates ± SE, Different letters in the same column indicate significant differences according to Duncan's multiple range test at $P \leq 0.05$.

TABLE 4
Effect of LED light ratios on biochemical contents in tomato seedlings (Jerry 201)

Nitrate content (mg.kg^{-1}FW)	Soluble protein content (mg.g^{-1}FW)	Soluble sugar content (%FW)
1100±28.87c	10.2±0.12c	2.50±0.06g
1300±28.87b	11.0±0.12b	2.70±0.12fg
1420±11.55a	12.0±0.12a	3.00±0.12f
860±34.64e	7.5±0.29ef	3.80±0.12de
940±23.09de	7.8±0.29e	4.20±0.12cd
1000±23.09d	8.4±0.23d	4.50±0.17c
580±34.64g	4.8±0.23h	5.57±0.26b
650±28.87fg	5.2±0.12h	6.20±0.12a
700±17.32f	6.0±0.17g	6.40±0.23a
960±34.64d	7.0±0.06f	3.60±0.17e

Values are means of four replicates ± SE, Different letters in the same column indicate significant differences according to Duncan's multiple range test at $P \leq 0.05$.

Biochemical contents. Table 4 presents the contents of nitrate, soluble protein, and soluble sugar of cucumber seedlings under different LED light modes. LM3 gave the highest nitrate and soluble protein contents; this shows the importance of red light in improving nitrate and soluble protein content. Also, LM9 exhibited the highest accumulation of soluble sugar in the cucumber seedlings (6.40 %FW).

DISCUSSION

For proper growth and development, plants are grown under constantly changing light environments. Some light wavelengths are critical for plant growth and development. Plants are observed to detect the subtle changes in light quality by light receptors. These light receptors can initiate signal transduction using different approaches thereby changing the appearance of plants [32-35]. It has been observed that the photosynthetic active radiation (PAR, 400–700 nm) has a direct role in the photosynthetic processes of plants. Red light in the range of 610–700 nm and blue at 425–490 nm is the optimum light spectrum for photosynthesis of plants. Recently, shreds of evidence abound on researches that focused on the impact of LED lights on morphogenesis and photosynthesis. They observed that red and blue light improved crop production when the light intensity and quality were controlled [36-39].

Growth parameters. Photosynthetic processes in leaves require the capturing of lights, The light utilized by the leaf is affected by the wavelength, intensity and the angle of incidence [38] as well as the total area of the leaves. The cucumber seedlings were observed to react strongly to LM9 with respect to Plant height, Stem diameter, total leaf area, hypocotyls length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight and percentage of water content (Table 2). This finding was in agreement with the findings of Naznin, Lefsrud [39], Yang, He [40] in pepper seedlings and in tomato seedlings [41] they have observed that a mixture of red and blue LED light was efficient in the production of strong seedlings.

Effects of LED light on chlorophyll contents. Chlorophyll directly affects photosynthetic ability and primary production [42, 43]. Moreover, the chlorophyll contents of plants are affected by the quality of light. Studies have explained the beneficial effects of using blue lights [37, 41, 44, 45]. From the results obtained from the cucumber seedlings, a combination of red and blue LED light with high red light (LM9) was observed to be favorable for chlorophyll-a, chlorophyll-b, and carotenoids (Table 3). This is in agreement with the findings of Yang, Xu [41] on tomato seedlings and pepper seedlings [39, 40]. They have observed that chlorophyll-a, chlorophyll-b, and carotenoid were more when seedlings were exposed to a mixture of red and blue LED lights than white fluorescent lights exposure.

Effect of ratios LED light on biochemical contents. The study presented the importance of using red light in metabolites accumulation in cucum-

ber seedlings. LM3 was effective in increasing nitrate and soluble sugar content levels in cucumber seedlings, while WL was observed to increase the soluble protein content level. The study of Bian, Cheng [46], has proved that soluble sugar was higher in lettuce when exposed to red, green and blue LED light (4:1:1) than under other types of the LED light. Also, Xiaoying, Shirong [47] has observed that tomato seedlings grown under blue LED light gave higher soluble sugar levels than under other types of LED light [48], they further have observed that soluble sugar levels were more in pepper, tomato and cucumber seedlings were grown under red LED light and (red: blue). Their findings proved that soluble sugars and proteins respond to different light quality in vegetable crops grown under a controlled environment and it also varies in species and cultivars. From the present study, it was observed that under light intensity of (80±2 $\mu mol.m^{-2}.s^{-1}$) and 14 h/10h photoperiods, a combination of R70: B30 LED light for cucumber seedlings were more effective in nitrate concentrations reduction than the white fluorescent light. These results were in tandem with the findings of Bian, Cheng [46], they have observed a mixture of red and blue LED light to be more effective in reducing the nitrate concentrations in lettuce grown hydroponically.

CONCLUSION

Light is a very important factor for plants to the process of photosynthesis, so countries whose environmental conditions do not have natural sunlight have come to rely on industrial lighting to produce their food and from crops of strategic importance are cucumber. The influence of light intensities, light qualities and photoperiod on the growth characteristics, chlorophyll contents and biochemical contents of cucumber seedlings were studied. Referring to our research results the best treatment for all morphological parameters and chlorophyll contents was LM9. The differences in these characteristics suggest the urge for a more in-depth investigation into the optimum requirements for each parameter studied as the basic molecular mechanisms are yet to be exploited fully. The need for further investigation is therefore imperative to provide a full understanding of these phenomena with a view to improving crop productivity and quality.

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Conflict of interest. The authors declare that they don't have any conflicts of interest with the contents of this research.

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NUTRITIONAL VALORIZATION AND HEALTH RISK ASSESSMENT OF METAL POLLUTION OF NILE TILAPIA FISH CULTURED IN BECHAR REGION, SOUTHWEST OF ALGERIA

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ABSTRACT

The present work aims to study the nutritional value and the level of metal pollution of Nile Tilapia fish flesh, this species is reared in south-west Algeria more precisely, in Bechar city. The nutritional analyses relate to the pH dosage, humidity, ash rate, proteins and lipids. However, alteration indices were studied namely, total volatile basic nitrogen (ABVT), the Trimethylamine (TMA) and the peroxide index. As for metal pollution, nine elements of heavy metals are dosed by SAA (Atomic Absorption Spectrometry) on the samples of flesh as well as on the drilling and farming waters. The results of the nutritional valorization show acceptable levels of all dosed compounds except for the ash content which exceeds the regulatory standards (2,116±0,0152%). However, the alteration indices show high rates which still always satisfying except for the peroxide index (25,6±0,1 meq O_2/100g of flesh). Metal pollution analysis shows good results for necessary heavy metals both in fish flesh and water samples, for example, (4,7804±0,0001 and 0,088833±0,000252 ppm), means of fish flesh respectively for Calcium and Iron. As for not necessary ones, the results are much lower than the thresholds (0,108±0,003, 0,0026±0,0001 and 0,0932±0,0001 ppm), means of fish flesh respectively for Lead, Cadmium and Chromium.

KEYWORDS:
Nutritional valorization, metal pollution, Nile Tilapia fish, south-west Algeria, fish flesh.

INTRODUCTION

Whether it comes from fresh water or the sea, fish has always been like an abundant and inexhaustible food. However, the reality is completely different and we are faced with the fact that many marine fish stocks are overexploited. In view of this, a new activity that has emerged is aquaculture, which comes with the aim of increasing production in order to maintain a balance between fish production and human consumption, ensure the production of food proteins of animal origin and reduce the pressure on natural stocks [1].

A strategy for the introduction of fish species of high nutritional and commercial value has been adopted. Tilapia is of major importance in the international trade of aquaculture products [2].

On the Algerian market, Tilapia is classified as a second product among fish imported from Egypt or Asia [2, 3, 4]. In Africa, by far the bulk of fish farming production remains Tilapia [1]. Among the Tilapia, the Nile Tilapia (*Oreochromis niloticus),* is the main species farmed. It is generally considered to be the candidate species for freshwater fish farming [5].

However, the pollution of freshwater by heavy metals is a topical issue of concern to all communities wishing to maintain their water heritage at a certain level of quality [6]. Metals are normal trace constituents of the environment and they are all toxic above a certain threshold [7].

In contrast, their bioaccumulation varies enormously depending on the organs and species. As reported by various authors [8, 9, 10, 11, 12]. They may also accumulate contaminants due to their high contents for fat and proteins [13].

The present work attempts to determine the nutritional value and the level of metal pollution of Nile Tilapia fish flesh frequently consumed by the Algerian southwest population.

MATERIALS AND METHODS

Study area. General information. Boukais (31° 55′ 55,272″N, 2° 27′ 27, 179″W) (Figure 1) is located in the northwest part of the Wilaya of Bechar (31° 37′7572 ″N, 2° 12′ 36,395″ W), at a distance of 50 km from Bechar city. It is an open landscape characterized by the agricultural and mining activities and the presence of a lake surrounded by vegetation. From a climatic point of view, it is a semi-arid region due to climatic variations: very

high temperatures in summer (42,2°C) and low ones in winter (2,3°C) with an average annual precipitation of 150 mm (2000–2010).

Sampling. The fish are transferred to the laboratory in ice boxes then anaesthetised using ethylamino-benzoatemethane-sulphonate (MS222). After sacrifice, the fish are washed under a gentle current of water while being rubbed with fingers. They are then dried in filter paper, the head is sliced off, the abdominal wall opened and the viscera removed; the visceral cavity is washed with a thread of water. The bones and skin are removed; the flesh alone is crushed to obtain a homogeneous sample [15]. Approximately 300g of flesh was aseptically sampled in front of a Bunsen burner and stored in sterile Petri dishes at -20°C for different examinations.

As for water samples, drilling water was sampled from the borehole of the aquaculture farm while farming water was sampled from a rearing pond that contains Nile Tilapia fish.

Determination of pH, humidity and ash content. It should be noted that the pH was measured directly from the Tilapia muscle using a pH meter after being calibrated and before preparing the fish flesh sample. Then, humidity and ash content were estimated using a muffle furnace [16].

Determination of proteins, lipids. The percentage of crude protein in the fish flesh shall be calculated from the nitrogen content determined by the Kjeldahl method using Kjeltec 2400 FOSS (Distillation and titration unit with an autosampler) and digestion unit FOSS with programmer. Thus, the quantity of total lipids was obtained by Soxhlet extraction, according to the method NF EN ISO 734-1, 2000 described by AFNOR [17].

FIGURE 1
Geographical situation of Boukais region [14]

TABLE 1
Mean of ph, ash, humidity, proteins and fat of fish flesh

	pH	Ash (%)	Humidity (%)	Proteins (%)	Fat (%)
Fish flesh	6,403±0,0115	2,116±0,0152	76,02±0,01	15,803±0,0152	0,126±0,00577

Values are means ± SD (triplicate)

Determination of ABVT, TMAand peroxide index. Moreover, the determination of ABVT and TMA was carried out according to the method of Belaiouer *et al* [14]. However, the determination of the peroxide index was carried out by the "Soxhlet" method using “Hexane” as solvent [17].

Determination of heavy metals. SAA conditions. The dosage of heavy metals was carried out using an atomic absorption spectrophotometer apparatus with graphite furnace and domain flame SAA Shimadzu AA7000.

Preparation samples and standards. Fish flesh samples were prepared according to the technique established by Dergal [20]. 1g of the fresh flesh was mineralized using 3 volumes of an HNO_3/H_2O_2 (1V/1V) mixture. The mixture was placed in a hot plate at 160°C for 30 minutes, after cooling the contents were filtered through filter papers and diluted in 25ml of distilled water. As well, the water samples were acidified and filtered through a 0.45µm [21].

The various heavy metal standards were prepared in parallel with the samples; we have chosen a range of concentrations for each metal according to the detection range of the SAA which allowed us to plot the calibration curves.

Statistical analysis. The mean values and standard deviation were analyzed statistically using Microsoft Excel 2016. One-way ANOVA was carried out to test for any significant difference. Difference between means at *P-value*< 0.001 level were considered highly significant.

RESULTS AND DISCUSSION

The mean of pH value complied with the national standards: pH 6,5-8,5. It is little acidic and can lead to bacterial growth which will later lead to the formation of undesirable products resulting from microbial spoilage of fish flesh. As it is known, pH, humidity and ash content are intrinsic factors influencing bacterial growth. Therefore, a microbial analysis must be carried out on the fish flesh in order to properly study its quality. The Table 1 shows mean results of pH, ash, humidity, proteins and fat.

The obtained results showed a significant proportion of proteins in the Tilapia studied, the lipid proportion is low.

The contents of all dosed compounds in fish flesh are within the standards set by the regulations and those set by Dergal [20] (0.33±0.03, 17.3±0.40% and 80.7±0.40%) respectively for humidity, proteins and fat. with the exception of the ash content which exceeds the threshold of 0.59±0.12 %.

In fact, ash is the inorganic residue obtained after calcination of organic matter and it gives an idea of the amount of mineral elements present in the fish flesh [21]. For that reason, the obtained result indicated that there is a moderately high quantity of mineral elements in fish flesh.

The results of the ash content are in agreement with those obtained by Hocine *et al* [22] which found 02,29 % in Tilapia fish flesh reared in an aquaculture farm in Algiers. They are, however, lower than those obtained by Barnabas *et al* [23] which found a mean of 5,93±0,17 in flesh of the Nile Tilapia fish.

The results of the protein level are lower than those obtained by Hocine *et al* [22] Which found 18,38% in Tilapia fish flesh. These results are lower than the national standards (17,4-19,4%) and also than those obtained by the studies of Dergal [20](17,3±0,40%).

Humidity results are in agreement with those obtained by Hocine *et al* [22] which found 78.09% in the Tilapia fish flesh from the aquaculture farm, these values are included in the ranges cited in the bibliography (77-80% water) and also in the studies of Dergal [20] (80,7±0,40%).

However, the lipid rates recorded in this work are lower than those obtained by Hocine *et al* [22] which found 1,24%. Note that, lipid content is always inversely proportional to water content. Fish are thus classified as lean (<5%), semi-fat (5 à 8%) and fat (8 à 25%) [24]. From this, we deduce that the Nile Tilapia is a lean fish.

On the contrary, alteration indices of fish flesh are considered as chemical analyses based on criteria of loss of freshness and quality. These analyses are based on the determination of compounds formed following the evolution and degradation of nitrogenous materials (ABVT and TMA) or lipids (peroxide value).

TABLE 2
Mean of alteration indices in fish flesh

	ABVT (mg/100g of flesh)	TMA (mg/100g of flesh)	Peroxide index (meq O_2/100g of flesh)
Fish flesh	10,25±0,01	0,35±0,01	25,6±0,1

Values are means ± SD (triplicate)

TABLE 3
European standards of alteration indices of fish flesh

	ABVT (mg/100)	TMA (mg/100g)	State of Freshness	Peroxide Index (meq of O2/100g)
Parameteres	<20	<17	Satisfying	<20
	20-25	17-40	Acceptable	/
	25	40	Not Satisfying	/

ABVT and TMA analysis was used to assess bacterial and enzymatic proteolysis. For example, the trend in TMA production during transportation or storage depends on the growth of spoilage bacteria. These bacteria use TMAO as a substrate under anaerobic conditions and reduce it to TMA inducing unpleasant smells in fish [25].

The Table 2 shows the results found for each index. In many cases, the ABVT criterion is difficult to interpret; the use in addition to the TMA assay makes this test more reliable for assessing product alteration [26]

The Table 3 shows European ranges of standards for alteration indices:

It can be noted that the average concentrations of ABVT and TMA are well below the standards set by JORADP [27]. As a result, the flesh is of satisfactory quality.

These results corroborate with those obtained by Dergal [20]**.** The latter, in the framework of the evaluation of a safety and quality management system for Nile Tilapia from aquaculture, had retained the thresholds for ABVT and TMA in the order of 35mg/ 100g and 8g/100g of flesh. The obtained values in this work correspond to the freshness values reported by this author.

The increase in ABVT concentrations is due to the accumulation of these major constituents (NH_3, DMA and TMA) in the altered Tilapia. Ammonia (NH_3) is generated from the bacterial deamination of amino acids as well as from the autolysis of adenosine monophosphate (AMP) [28].

The fish becomes non-consumable when the ABVT and TMA successively exceed 35 mg/100g and 15 mg/100g of flesh. According to the Table 03 above, the average levels of ABVT and TMA in Tilapia fish flesh are well below European standards.

It is well known that some heavy metals can be considered as co-factors in the oxidation of fish flesh, which is why the peroxide value exceeds the standards. The peroxide value is a good indicator of the state of preservation of a fat, it measures the total hydro peroxides which are the first oxidation products. It makes it possible to monitor the rancidity of the flesh by measuring peroxide compounds due to the fixation of oxygen on the unsaturated fatty acids. The higher the index, the more the fat is oxidized. However, this index is only an indicator of the beginning of oxidation; it increases to reach a peak and then decreases with the advanced state of oxidation [29].

The peroxide index exceeded the standards set by the regulations (20 meq O_2/kg), these results corroborate with those obtained by Hocine *et al* [22] which found 34,78 meq of O_2/ kg for Tilapia reared in the aquaculture farm, this can be partly explained by the fact that due to lack of means, the analysis was not carried out on the fresh product but after one week of freezing at -20°C.

As for metal pollution, the pollution assessment of nine heavy metals (Calcium (Ca), Lead (Pb), Iron (Fe), Zinc (Zn), Chromium (Cr), Cadmium (Cd), Magnesium (Mg), Copper (Cu), Cobalt (Co)) in 02 water samples (drilling and farming waters) and in fish flesh sample from the south west of Algeria was investigated.

Generally, metals are divided into two categories according to whether they are essential for living beingsor no. They can prove to be essential to the development of biological processes (trace elements), this is the case of Iron (Fe), Copper (Cu), Calcium (Ca), Magnesium (Mg) and Zinc (Zn). This causes a lack or excess of these essential elements that may have deleterious effects. Otherwise, other metals are not necessary for life, and may even be harmful such as lead (Pb), Cadmium (Cd), chromium (Cr) and Cobalt (Co). For example, previous studies demonstrated that Arsenic (As), Cadmium (Cd), lead (Pb) and chromium (Cr) have harmful effects on human health even at low concentrations ([30] ; [31]). As could cause central and peripheral nervous system damage, cardiovascular disease, birth defects, placental development disorders and other reproductive problems ([32] ;[33] ; [34]). In this study, the comparison should be made along two lines; one comparison with regulatory thresholds and another comparison between the rates found for each type of sample more precisely, if the heavy metal content found for drilling water, considered as water source, increases or decreases reaching the flesh of the farmed fish.

TABLE 4
Mean of heavy metals in drilling and farming waters and in fish flesh samples (ppm)

	Drilling water	Farming water	Fish flesh
Ca	112,8577±0,001	113,5248±1,74×10^{-14}	4,7804±0,0001
Pb	0,1512±0,0002	0,1152±1,699×10^{-17}	0,108±0,003
Fe	0,2523±0,0002	0,063133±0,000057	0,088833±0,000252
Zn	0,8138±0,0002	0,03946667±0,0000573	0,007767±0,000153
Cr	0,0848±0,0001	0,0933±0.00	0,0932±0,0001
Cd	0,000167±0,000057	0,0014±0.00	0,0026±0,0001
Mg	2,0378±0,0001	2,0233±0.00	1,2767±0,0001
Cu	0,000533±0,000057	0,0012±0.00	0,000533±0,000057
Co	0,0117±0,0001	0,0109±00	0,0138±0,0001

Values are means ± SD (triplicate)

The results showed that the pollution of heavy metals in water and fish samples was at moderate levels. The concentration range and mean levels of heavy metals in drilling and farming water and fish samples are listed in Table 4.

The mean concentration of Ca in water and fish samples was the highest, which was followed by Mg, Zn than Fe, which is a good sign for the fish flesh quality. We noticed that the concentration of Ca, Zn, Fe, Cu and Mg have decreased in fish flesh comparing with drilling water especially for Ca which has decreased dramatically in fish flesh. These results were in accordance with those observed by Wenjue *et al* [35] (48,5 ±18,8) (4,84±1,92) (means in ppm± SD for Zinc and Cu in water samples), (8,75±6,91) (0,45±0,40) (means in ppm± SD for Zinc and Cu in fish flesh samples).

It indicated that farming waters in Algerian southwest were not contaminated with more heavy metals, although some literatures reported that heavy metals in feed perhaps affect the water quality of farming water [37].

According to Sameckacymerman *et al* [37], Cu in water might be from mining; this can be compatible with the nature of the region of study (Boukais- Bechar, Algeria) which is known by mining, especially copper and iron. Also, leaching from agricultural soils was a main source of zinc in water according to the report of Bonten *et al* [38] which can be also the case of the region of study known as an important area for agriculture in the Algerian southwest. The study of Bryan [39] showed that copper and zinc concentrations were related to their levels in the blood, particularly zinc, which is associated with blood proteins.

According to the Food and Agriculture Organization of the United Nations (FAO), there is no set regulations for concentrations of Cu, Zn and Fe in fish organs [40], however, by comparing the concentrations of Cu, Zn and Fe to Canadian thresholds (Cu : 100 µg/g, Zn : 100 µg/g, Fe= 200 µg/g) reported by Papagionnis *et al* [40], they do not agree with the studies conducted by Wang *et al* [41] and Papagionnis *et al* [40] which revealed fairly high levels of Cu, Zn and Fe in the gonads of dam fish.

The concentrations of Zn and Cu in this study were lower than those found in fish flesh [35] (0.37–22.5) and (0.17–0.89) minimum and maximum values in ppm respectively for Zn and Cu. They were also lower than those found in fishes by Abdul Baki *et al* [42] (3.34–12.10), (0.3–2.23) and (1.19–165.2) minimum and maximum values in ppm respectively for Zn, Cu and Fe.

As for the other type of heavy metals, the mean levels of the not necessary heavy metals (Cd, Pb, Cr and Co) in this study were similar to each other without significant differences and they were lower than the regulatory thresholds (Cd : 0,050-0,3 mg/kg), (Pb : 0,3 mg/kg) (according to standards fixed by Codex alimentarius [43].

Comparing our results to other studies done on the Nile Tilapia species, our study showed Cr and Cd concentrations lower than those found by Barnabas *et al* [23] done on Tilapia fish farmed in lake Tchad who found (0.00-2,28) (26,56-51,93) minimum and maximum values in ppm respectively for Cd and Cr. Also, Allinson *et al* [44] found Cd concentration higher than those found in our study 0.00-0,50 µg/g. Pb and Cd concentrations were lower than those found by Chale [45] which studied metal pollution of *Oreochromis spp* farmed in Lake Tanganyika (Tanzanie), 2,70 and 0,20 µg/g mean levels respectively for Pb and Cd.

The results for Cd and Cr were much lower than those found by Barnabas *et al* [23] which found (0.00-2,28 µg/g), (26,56-51,93 µg/g) minimum and maximum values respectively for Cd and Cr in the Nile Tilapia fish flesh at Lake Chad.

Cr and Cd results were lower than those found in fishes by Abdul Baki *et al* [42] (0.18–1.87), (1.52–

14.09) minimum and maximum values respectively for Cr and Cd. Pb has recorded almost the same value as the last study mentioned (0.11–8.92).

In the same vein, Cd and Pb concentrations were higher than those found by Wenjue *et al* [35] (0.00071–0.075) (0.03–0.29 mg/kg) minimum and maximum values respectively for Cd and Pb while Cr recorded a lower value than that of the same study (0.44–4.61 mg/kg).

Heavy metals are one of the most significant pollutants in aquatic ecosystems due to their toxicities, persistence and bioaccumulation potentials. It is well known that heavy metals pose significant health risks when human exposure dose exceeds safe consumption levels [46, 31, 48, 49].

Furthermore, farming water and feed qualities, transportation and storage of the samples studied played a big part in the obtained results and they are essential factors to preserve the nutritional quality of the fish flesh and also to ensure the fish farming of this species in the southwest region, species considered as a main source of protein especially for Saharan population.

CONCLUSION

The results in this study indicated that the Nile Tilapia fish in Algerian Southwest is a nutritionally rich species, it can be considered as an important source of protein and essential fatty acids. Also, the heavy metals in this freshwater fish are at relatively low level and they don't cause considerable human health risks.

The daily consumption of Nile Tilapia fish from aquaculture farm of Bechar cannot expose consumers to high health risks because the levels of heavy metals in fish flesh still below the mentioned standards, however, the risk of accumulation of these metals in the body is real and the adverse effects to be feared.

Factors such as species physiology, trophic level and metal specificity are predominant in the bioaccumulation of metals in aquatic organisms [50, 51, 52].

Finally, other types of analyses such as microbial and physico-chemical analyses of water samples, also, vitamin dosage and microbial analysis must be carried out on both fish flesh and feeding samples to make sure of the contamination source of Nile Tilapia fish which proves to be an enormous nutritional richness in the regions of the Algerian southwest extremely far from the Mediterranean Sea.

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QUALITY ASSESSMENT OF LOW CALORIC READY TO SERVE GRAPEFRUIT DRINK WITH DIFFERENT CONCENTRATION OF D-SORBITOL

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ABSTRACT

This study investigated the effects of low caloric sweetener (D-sorbitol) on the quality parameters of grapefruit ready to serve drink. The chemical characteristics and sensory attributes of the grapefruit drinks with D-sorbitol were evaluated at each 15days interval for a total period of 90 days at ambient temperature. Results from each independent experiment revealed an increase in pH, TSS and reducing sugar content during storage while a decreased in the % acidity, ascorbic acid, non-reducing sugar, total sugar, color, taste, settling and overall acceptability. During storage TSS significantly increased from 16.47 to 16.52°brix, pH from 3.25 to 3.31 and reducing sugar from 1.47 to 2.40%. The highest score was noted initially for control in terms of color, taste, settling and overall acceptability during storage. As the concentration of sorbitol was increased, a significant decline was observed in all the organoleptic properties of the drink during storage in addition to a decrease in the % acidity from 0.37% to 0.31%, ascorbic acid from 9.13 mg/100g to 8.10 mg/100g, total sugar from 7.57 to 6.93, non-reducing sugar from 10.06 from 6.12. The score for color, taste, settling and overall acceptability was also decreased from 7.23 to 5.00, 7.57 to 5.79, and 7.29 to 5.14, respectively. The finding of this study further revealed that D-sorbitol concentration and storage significantly affect the overall quality of grapefruit low caloric drink and hence these results can be used as a guide for the development of D-sorbitol based diet grapefruits drink with higher organoleptic and physicochemical attributes.

KEYWORDS:
Grapefruit, low caloric drink, RTS, D-sorbitol, Storage, acceptability, quality, Vitamin C, pH, acidity.

INTRODUCTION

Grapefruit (*Citrus Paradisi*) belong to the family of *Rutaceae* and a member of *Citrus* genus. Grapefruit is an important citrus fruit in Pakistan with gradually increasing production in the citrus growing regions of the country. It is also an essential constituent in the production of perfumes, detergents, soap and cosmetics [6]. Grapefruit contains vitamin C and also dietary fiber, vitamin A, folate, potassium and vitamin B_5. Grapefruit is rich source of phytochemical such as lycopene and limonoids. The interest for using grapefruit is mainly due its lipid lowering ability and high pectin content. Grapefruit also includes the soluble fiber which also helps in lowering the cholesterol level of the human body. It is rich in phytochemicals that helps in the protection against various cancers and cardiovascular diseases [14].

Soft drinks are usually consumed to satisfy thirst, but these drinks always have bad effect on the health due higher contents of sugar and other chemicals. Worldwide Ready to serve (RTS) drinks gains popularity day by day increasingly because of their beneficial properties such as its nutritional, refreshing and its digestibility characteristics. In Pakistan, long summer season attracts the consumers to these beverages to satisfy their thirst. Till date a number of different beverages are developed with the addition of minerals, protein and vitamins [8]. These products are thirst smother and instantly nourish the energy requirement of body. Addition of medicinal plant extract further improve the beneficial health effects of such products on human health [19].

Fruit beverage products are consisting of pulp/juice, water as well as flavors, colors, preservatives and sweetener (sucrose) for energy. Sucrose is not a superlative sweetener for all function because consuming sucrose will add calories and calories will increase weight. Grapefruit is invariably suggested for diabetic and high blood pressure patients. Added sugar may reduce intakes of nutrient-rich, recommended foods, such as yogurt, whole grains, and tart fruits including cranberries, cherries, and grapefruit [4]. Alternatives of sugars are used in the food items

TABLE 1
Plan of study

Treatments	Water(%)	Juice(%)	Sugar(%)	Sorbitol(%)
GF_0 (Control)	72%	14%	14%	-
GF_1	72%	14%	-	14%
GF_2	71%	14%	-	15%
GF_3	70%	14%	-	16%
GF_4	69%	14%	-	17%
GF_5	68%	14%	-	18%
GF_6	67%	14%	-	19%

in small quantities to sweeten the beverages and juices but with fewer calories for diabetics and overweight consumers. Sorbitol is also known as D-glucitol which is six carbon polyol and are found in plants especially in berries, cherries, apples, pears [24]. Sorbitol is a colorless, fragrance-free, and sweet taste liquid that is ranked as 60% compared to the sweetness of sucrose, and it provides energy of 2.6 kcal.g-1. The human body consumes sorbitol deliberately. Sorbitol provides sweetness and functionality to food products but not as much sweetness as compare to sucrose [13]. In order to maintain blood sugar level, sucrose is replaced by low calorie sweetener Sorbitol. By the uptake of Sorbitol, D-glucose is produced and the metabolism of Sorbitol is very slow, consequently hyperglycosemia is rarely detected [8]. Hence, the present study is aimed to evaluate the effect of low caloric sweetener on the overall quality parameter of grapefruit ready to serve low caloric drink.

MATERIALS AND METHODS

Chemical and reagents. Sodium Benzoate (Analytical grade-Merck Germany), 2,6-Dichloroindophenol (Analytical grade-Merck), Sodium hydroxide (Analytical Grade-Sigma), Copper sulphate (Analytical Grade-Merk Germany), Oxalic Acid (Analytical Grade- Sigma), Potassium hydroxide (Analytical Grade-Sigma), Methylene Blue (Sigma), Phenolphthalein (Analytical Grade-Merk). Sodium Potassium tartrate (ChemPol England).

Preparation of low caloric grapefruit drink. The fresh, healthy and sound grapefruit were provided from the orchard of Agricultural Research Institute (ARI) Tarnab Peshawar Pakistan. The fruits were brought to the laboratory of Food Science &Technology Section, where the grapefruits were first thoroughly washed with tape water to remove the dust and adhering residues. After washing the juice was extracted from the fruit by citrus extractor machine. The sample was prepared by the addition of water, juice and sweetener in the ratio of 5:1:1 respectively as mentioned by [16]. A control sample was prepared with sucrose as sweetener by using the formula. Low caloric grapefruit drink was prepared by replacing sucrose with low caloric sweetener sorbitol (liquid) in increasing trends for the preparation of drink as shown in Table 01. All the treatment were preserved with potassium metabisulfite [17]. The sweetness of sucrose was taken as a standard. The grapefruit RTS drink were filled in the 250ml plastic bottles. These bottles were stored at room temperature in the laboratory and were analyzed for physicochemically characteristics (pH, total soluble solids (TSS), titratable acidity, vitamin C, reducing sugar, non-reducing sugar) according to standard methods [1]. The organoleptic analysis was recorded by using the nine point's hedonic scale [12] at interval of 15 days, for the total period of 90 days.

Statistical analysis. All the data were analyzed statistically by using complete randomized designed (CRD) with two factors and means were separated by applying least significant difference (LSD) test as described by [22]

RESULTS AND DISCUSSION

Physicochemical properties of grapefruit drink. Total soluble solid (TSS). Total soluble solids of a product usually represent the percentage of sugar content. Total soluble solids of low caloric grapefruit RTS drink samples with sugar and different levels of sorbitol are shown in Figure (4.1). The present study showed that the replacement of sucrose with sorbitol significantly (p<0.05) increased the TSS of the grapefruit drink. There was no significant effect of storage on the mean value of total soluble solid of the grapefruit drink after 90 days of storage. The treatment GF_6 has shown highest mean value

(20.39) for TSS while GF_1 showed the lowest TSS value (12.44). The highest change during storage was found in GF_0 (0.50%) and lowest change were occurred in GF_3 (0.18%). The increased in TSS content of samples might be due to the solubility of content (hydrolysis of polysaccharide to monosaccharide) during storage in the presence of acid and temperature. These results are in lined with finding of [7], observed increased in TSS contents from 12 to 15 ^{0}Brix at ambient temperature due to the hydrolysis of polysaccharide into monosaccharide and soluble disaccharide in mulberry RTS. [3] showed slight increase in TSS range from 15.0 to 15.3 ^{0}Brix at 120 days of storage during development of drink based on orange, grapefruit and pineapple. [2] showed that TSS increased (10.00 to 15.00) significantly of bitter guard RTS drink, due to the increase in relative viscosity of sorbitol and higher refractive index of sorbitol.

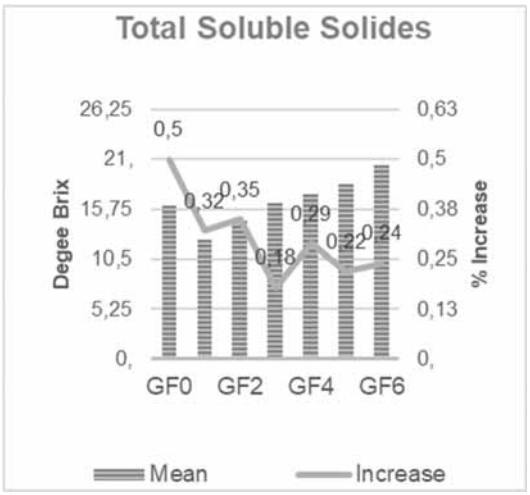

FIGURE (4.1)
Total soluble solid (^{0}brix) of low caloric grapefruit drink

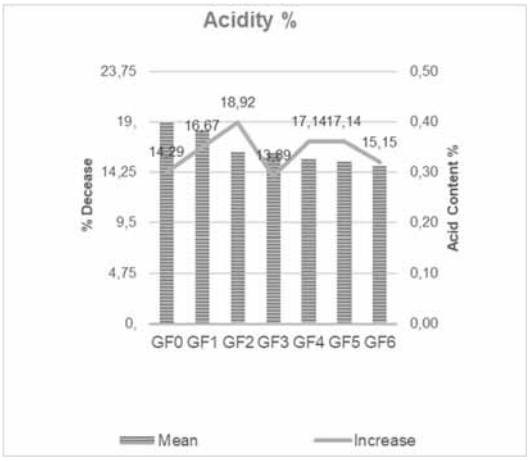

FIGURE (4.2)
Titratable (%)of low caloric grapefruit drink

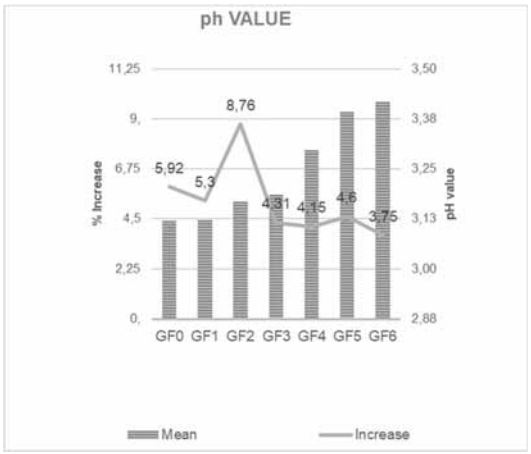

FIGURE (4.3)
pH of low caloric grapefruit drink.

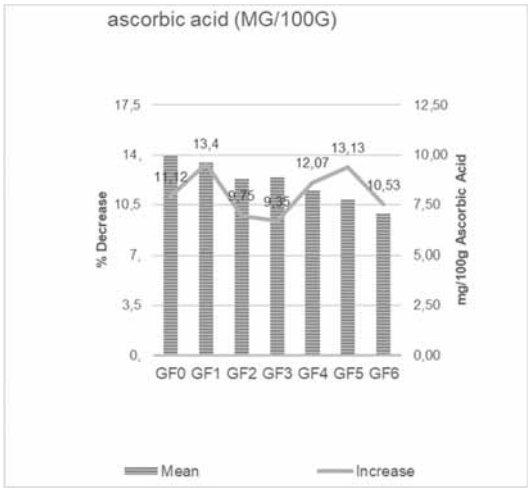

FIGURE (4.4)
Ascorbic acid (mg/100ml) of low caloric grapefruit drink

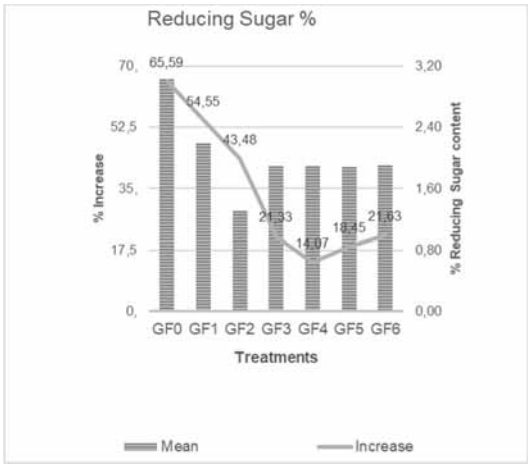

FIGURE (4.6)
Reducing sugar (%) of low caloric grapefruit drink

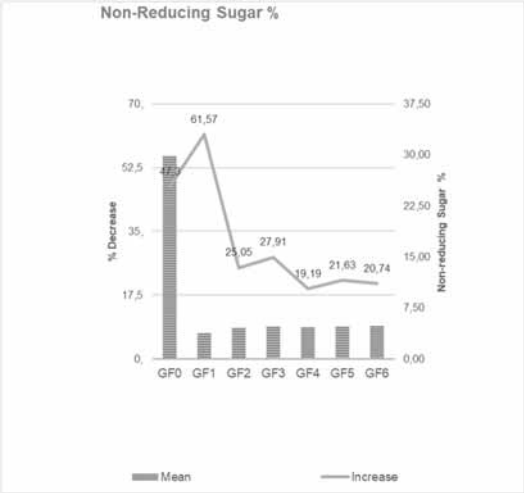

FIGURE (4.7)
Non-reducing sugar (%) of low caloric grapefruit drink

Titratable acidity (TA). Titratable acidity of grapefruit RTS drink samples with sugar and different level of sorbitol are shown in Figure 4.2. The study shows that the replacement of sucrose with sorbitol significantly (p<0.05) decreased the titratable acidity of the grapefruit drink. The mean value for storage of titratable acidity of the grapefruit drink significantly decreased from 0.37 to 0.31% during storage. GF_0 had highest mean TA value 0.40% while GF_6 was found lowest mean value (0.31%). The highest change during storage was found in GF_2 (18.92%) and lowest change were occurred in GF_4 (13.89%). The decreased in acidity of grapefruit RTS drink was due to the consumption of acid content in the hydrolysis of polysaccharides to monosaccharides during storage. In agreement to this data [7] also reported decrease in titratable acidity from 0.30 to 0.25% during storage in mulberry RTS drink due to co-polymerization of organic acids with sugars and amino acids. During a previous study, [21] prepared bitter gourd drink with the addition of sorbitol, as low caloric sweetener which also revealed a decrease in the titratable acidity (0.4 to 0.39%) because of the organic acid copolymerization with amino acid and sugars. Similarly, [3] showed slight decrease in acidity range (0.133 to 0.097) due to loss or degradation of vitamin C contents of drink based on orange, grapefruit and pineapple

pH value. pH plays an important role in maintaining shelf-stability of food. pH of grapefruit RTS drink samples with sugar and different levels of sorbitol are shown in Figure 4.3. The study showed that the replacement of sugar with sorbitol increased the pH of the grapefruit drink significantly (p<0.05) by treatments and storage. The highest pH mean value was recorded forGF_3 (3.33) while the lowest mean value of pH was recorded inGF_5sample (3.15). The highest increase value was found in treatment GF_6 (8.76%) while the lowest one was observed in treatmentGF_3 (3.75%). Reason of increasing pH during storage might be due the maximum degradation of acid content of product, that may contribute to pH value. Increase in pH might be due to change in chemical characteristics which were affected by condition of storage [20]. [9] reported increased in pH of RTS drink prepared due to decrease in percent acidity as it has inverse relation.

Ascorbic acid (mg/100g). Ascorbic acid is involved in the metabolism of protein, synthesis of collagen and plays an important role as physiological antioxidant. Ascorbic acid of grapefruit RTS drink samples with sugar and different level of sorbitol are shown in Figure(4.4). The maximum value of ascorbic acid mean was found in GF_2 (9.13) followed by GF_6 (9.63) while GF_0 contained the lowest mean value of ascorbic acid (7.04) followed by GF_3 (7.76).The highest % decrease was found inGF_6 (13.40%) which were followed by GF_3 (13.13%) while the lowest % decrease was observed in GF_5 (9.35%) which were followed by GF_1 (9.75%).The ascorbic acid L- isomer is unstable in light, high temperature and oxidized with changes in pH of the media. According to [7] in mulberry RTS drink during 90 days of storage the ascorbic acid were decrease from 1.88 to 1.10 and 0.86 mg/100 ml which might be due to degradation of furfural or dehydro-ascorbic acid and ascorbic acid was sensitive highly to heat hence the degradation was more at room temperature. Similar result was found by [15] in pineapple RTS drink by using artificial sweetener. [3] showed decreased in vitamin C contents in prepared RTS drink of grapefruit, orange and pineapple that ranged from 9.95 to 10.2 mg/100ml. The decrease noticed might be attributed to the factors such as temperature, oxidation, acidity and light.

Reducing sugar (%). Reducing sugar of grapefruit RTS drink samples with sugar and different levels of sorbitol are shown in Figure (4.6). The study showed that the replacement of sugar with sorbitol as a low caloric sweetener significantly (p<0.05) increased the reducing sugar of the grapefruit drink for treatments and storage. The maximum reducing sugar mean value were found inGF_0 (2.79) while minimum mean was at GF_1 (2.19).The highest % increase was found in GF_0 (65.59%) which were followed by GF_1 (54.55%) while the lowest % increase was found at GF_4 (14.07%) which were followed by GF_5 (18.45%). The increase in reducing sugar of RTS drink during storage was due to the non-reducing sugar conversion. The increasing amount of sorbitol in the bitter guard RTS drink shows increased in reducing sugar during analysis which might be due to the inversion or hydrolysis of non-reducing sugar to reducing sugar [2].

Non-reducing sugar (%). Non-Reducing sugar of grapefruit RTS drink samples with sugar and different level of sorbitol are shown in Figure (4.7). The study shows that the replacement of sugar with sorbitol significantly ($p<0.05$) decreased the non-reducing sugar of the grapefruit drink. The maximum non-reducing sugar mean were found at GF_0 (29.83) followed by GF_6 (4.81) while minimum mean was found at GF_1 (3.76) followed by GF_2 (4.56).The highest % decrease was GF_1 (61.57%) followed by GF_0 (47.30%) while the lowest % decrease was at GF_4 (19.19 %) which were followed by GF_5 (21.63%).The decreasing trend might be due to the hydrolysis and inversion of non-reducing sugar during storage, which rise in level of reducing sugar by the acid of drink. Similar observation was reported by [2] showed decrease in non-reducing sugar which might be due to the hydrolysis of reducing sugar into non-reducing sugar. [18] also confirm the same result of increasing reducing sugar with hydrolysis of non-reducing sugar in fruit juices concentrates.

Organoleptic properties. Color. Color of grapefruit RTS drink samples with sugar and different level of sorbitol are shown in the table. The study showed that the replacement of sugar with sorbitol significantly ($p<0.05$) decreased the color of the grapefruit drink. The maximum color mean was found inGF_0 (6.00) followed by GF_1 (5.93) while minimum mean was at GF_3 (5.70) followed by GF_2 (5.76).The highest % decrease was in GF_6 (38.03%) followed by GF_0 (34.67%) while the lowest % decrease was observed in GF_2 (29.58%) followed by in GF_4 (29.73%). Similar result trend of decreasing in color score of drink (pineapple, grapefruit and orange) was due to the different concentration of sugar and little effect of acids [3]. Development of drink based on pineapple, orange and grapefruit. Bangladesh Res. Pub. J. 7(2): 170-174.3]. Same result of decrease was found in bitter gourd RTS drink which score decreased from 7.60 to 6.00with the passage of time during storage [2].

Taste. All the samples analyzed with increasing trends of sorbitol were observed at 15 days of storage intervals for 90 days of storage. The study showed decreased in the taste score of the grapefruit drink. The maximum taste mean was recorded for GF_4 (7.23) followed by GF_0 (7.13) while minimum mean was observed in GF_3 (6.19) followed by GF_2 (6.19).The highest % decrease was GF_3 (27.27%) followed by GF_1 (26.56%) while the lowest % decrease was GF_4 (18.75) which were followed by GF_5 (22.50%). The decreased in taste score was the development of minute medicinal taste during the last intervals of storage. The slight bitter taste was found in the samples based on stevia (Bio-sweetener) was due to the flavonoids, essential oil and tannins reviewed by [5]. Usually, sorbitol sweetened drinks at higher concentration are better taste and flavor score, which might be due to their stability to thermal and non-enzymatic browning and low tendency to undergo fermentation [2]. Change in taste of prepared orange dink during physico-chemical and sensory properties, was found by [11].

Overall acceptability. The influence of treatments and storage on the overall acceptability of value-added grapefruit RTS drink samples with sugar and different level of sorbitol are shown in the table 02. The study concluded that the replacement of sugar with sorbitol as a low caloric sweetener significantly ($p<0.05$) decreased the overall acceptability of the grapefruit drink The maximum overall acceptability mean score was found in GF_4 (6.58) followed by GF_5 (6.41) while minimum mean score was recorded in GF_1 (55.84) followed byGF_3 (5.98).The highest % decrease was GF_3 (30.61%) followed by GF_0 (29.94%)while the lowest % decrease was observed in GF_4 (24.03) which were followed by GF_2 (24.64%). The research found same result in overall acceptability which was due to the processing condition including time of storage and temperature in orange RTS drink [10]. [20] reported that same decrease in overall acceptability of RTS drink of aloe Vera and anole fruit with a decreased in the overall acceptability from 8.50 to 7.81.

CONCLUSION

This study concludes that sorbitol can be used in preparation low caloric grapefruit RTS drink. The results further revealed that minimal processing cause significant loss of chemical constituents to the drink during storage. From this study the formulation of treatment GF_4 (17% sorbitol) was highly accepted among the other treatment regarding physio-chemical and sensory attributes during 90 days of storage. Hence, the availability of low caloric grapefruit drinks in the market will benefit the health conscious and obese persons.

TABLE 2
Effect of sorbitol and storage on mean score of judges for organoleptic evaluation of low caloric grapefruit drink

Treatments	Storage Intervals							% Decrease	Mean
	0 Day	15 Day	30 Day	45 Day	60 Day	75 Day	90 Day		
					Mean Score Color				
GF_0	7.5	6.7	6.3	5.9	5.5	5.2	4.9	34.67	6.00a
GF_1	7.3	6.6	6.1	5.7	5.5	5.3	5	31.51	5.93b
GF_2	7.1	6.5	6	5.5	5.2	5	5	29.58	5.76ab
GF_3	7	6.4	6	5.5	5.4	5	4.6	34.29	5.70ab
GF_4	7.4	6.4	6.2	5.5	5.4	5.1	4.7	36.49	5.81ab
GF_5	7.2	6.4	5.7	5.6	5.4	5	4.8	33.33	5.73ab
GF_6	7.1	6.4	5.7	5.6	5.4	4.6	4.4	38.03	5.60c
Mean	7.23a	6.49b	6.00c	5.61d	5.40e	5.03f	4.77g		
					Mean Score Taste				
GF_0	8.2	7.7	7.5	7.2	6.7	6.5	6.1	25.61	7.13a
GF_1	6.4	6.4	6.3	5.7	5.5	5.3	4.7	26.56	5.76d
GF_2	6.7	6.6	6.6	6.5	6	5.5	5.4	19.4	6.19c
GF_3	7.7	6.6	6.3	6.2	5.7	5.7	5.6	27.27	6.26c
GF_4	8	7.7	7.6	7.5	6.7	6.6	6.5	18.75	7.23a
GF_5	8	7.6	7.4	7.2	6.7	6.6	6.2	22.5	7.10a
GF_6	8	7.7	7.2	7	6.5	6	5	37.5	6.77b
Mean	7.57a	7.19b	6.99bc	6.76c	6.26d	6.03d	5.64e		
					Mean Score Overall acceptability				
GF_o	7.85	7.2	6.9	6.55	6.1	5.85	5.5	29.94	6.56a
GF_1	6.85	6.5	6.2	5.7	5.5	5.3	4.85	29.2	5.84d
GF_2	6.9	6.55	6.3	6	5.6	5.25	5.2	24.64	5.97c
GF_3	7.35	6.5	6.15	5.85	5.55	5.35	5.1	30.61	5.98c
GF_4	7.7	7.05	6.9	6.5	6.05	5.85	5.6	27.27	6.52a
GF_5	7.6	7	6.55	6.4	6.05	5.8	5.5	27.63	6.41a
GF_6	7.55	7.05	6.45	6.3	5.95	5.3	4.7	37.75	6.19b
Mean	7.40a	6.84b	6.49bc	6.19c	5.83d	5.55d	5.24e		

Values having different alphabetical letters are significantly ($p<0.05$) different from each other.

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QUALITY ATTRIBUTES OF BEEF BURGERPREPARED FROM MEAT AND MILLETFLOUR

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ABSTRACT

Physical and chemical properties of pearl and proso millet were evaluated. The flour of both millet varietieswas used in different combinations(10 % and 20 %) with extruded soy to develop beef burgersin comparison with control (20 % soy). Quality characteristics of burger treatments were evaluated during storage at - 18 ºC for 4 months.Pearl millet had highercontents of oil, total phenol and antioxidant than proso millet.Both millet varieties are rich source of phosphorus, magnesium and potassium. Leucine was the dominant essential amino acid in both millet varieties. Generally, burgers formulated with the flour of both millet varieties had a good acceptability.Cooking and texturecharacteristics improved with increasing millet flour in the burger formulations.After 4 months of frozen storage,the total volatile nitrogencontent of all treatments was in the range of permissible level. Thiobarbituric acid content and microbial criteria of all treatments were within the permissible levels till the end of the third month of storage period. The obtained results herald a promising future for incorporationof millets into the meat products industry.

KEYWORDS:
Beef burger, millet, amino acids, fatty acids, sensory properties,quality characteristics.

INTRODUCTION

Recently, the world is undergoingchanges in climatic conditions and lack of water resources. According to the UN world water development report 2015, world will be facing 40% water deficit by 2030, which means the world will not have enough water to meet its needs in just 10 years [1]. As Egypt passed through the consequences of the construction of the Al Nahda dam and the consequent exposure to the conditions of lack of water resources of the River Nile, there was a need to focus on growing crops that does not require large quantities of water. Among these crops is the millet. The world agriculture production report stated that millets are one of the most important droughtresistant crops and depict 6th rank among cereal crops [2].

Milletisan important crop in the semiarid tropics of Africa and Asia with 97% of millet production in developing countries [3]. This crop is preferred because of its high productivity and short growing season under dry, high-temperature conditions. Due to their high ability to grow under harsh climatic conditions with poor soil fertility, millets are the future of food and farming [4]. Unlike main cereals such as rice and wheat, no millets attract any pest during their growth or storage. While season specific for wheat or rice might provide only food security, season specific formillets can provide food, animal feed, livelihood and ecological securities [5]. Many authors reported that millet has many nutritious and medical functions [6 and7]. Moreover, millets are rich in phytochemical such as tannins, phenolic compounds, flavonoids, alkaloids and terpenoids. These phytochemicals play an important role in stimulating detoxification enzymes, removing free radicals, inhibiting the formation of nitrosamine, preventing the formation of tumor factors, and reducing carcinogens, among others [8]. Millets contents of crude protein, fat, crude fiber, carbohydrates, minerals and vitamins are comparable to other cereals such as rice and wheat. Millets are unique among the cereals because of their richness in calcium, dietary fiber and polyphenols [2].Due to its low cost, health benefits, antimicrobial and antioxidant activities,there is increased interest in millet in food industry [9]. Furthermore, as millets are free gluten and are known for their low carbohydrate concentration and low glycemic index, some studies developed bakery products using millet flour [10]. The acceptability of biscuit dough and breads formulated with millet flour is reported to be very good [11].

Pearl millet (*Pennisetumamericanum*) and proso millet (*Panicummiliaceum*) are essential millet varieties that are cultivated widely in the African and Asian countries. Millet's content of fiberis higher than that in rice and wheat (up to 3 to 5 times more). This high content of fiber has a beneficial effect on reducing blood sugar, body weight, insulin resistance and diabetes [12]. The high fiber content acts as prebiotics that helps in maintaining the health of the gut microbiome [13]. A plenty of nutritious

food products can be developed using *Pennisetumgluacum* and there is an increasing interest in pearl millet cultivation due to its availability and its capability to adapt to changes in climatic conditions [14]. Different varieties of millet are used in Africa, Europe and Asia, but no inclusive study has been made to show their characteristics in the food system. Therefore, further studies are required to evaluate the whole nutritional values, amino acid composition, and their thermal properties for its utilization in meat products [12].

On the other hand, consumers demand to provide a variety of meat products that meet their acceptance at reasonable prices are increasing. This entails searching for reduced formulation cost procedures without compromising the quality and sensory properties of the products. Varieties of non-meat ingredients are used as extenders in processed meats to provide diversity, improve sensory properties, enhance storage stabilityand reduce production costs. Meat extendersare non-meat ingredients which are added to low-quality meat products for economic reasons but they were later used to make meat products healthier by adding dietary fiber, or to improve the texture.Beef burger is one of the most popular meat products and the most widespread and beloved among the Egyptians. To meet the wide demand for this product, one type of extender material is added during manufacturing, and the most commonly used is extrudedsoy. Unfortunately, no meat products that employ the use of millet or millet flour have been developed in Egypt. The aim of the present study is to shed light on the millet crop as an economically unexploited crop in the human diet, which can take advantage of its suitability to withstand water and climate conditions in Egypt to fill the food gap through the evaluation of the physicochemical parameters of two millet varieties (pearl and proso millet). In addition, to develop different beef burger formulations using millet flour and evaluate their sensorial and technological quality following freeze storage (4 months at –18 °C), in order to replace extrudedsoy and provide novel and alternative products.

MATERIALS AND METHODS

Materials. Two millet varieties were used in this study. The pearl millet (*Pennisetumamericanum*), var.Shandawel 1, was obtained from Crop Research Institute in 2019, Agricultural Research Center, Giza, Egypt.Proso creamy millet (*Panicummiliaceum*), extruded soy flour, frozen imported meat,salt, onion, eggs and spices were purchased in 2019 from the local market in Giza, Egypt. The seeds were manually cleaned then stored in polyethylene bags at 21º ± 2ºC.A mixture of powdered spices was prepared as follows: 0.07% nutmeg; 0.07% ginger, 0.07% Coriander, 0.27% black pepper, 2.5% Cardamomim and 2.5% Curry. Sodium tripolyphosphate and ascorbic acidwere obtained from Adwic Laboratory Chemicals Co., Cairo, Egypt.

Methods. Preparation of millet flour. Milletseeds were washed with running water,driedat 50° ±2°C/ 2 hr. in an air oven dryerand then milled into flour by using a laboratory disc mill (Braun AG Frankfurt Type: KM 32, Germany).The resulting flourwaspassing through 250 - 300 micron sieve and kept in polyethylene bag till analysis.

Beef burger preparation. The meat was defrosted overnight at 4±1°C, ground and mixed for homogeneity. The beef meat 62% was mixed with salt 1.5%, spices1%, Onion 7%, egg 2%, sodium tripolyphosphate 0.2%, ascorbic acid 0.3% and chilled water 6%. Burger treatments were prepared by adding rehydrated extruded soy (1g soy: 2ml water) or millet flour to the formula as follows: 20% rehydrated extruded soy (control), 10% of rehydrated extruded soy and 10 % proso millet flour (T1), 20% of proso millet flour (T2), 10% of rehydrated extruded soy and 10 % pearl millet flour (T3). 20% of pearl millet flour (T4). Each treatment was mixed by hand under hygienic condition, subjected to final grinding (0.4 cm plate) and processed into beefburgers (80 g weight, 1 cm thick and 10 cm diameter). The burgers were placed on foam trays, wrapped with polyethylene bags, sealed and kept frozen at -18 °C for 4 months until analysis. Samples were periodically taken for analysis every month. All measurements were performed in triplicates.

Proximate composition. Moisture, crude protein (Nx6.25), fat, ash, and crude fiber contents of raw materials were determined using the methods of the AOAC [15]. Carbohydrates content was calculated by difference [16]. Total calories (Kcal) of uncookedburgers was estimated by multiplying the crude protein, fat and carbohydrates by calculation as its basis of 4, 9 and 4 kcal/g, respectively according to the method of James [17].

Determination of antioxidant components. Total phenols content was estimated using the method of Folin-Ciocalteu according to the Singleton et al.[18]. Gallic acid was chosen as a standard to prepare the standard curve.Total flavonoids content was determined according to the methods of Zhishen et al. [19]. Quercitin was used as standard compound. Tannins were determined as described by Price et al.[20].Catechin was used to prepare the standard curve.

Determination of antioxidant activity. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Brand-Williams et al.[21]. 2,2-diphenyl-1-picrylhydrazyl

method (DPPH) solution (3.9 ml, 25 mg/l) in methanol was mixed with the methanol samples extract (0.1 ml). The reaction progress was monitored at 515 nm until the absorbance was stable.

% Antioxidant activity = ($A_{Control}$- A_{Sample}) × 100 / $A_{Control}$

Minerals content. Mineral contents of millet flour (Fe, Zn, Cu, Mn, Ca, K, Mg and Na) were determined according to AOAC [15] using the Perkin Elmer (Model 300, USA) Atomic Absorption Spectrophotometer. Total phosphorus was determined by the colorimetric method of Trough and Mayer[22].

Amino acids composition. Amino acids contentsof millet flour were determined according to AOAC [15]. The analysis was performed using high performance amino acid analyzer (Biochrom 30) instruction manual. The amino acids profile was carried out on the precipitated protein from defatted millet flour after hydrolysis by 6.0 N HCl for 24 h at 110˚C in evacuated ampoules. Quantitative determination of amino acids were carried out by Biochrome 30 instruction manual (Analyzer used), 2005. EZ chrome manual (software for data collection and processing).

Fatty acids analysis . Lipid was extracted according to the method described by Bligh and Dyer [23]. Fatty acid methyl esters were prepared according to ISO 12966-2 [24]. Fatty acid methyl esters were injected into (HP 6890 series GC) apparatus provided with a DB-23 column (60m × 0.32 mm × 25 µm). Carrier gas was N_2 with flow rate 2.2 ml/min, splitting ratio of 1:50. The injector temperature was 250 ° C and that of flame Ionization Detector (FID) was 300 °C. the temperature setting was as follows: 150 °C to 210 °C at 5 °C /min, and then held at 210 °C for 25 min. peaks were identified by comparing the retention times obtained with stander methyl esters.

Water and oil absorption capacity. Water absorption capacity (WAC) and oil absorption capacity (OAC) of millet flour were determined according to AACC (2000)[25].

Chemical quality properties. The total volatile nitrogen (TVN) content in burger treatments was determined by macro distillation method as described by Pearson [26].

Thiobarbituric acid (TBA) values (mg malonaldehyde/kg sample) of burger treatments were estimated by colorimetric method at 538 nm using BECKMAN DU 7400 spectrophotometer according to Pearson [26].

Physical properties. The pH value for burger samples was estimated using a calibrated pH meter (Jenway, 3510, UK) according to Fernández-López et al.[27].

Water holding capacities (WHC) and plasticity of burger treatments were measured according to the filter-press method described by Kauffman et al.[28].

Cooking characteristics. Five units of each beef burger treatments were weighted before and after cooking. Beef burgers were cooked in preheated sunflower oil (148 °C) for 4 min on one side, turned over, cooked for 6 min, turned again and cooked for 4 min. Cooking yield, fat retention and moisture retention were determined as described by Aleson-Carbonell et al. [29] and calculated as follows:

% cooking yield = cooked burger weight/ uncooked burger weight 100

Fat retention % = [(cooked burger weight % fat in cooked sample)/ (uncooked burger weight % fat in uncooked sample)] 100

Moisture retention % = [(cooked burger weight % moisture in cooked sample) / (uncooked burger weight % moisture in uncooked sample)] 100

The shrinkage of beef burgers was determined as described by Carvalho et al. [30] and calculated as follows:

Shrinkage % = (diameter (mm) before cooking - diameter (mm) after cooking) / diameter (mm) before cooking 100

Texture analysis. Texture analysis was performed using a texture analyzer (Brookfield Engineering, CT V1.6, Middleboro, MA, USA) coupled to a computer interface. Cubes (2 cm^3) of cooked samples at room temperature were compressed axially in two consecutive cycles of 50% compression using knife-edge probe TA-PFS-C. Data collection and calculations were performed using the Texture Pro Software (Brookfield Engineering, CT V1.6, Middleboro, MA, USA). Force-time deformation curves were obtained with a 10 kg load cell applied at a cross-head speed of 2.0 mm/s. A trigger load of 4N was applied. Analyses of hardness, chewiness, springiness, and cohesiveness were performed as described by Bourne [31]

Color . Samples color was measured as described by Mc Gurie [32] using a hand- held colorimeter Minolta chromameter (Konica Minolta CR-400. Ramsey, Japan), which provided CIE L^*, a^*, b^*, *Chroma*$[(a^{*2} + b^{*2})^{1/2}]$ which indicates the color saturation, and hue angle $[\tan^{-1}(b^*/a^*)]$.This measurement was repeated at five randomly selected locations for each beef burger exposed at room temperature.

Microbiological examinations. Ten grams of representative burger samples were mixed with 90 ml of sterile buffered (0.1% peptone in water) under

sterile conditions, to give 1/10 dilution. Serial dilutions were prepared and total bacterial count (TBC), psychrophilic bacteria, Coliform bacteria and yeast & mold counts of burger samples were determined according to Difco-Manual [33]. Incubations were carried out at 37 °C/48 h for TBC; at 7 °C/10 days for psychrophilic;at 37 °C/24 h for Coliform bacteria and 25 °C/5 days for yeasts & molds count.

Organoleptic evaluation. Organoleptic evaluation of burgersamples was carried out after cooking according to Watts et al.[34] by aid of ten well trained members of the Meat and Fish Res. Dep. stuff, Food Technology Research Institute. Judging scale for each attribute was as follows:Excellent (8-9), Very good (7-<8), Good (6-<7), Fair (5-< 6), Poor (4-<5) and Rejected (<4).

Statistical analysis. Data were subjected to Analysis of Variance (ANOVA). An analysis of variance was conducted using Costat version 6.311 (Copyright 1998– 2005, CoHort software). Means comparison was performed using Duncan's test at the 5% ($p < 0.05$) level of probability as reported by Snedecor and Cochran [35]. Data were represented as average of three repetitions for all the experiments ± standard deviation (SD).

RESULTS AND DISCUSSION

Chemical and physical proprieties of millet flour. Results in Table 1pointed out thatproso millet recorded highermoisture content (10.9%) than pearl millet (9.04%). Whereas, no significant($p > 0.05$)differences were found in fiber and ash contents between both millet varieties. Meanwhile, pearl millet had higheroil and protein contents than proso millet. Higher carbohydrate content was observed in proso millet flour (84.75%).These results are in agreement with those obtained by Kulkarni and Naik [36] who reported that moisture content ranging from 10.60 to 15.00 % in proso millet. Anitha et al.[37] found that the pearl millet has protein and fat contents ranged from 8 % to 19% and from 2.4% to 5%, respectively.

Data also revealed thatpearl millet has higher WAC and OAC values than those of proso millet. These results are confirmed by Gull et al. [38] who studied oil absorption capacity (OAC) of different millet varieties and found that OAC of pearl millet flour recorded 1.60 g/100g.

The phenolic compounds content that present in grains are of particular importance because of their ability in scavenging free radicals and prevent cells from the oxidative damage. It has been established that phenols and tannins present in cereals are also good sources of natural antioxidants important in health, aging and metabolic diseases [39]. No significant differences ($p > 0.05$) were found in phenols and flavonoids contents between both millet varieties, while pearl millet flour has high tannin content. It was stated that millet varieties with dark color dyed pericarp showed higher content of soluble phenolic fragments than those found in light color such as proso millet [40]. Zhang et al.[41] reported that proso millet has total phenolic content around 456.95 mg gallic acid/100g.

In the DPPH assay, the color stable DPPH radical is reduced in the presence of an antioxidant which donates hydrogen to non-radical DPPH–H (Ragaee et al.[42]. Regarding antioxidant activity, results showed that there were no significant differences ($p > 0.05$) between both millet varieties (Table 1).

Both millet varieties are rich sources of Pand K (Table 1). Moreover, higher contents of Ca, Fe and Zn were observed in pearl millet than in proso millet. Phosphourus is an important mineral for the skeleton and energy production. It is an essential component of ATP – the energy currency of the cell, It also forms a part of the nervous system and cell membranes. Both millet varieties contain high amount of magnesium.Magnesiumhelps in relaxing blood vessels, keeps up the blood pressure, improves nutrient delivery by enhancing the blood flow and thus further protects the cardiovascular system. It also increases insulin sensitivity,lowers triglycerides and acts as a cofactor for more than 300 enzymes. Millet is such a grain that ought to be listed as heart-healthy choices due to its importance as a good source of magnesium [43].

Amino Acids and Fatty acids profile of millet flour. Amino acid composition (g/100 g protein) of both millet varieties are presented in Table 2. Leucine was the dominant essential amino acid in both millet verities. This result is confirmed by Obadina et al.[44] who stated leucine to be the principal amino acid in pearl millet. The lysine content was 0.14 and 0.34 (g/100 g protein) for proso and pearl millet flour, respectively. Methionine and cysteine, which are sulphur containing amino acids, were 0.58 and 0.62 g/100 g protein for pearl and proso millet flour, respectively. Obilana and Manyasa [7] reported that millets contain significant amounts of essential amino acids particularly the sulphur containing amino acids (methionine and cysteine).

The results of saturated and unsaturated fatty acids ofboth millet varietiesare presented in Table 2. It could be noticed that proso and pearl millet oil had a high content of unsaturated fatty acids. Proso millet had a higher amount of linoleic acid $C_{18:2}$ (62.72%) than pearl millet (44.65). While, pearl millet had a higher percentage of olic acid than proso millet.Among the saturated fatty acids (SFA), pearl millet oil has high values from palmitic acid $C_{16:0}$(20.16%).Furthermore, other fatty acids were identified in trace amounts (arachidic acid C20: 0,

behenic acid C22: 0, erucic acid C22:1) in proso millet oil only. The fatty acid profile showed the total amount of saturated fatty acids present is 12.19–25.61% while unsaturated fatty acids content is 87.81–74.39% for proso and pearl millet, respectively. The prevalent fatty acids in the free lipids of millet oil were linoleic, oleic, and palmitic acids [45].In addition, examination of some millet varieties from Tunisia and Mauritania by Ibrahima et al.[46] showed that the fatty acid profile of the millet lipid is mainly characterized by the presence of high levels of linoleic, oleic and palmitic acid. However, palmitoleic acid (C16: 1), stearic acid (C18: 0) and omega-6 fatty acid (C18: 3) were less represented. Other fatty acids are identified in trace amounts (arachidic acid C20: 0, behenic acid C22: 0, anderucic acid C22:1).

Sensory evaluation of beef burger. Sensory evaluation results of different beef burger treatments are presented in Table 3.Non-significant ($P > 0.05$) difference was found in all sensory attributes between beef burger formulated with 20% pearl millet flour (T4) and control sample. While, a significant difference ($P < 0.05$) were found between T1, T2 and T3treatments and the control sample in appearance, taste, odor and overall acceptability.The convergent L^* values of cooked burgers formulated with 20 % pearl millet and 20% soy might be contributing factors to the higher appearance scores of T4 and control. As for texture attribute, no significant differences

TABLE 1
Proximate composition, bioactive compounds, WAC and OAC and minerals contents in both millet varieties.

Chemical composition	Proso millet	Pearl millet
	(% on dry weight basis)	
Moisture	10.9 ^{a} ±0.09	9.04 ^{b} ±0.03
Crude protein	10.07 ^{b} ± 0.95	10.6 ^{a} ±0.02
Ether extract	3.41 ^{b} ±0.45	5.5^{a} ±0.23
Crude fiber	2.35 ^{a} ±0.18	2.5^{a} ±0.09
Ash	1.77 ^{a} ±0.02	1.81 ^{a} ±0.04
Carbohydrates	84.75 ^{a} ±0.48	82.09 ^{b} ± 1.40
Water absorption capacity (g/100g)	1.62 ^{b}±0.018	1.84^{a} ±0.01
Oil absorption capacity (g/100g)	1.45^{b} ±0.04	1.66 ^{a} ±0.02
Total phenols (mg/100g)	326.4 ^{a} ±15.57	340.14 ^{a} ±11.82
Total flavonoids (mg/100g)	79.09^{a}±1.36	83.49^{a} ±1.69
Tannins (mg/100g)	135 ^{b} ±3.53	160^{a} ±4.24
Antioxidant activity (%)	29.92 ^{a} ±1.97	31.8 ^{a} ±2.53
Minerals (mg/100g)		
Ca	13.65	0
Na	6.03	20.16
Mg	117	0.29
K	320	0
P	280	0
Fe	2.56	5.45
Zn	2.4	27.31
Mn	0.9	44.65
Cu	0.83	1.63

Values are means and SD (n=3); where: Mean values in the same row with the same letter are not significantly different at 0.05 level.

TABLE 2
Amino acids (g\100g protein) and fatty acids composition of both millet varieties.

Amino Acid(g\100g protein)			F. A (%)		
	Proso millet	Pearl millet		Proso millet	Pearl millet
Essential amino acids					
Lysine	0.14	0.34	C14:0	0.03	0
Leucine	1.17	1.01	C16:0	8.55	20.16
Isoleucine	0.40	0.43	C16:1	0.14	0.29
Methionine +	0.62	0.58	C17:0	0.06	0
Phenylalanine	0.53	0.54	C17:1	0.03	0
Therionine	0.29	0.38	C18:0	2.15	5.45
Tyrosine	0.39	0.37	C18:1	23.24	27.31
Valine	0.52	0.58	C18:2	62.72	44.65
Tryptophan	ND		C18:3 n3	1.04	1.63
Non-Essential amino acids			C20:0	0.85	0
Glutamic	2.04	1.82	C20:1	0.63	0
Aspartic	0.74	0.85	C22:0	0.55	0
Proline	0.69	0.65			
Alanine	1.03	0.93			
Glycine	0.19	0.35			
Serine	0.45	0.44			
Arginine	0.33	0.55			
Histidine	0.22	0.26			

ND = not determined

TABLE 3
Sensory properties of burger treatments.

Attribute	Control	T1	T2	T3	T4
Appearance	$8.4^{a} \pm 0.27$	$8.15^{b} \pm 0.37$	$8.01^{b} \pm 0.46$	$8.0^{b} \pm 0.51$	$8.51^{a} \pm 0.25$
Taste	$8.25^{ab} \pm 0.48$	$7.95^{c} \pm 0.45$	$8.05^{bc} \pm 0.28$	$7.9^{c} \pm 0.32$	$8.3^{a} \pm 0.36$
Texture	$8.35^{a} \pm 0.35$	$8.15^{b} \pm 0.38$	$8.25^{ab} \pm 0.22$	$8.40^{a} \pm 0.24$	$8.35^{a} \pm 0.30$
Odor	$8.5^{a} \pm 0.41$	$7.8^{c} \pm 0.49$	$7.9^{c} \pm 0.31$	$8.2^{b} \pm 0.29$	$8.3^{ab} \pm 0.44$
Overallacceptability	$8.4^{a} \pm 0.18$	$8.11^{cd} \pm 0.35$	$7.95^{d} \pm 0.40$	$8.2^{bc} \pm 0.43$	$8.35^{ab} \pm 0.20$

Values are means and SD (n=10); where: Mean values in the same row with the same letter are not significantly different at 0.05 levels.

($P > 0.05$) were found among all treatments except for treatment T1 which recorded the lowest score. When comparing the results of burgers formulated with the flour of two millet varieties, it was found that beef burgers formulated with 20 % pearl millet (T4) recorded significantly ($P < 0.05$) higher scores in appearance, taste, odor and overall acceptability than beef burgers formulated with 20 % proso millet (T2). In general, results indicated that pearlmillet flour could be added up to 20 % without significant

loss in palatability. These results are in the line with those obtained by Naveena et al. [47] who found that the addition of finger millet (*Eleusinecoracana*) flour into chicken patties formulations showed no difference ($p > 0.05$) between control and finger millet flour-added chicken patties for all the sensory attributes.Among both millet varieties, high ($p< 0.05$) scores for taste and odor was recorded for beef burgers containing 10% (T3) and 20% (T4) pearl millet flour which can be attributed to the high ability of pearl millet flour to absorb and hold water and fat compared to proso millet. Yadahally et al. [48] declared that there is a positive correlation betweenhigh water and oil absorption capacity of the flour and organoleptic characteristics of food. An increase in the juiciness of the meat products formulated with millet was observed by Vijayakumar et al.[49], which was attributed to the water holding capacity of millet flour. Talukder and Sharma [12] reported that millets have a unique grainy and gritty texture which can convey a different and acceptable texture to the meat products. Nirmala et al. [50] noticed a general improvement in the flavor of the chicken patties formulated with finger millet flour.Although soy flour contains high amounts of phenols, flavonoids and tannins, it contains high fat content that masks the taste and flavor of these compounds. Moreover, Brewer et al.[51] and Su et al.[52]. reported that soy proteins has undesirable soy and beany flavor, bitter taste and astringent mouth feel which decreases the sensory attributes of the final product, thus limits its use in meat products. Pearl millet seeds are distinguished by a mixture of color and flavor, if added to a product formulation, it can transfer the distinctive color and flavor to the product. Generally, the products formulated with pearl millet flour possessed darker color [49]

Chemical composition of beef burger. Gross chemical compositionofdifferent burgertreatments are shown in Table 4. The results showed significant differences ($p < 0.05$) between all tested samples. These differences might be attributed to the added amount of soy, proso and pearl millet flour. Through all treatments, moisture content was significantly ($p < 0.05$) decreased as soy flour levelsdecrease. The protein, ash and fiber contentsof treatments containing different amounts of millet flour were significantly ($p < 0.05$) lower than that of control sample. Furthermore, protein content significantly ($P < 0.05$) decrease with the increase of millet flour levels.These results are in agreement with those reported by Naveena et al.[47] and Dzudie et al. [53] who found lower protein content in chicken patties and beef patties formulated with different levels of ragi millet flour and common bean flour, respectively.Results of crude fat content had the same trend of moisture and protein contents.This may be due to the high fat content of soy than that of both millet varieties. On the contrary, carbohydratescontent increased significantly ($P < 0.05$) as the amount of millet flour increased.

Total energy calculations showed that, as the fat content was decreased, total energy (kcal / 100 g) was declined. Being the highest treatment in the fat content, control samples had the highest total energy (475.14 kcal / 100 g) in comparison with the other treatments. These results are in line with Mansour and Khalil [54] who reported that caloric reduction correlated positively with fat reduction.

The estimated production cost (per kilogram) ofdifferent burger treatments were calculated according to the prices at the processing time (Table 4). Among all treatments, minor differences in the production cost were found. It is worth mentioning that the main goal of incorporating millet into meat products is not only because of its cheap price, but.

TABLE 4
The proximate composition (% on dry weight basis) and production cost of different burger treatments.

Constituents	Control	T1	T2	T3	T4
Moisture	$65.68^{a}\pm 0.36$	$64.82^{b} \pm 0.34$	$61.33^{c} \pm 0.12$	$64.41^{b} \pm 0.23$	$60.68^{d} \pm 0.47$
Protein	$59.93^{a} \pm 0.25$	$56.56^{b} \pm 0.46$	$53.28^{d} \pm 0.25$	$57.02^{b} \pm 0.3$	$54.1^{c} \pm 0.32$
Fat	$23.29^{a} \pm 0.44$	$21.56^{b} \pm 0.17$	$19.83^{d} \pm 0.22$	$21.77^{b} \pm 0.31$	$20.26^{c} \pm 0.26$
Ash	$9.03^{a} \pm 0.27$	$8.15^{b} \pm 0.08$	$6.37^{c} \pm 0.20$	$8.24^{b} \pm 0.16$	$6.21^{c} \pm 0.24$
Fiber	$1.29^{a} \pm 0.04$	$0.94^{b} \pm 0.11$	$0.59^{c} \pm 0.08$	$0.96^{b} \pm 0.06$	$0.62^{c} \pm 0.13$
Carbohydrates	$6.46^{e} \pm 0.07$	$12.78^{c} \pm 0.05$	$19.93^{a} \pm 0.01$	$12.02^{d} \pm 0.01$	$18.82^{b} \pm 0.03$
Energy, kcal/100 g	475.14	471.43	471.3	472.08	473.96
Production cost (EP	74.52	72.27	70.02	72.27	70.02

Values are means and SD (n=3); where: Mean values in the same row with the same letter are not significantly different at 0.05 levels.

TABLE 5
Physical properties of different burger treatments.

Treatments	WHC	Plasticity	Cooking yield %	Moisture retention %	Fat retention %	Shrinkage %
Control	$0.5^{ab} \pm 0.12$	$3.2^{ab} \pm 0.14$	$82.04^{c} \pm 0.26$	$70.10^{b} \pm 0.16$	$123.96^{a} \pm 0.51$	$20.49^{a} \pm 0.37$
T1	$0.6^{a} \pm 0.06$	$2.85^{d} \pm 0.03$	$80.24^{d} \pm 0.32$	$63.05^{e} \pm 0.07$	$82.04^{d} \pm 0.33$	$18.75^{b} \pm 0.43$
T2	$0.5^{ab} \pm 0.02$	$3.0^{cd} \pm 0.15$	$80.41^{d} \pm 0.25$	$63.92^{d} \pm 0.27$	$67.88^{e} \pm 0.30$	$15.8^{c} \pm 0.24$
T3	$0.4^{b} \pm 0.05$	$3.05^{bc} \pm 0.09$	$84.94^{b} \pm 0.26$	$67.57^{c} \pm 0.36$	$118.80^{b} \pm 0.28$	$15.63^{c} \pm 0.49$
T4	$0.2^{c} \pm 0.08$	$3.35^{a} \pm 0.06$	$88.45^{a} \pm 0.40$	$76.55^{a} \pm 0.18$	$97.60^{c} \pm 0.45$	$14.24^{d} \pm 0.39$
	Hardness (N)	Resilience	Cohesiveness	Springiness (Mm)	Gumminess (N)	Chewiness (mJ)
Control	28.38	0.56	0.94	2.76	26.56	73.3
T1	21.51	0.51	1.01	2.67	21.79	58.2
T2	21.38	0.54	0.95	2.58	20.22	52.2
T3	21.81	0.52	0.9	2.59	19.6	50.8
T4	19.39	0.45	0.89	2.39	17.73	42.4

Values are means and SD (n=3); where: Mean values in the same column with the same letter are not significantly different at 0.05 level.

also to find an alternative commensurate with the successive circumstances that the country is going through, in terms of climate changes, the need to grow crops commensurate with the reclaimed land and the effects of Al Nahda Dam

Physical quality criteria of beef burger. The physical quality criteria of different beef burger treatments formulated with extruded soy flour, proso and pearl millet flour are presented in Table 5. Non-significant differences ($p > 0.05$) in WHC and plasticity were found among all burger treatments.At the same addition percentage (20%), treatment T4 recorded higher ($p < 0.05$) WHC and plasticit values than those of T2 or control samples.These results indicated the strength of water binding capacity of pearl millet compared to proso millet or soy.Treatments T4 and T3 presented higher cooking yield ($p < 0.05$) than control, T1 and T2, which shows an important influence of the pearl millet in reducing water loss, leading to an increased cooking yield. These results are confirmedby Naveena et al.[47] who observed an increment in the cooking yield of chicken patties processed with finger millet flour. Pearl millet increased cooking yield because of its ability to retain moisture in the burger structure. This is indicated by the results of moisture retention, where burgers formulated with 20 % pearl millet (T4) recorded the highest ($p < 0.05$) moisture retention value (76.55 %) compared to the other treatments. Regarding fat retention, control sample recorded the highest ($p < 0.05$) fat retention value (123.96 %). Thisis probably due to the high ability of soy flour to absorb oil. On the other hand, fat retention significantly ($p < 0.05$) decreased with the millet flour levels increased. Burgers formulated with 20 % proso millet (T2) recorded the lowest ($p < 0.05$) fat retention value (67.88%). Although cooking yield and moisture retention values of treatment T4 were higher ($p < 0.05$) than those of control sample, it was recorded lower fat retention value (97.6 %). Anderson and Berry [55] reported that increased cooking yield does not always result in high fat retention. In contrast, Naveena et al.[47] observed a positive relationship between increasing cooking yield and high fat retention in chicken patties formulated with finger millet.

Generally, about 25 percent shrinkage occurs when beef, poultry and fish are cooked. The amount of shrinkage will depend on its fat, moisture content, and the temperature at which the meat is cooked.Concerning shrinkage, treatments T4, T3 and T2 showed higher ($p < 0.05$) reductions than the control sample, confirming the role of millet in the improvement of cooking characteristics of the beef burger. This result could be related to the binding property of the pearl and proso millet, which detained the meat particles together and limited changes in the product moisture loss. In a study by Cannel et al.[56] they found that when extruded soy was added to beef patties (from 0 to 20 %), shrinkage of patties decreased from 29.95 to 17.87%. Whereas,

pearl millet applied in this study significantly ($p < 0.05$) decrease shrinkage with greater moisture retentions, where, burger contain 20% pearl millet recorded 14.24 % shrinkage. Borba et al. [57] investigated different methods for preparation of commercial beef burgers and they found a reduction in the diameter of 16.87%.

The texture analysis (Table 5) showed higher hardness value for control samples followed by burger samples formulated with a mixture of 10 % soy and 10 % millet (T1 and T3). While, burger samples formulated with 20 % pearl millet (T4) showed lower hardness value. This finding was confirmed by results of cooking yield, moisture retention and shrinkage. Increasing the concentration of pearl millet significantly ($p < 0.05$) reduced chewiness values. Springiness is associated with water and fat binding properties Horita et al.[58]. The data showed that springiness values of all burger treatments were very close. Cohesiveness, was slightly affected by the type and concentration of millet. Increasing the concentration of millet reduced the cohesiveness of the beef burgers. In general, it was obviously cleared that the control treatment was harder, springier, gummier, more chewy and cohesive compared to all the formulations with added millet. This is an indication that compared to the control, the use of millet in the burgers aided in moisture absorption and retention in the products during cooking. Hence, water holding capacity of burgers containing millet will increase with increasing levels of millet. Akwetey and Knipe [59] reported that consumers tend to buy beef burgers that are not hard, less springy and cohesive, less gummy and easy to chew because harder, gummier and chewy burgers mean more time wasted in masticating and completing a beef burger meal. Hardness reduced from 28.38 (control) to 19.39 (T4) while springiness ranged from 2.76 (control) to 2.39 (T4). Gumminess and chewiness were reduced from 26.56 to 17.73 and from 73.3 to 42.4 for control and T4, respectively. It is concluded that more water was retained in the burgers with more than 10% pearl millet compared to soy or even proso millet, and this resulted in a very soft beef burgers.

Color. Color parameters of raw materials, uncooked and cooked beef burger are presented in Table 6. Uncooked burgers showed that among all treatments, significant ($P < 0.05$) differences in all color parameters were found. These differences are contributed to a large extent with the significant ($P < 0.05$) differences in color elements between soy, proso and pearl millet flour used as raw materials. Uncooked control sample was significantly ($P < 0.05$) darker than burgers containing 20% pearl millet or proso millet flour. For both millet varieties, burgers containing 10 % millet and 10 % soy (T1 and T3) showed higher ($P < 0.05$) a^* values than burgers containing 20 % millet (T2 and T4). This is probably due to the interference effect of a^* value of raw soy flour. Uncooked burgers formulated with proso millet (T1 and T2) were more yellow (higher b^* value, $P < 0.05$) than burgers formulated with pearl millet (T3 and T4). For both millet varieties, as the millet addition ratio increased, the yellowness significantly ($P < 0.05$) increased.

Color analysis of cooked burger indicated that the lowest luminosity (L^*, $p < 0.05$) was observed in the control samples. High ($P < 0.05$) lightness values were found for burgers formulated with proso millet (T1 and T2). This can be attributed to the creamy color of proso millet which is similar to the color of beef fat. However, control sample was more red and yellow (higher a^* and b^* values) ($P < 0.05$) than burgers with 20% flour of both millet varieties. The values of chroma were high ($p < 0.05$) in the control and decreased with the addition of millet flour. High chroma points out to more intensity and fullness of the red color in the beef burger [52]. Significant ($p < 0.05$) differences were detected for hue values between all burgers treatments. Burgers formulated with 20 % proso millet (T2) showed the highest ($p < 0.05$) values of hue. In general, the variations in the lightness and redness values may not referee to a loss in the quality attributes, unless the consumer disagree this parameter when buying beef burgers Brasil et al.[60].

Storage stability. Physical and chemical quality. Physical and chemical quality criteria of burger treatments during frozen storage at – 18 °C are presented in Table 7.Initially, the addition of proso and pearl millet into
burger formulations resulted in a significant ($p< 0.05$) decrease in pH values compared with control sample. This may be due to the differencesin primary composition between the three types of extenders used in burgers formulation. During storage, pH value continuously increased in all beef burger treatments.This may be due to the protein deteriorates and breaks down to some basic compounds (such as volatile nitrogen compounds, amines and hydrogen sulfide), which results in an increase in the pH value Oroszvári et al.[61]

Regarding total volatile base nitrogen, no significant ($p > 0.05$) differences were recorded in TVBN values between all burger treatments at zero time. However, starting from the 1st storage time, significant ($p < 0.05$) differences were observed among burger treatments till the end of storage period. During storage, TVBN of all treatments was progressively increased.By the end of storage periods, control samples exhibited the lowest ($p < 0.05$) TVBN values. After 4 months of storage, TVBN of all treatments were in the range of permissible level reported by the Egyptian Organization Standardization, (EOS)[62] being not more than 20 mg/100 g. This indicates that the incorporation of the millet flour into meat products could retain the chemical quality criteria.

Meat products are rich-fat foods, which contain a high percentage of polyunsaturated fatty acids that is susceptible to oxidation resulting in low quality meat products. Fat oxidation can be prevented using natural antioxidants and thus maintain the quality of meat products for a longer period [12]. Data in Table 7 showed no significant ($p > 0.05$) differences were recorded in TBA values between burger treatments either at zero time or throughout the storage periods. Nevertheless, incorporation of millet into burger formulations resulted in a slight decrease of TBA contents than control sample. Although soy flour contained higher antioxidants compounds (phenols and flavonoids) than both millet varieties, TBA values were higher in control samples than other treatments. This might be due to the high fat content in soy flour. Talukder and Sharma [12] reported that millets contain a good ratio of phenols and tannins that act as antioxidants and its activity has beenproven in foods and biological systems. Thus the incorporation of millet flour in the meat products can enhance their storage stability by scavenging the free radical molecules [63].Thiobarbituric acid values of all beef burger treatments gradually increased during frozen storage up to 4 months. This increase could be basically related to the oxidation of lipids resulted in the formation of some TBA-reactive substances during the storage period as reported by Stahnke [64]. Generally, till the third month of storage periods, TBA values of all beef burger treatments were within permissible levels reported by E.O.S. [62], which recommend that the TBA contents in frozen beef burger should not exceed 0.9 mg malonaldehyde/kg sample.

Microbiological quality . Microbiological quality (cfu/g) of beef burgertreatments is shown in Table 8. It could be observed that at zero time storage,beef burgers formulated with millet flour showed lower counts for TBC, psychrophilic bacteria, coliform bacteria and mold & yeast than control sample. This may be due to washing of millet grains before processing that reduced the microbial load unlike extruded soy.

For all burger treatments, all microbial counts gradually increased throughout the storage time. At the end of storage period, control samples recorded

TABLE 6
Color values of different burger treatments.

Sample	*L**	*a**	*b**	*Chrom*	*hue*
Soy flour	80.84 ^{b}± 0.08	3.12 ^{a} ± 0.05	24.03 ^{a} ± 0.15	24.23 ^{a} ± 0.15	82.61 ^{c} ± 0.06
Proso millet flour	91.14 ^{a}± 0.92	-0.70 ^{c}± 0.1	20.65 ^{b} ± 0.79	20.66 ^{b} ± 0.79	91.96 ^{a} ± 0.35
Pearl millet flour	81.42 ^{b} ± 0.02	-0.05 ^{b} ± 0.02	12.95 ^{c} ± 0.01	12.95 ^{c} ± 0.01	90.22 ^{b} ± 0.06
			surface color of raw burger		
Control	50.26 ^{d} ± 0.39	8.46 ^{c} ± 0.24	16.26 ^{e} ± 0.13	18.33 ^{d} ± 0.3	62.48 ^{c} ± 0.24
T1	51.54 ^{c} ± 0.12	10.78 ^{a} ± 0.08	17.90 ^{b} ± 0.01	20.90 ^{a} ± 0.08	59.01 ^{e}± 0.08
T2	56.28 ^{b} ± 0.2	8.38 ^{c}± 0.18	19.12 ^{a} ± 0.31	20.89 ^{a} ± 0.04	66.32 ^{b} ± 0.06
T3	51.88 ^{c} ± 0.02	9.56 ^{b} ± 0.29	16.86 ^{d}± 0.15	19.38 ^{b} ± 0.14	60.43 ^{d} ± 0.14
T4	57.31^{a}± 0.47	7.44 ^{d} ± 0.16	17.54 ^{c}± 0.2	19.06 ^{c} ± 0.03	66.97 ^{a} ± 0.24
			internal color of cooked burger		
Control	56.35 ^{d} ± 0.06	6.57 ^{a}± 0.23	18.03 ^{a} ± 0.09	19.48 ^{a}± 0.01	70.26 ^{b} ± 0.11
T1	58.47 ^{b} ± 0.14	5.75 ^{b}± 0.23	14.64 ^{d} ± 0.07	17.37 ^{cd}± 0.04	69.18 ^{d} ± 0.07
T2	61.09 ^{a} ± 0.26	4.83 ^{c}± 0.41	16.60 ^{c} ± 0.36	17.17 ^{d} ± 0.23	73.56 ^{a}± 0.16
T3	53.07 ^{e} ± 0.05	6.77 ^{a}± 0.20	17.10 ^{b} ± 0.32	18.39 ^{b} ± 0.13	68.38 ^{e} ± 0.31
T4	57.64 ^{c} ± 0.18	6.06 ^{b} ± 0.21	16.45 ^{c} ± 0.12	17.54 ^{c} ± 0.07	69.76 ^{c} ± 0.04

Values are means and SD (n=5); where: Mean values in the same column with the same letter are not significantly different at 0.05 level.

TABLE 7
Physicaland chemical quality criteria of burger treatments during storage at -18 °C for 4 months.

Storage (months)	Control	T1	T2	T3	T4
			pH		
0	7.01^{a} ±0.05	6.53 ^{c}±0.01	6.54 ^{d}±0.04	6.78 ^{b} ±0.02	6.63 ^{d} ±0.03
1	7.05 ^{a}±0.02	6.55^{d}±0.11	6.58 ^{cd} ±0.06	6.82 ^{b} ±0.01	6.67 ^{c}±0.02
2	7.07 ^{a}±0.08	6.60 ^{c}±0.03	6.63 ^{c} ±0.02	6.85 ^{b}±0.1	6.71 ^{c} ±0.05
3	7.1 ^{a} ±0.12	6.65 ^{c}±0.07	6.69^{bc}±0.09	6.86 ^{b} ±0.13	6.76 ^{bc}±0.01
4	7.12 ^{a}±0.14	6.72 ^{c}±0.04	6.77^{bc}±0.11	6.92 ^{b} ±0.06	6.8 ^{bc} ±0.03
			TVN (mg/100g sample)		
0	9.45 ^{a}±0.33	9.30 ^{a}±0.23	9.06^{a} ±0.44	9.27 ^{a} ±0.39	9.17 ^{a}±0.42
1	10.30 ^{b} ±0.18	10.97 ^{a} ±0.36	10.33 ^{b}±0.27	10.25 ^{b} ±0.41	11.19 ^{a}±0.31
2	11.92^{b} ±0.47	13.08^{a} ±0.51	12.95 ^{a}±0.35	12.09 ^{b} ±0.32	13.23 ^{a}±0.28
3	14.64^{b} ±0.22	15.50 ^{a} ±0.16	15.67 ^{a} ±0.28	14.61 ^{b} ±0.40	15.35 ^{a} ±0.19
4	17.39 ^{c} ±0.13	18.44 ^{a}±0.43	18.60^{a} ±0.50	17.59^{bc} ±0.30	18.20 ^{ab}±0.45
			TBA (mg/kg sample)		
0	0.28 ^{a} ±0.09	0.23 ^{a} ±0.13	0.22 ^{a}±0.1	0.25 ^{a} ±0.08	0.24 ^{a} ±0.04
1	0.34 ^{a}±0.12	0.42 ^{a} ±0.1	0.48 ^{a} ±0.18	0.36 ^{a}±0.23	0.42 ^{a} ±0.15
2	0.54 ^{a} ±0.17	0.65 ^{a} ±0.27	0.69 ^{a} ±0.09	0.49 ^{a} ±0.14	0.58 ^{a}±0.21
3	0.72 ^{a} ±0.25	0.82 ^{a} ±0.07	0.87 ^{a} ±0.11	0.61 ^{a}±0.08	0.74 ^{a}±0.22
4	0.90 ^{a}±0.15	0.99 ^{a}±0.20	1.05 ^{a} ±0.31	0.77 ^{a}±0.26	0.89 ^{a}±0.30

Values are means and SD (n=3); where: Mean values in the same row at the same storage period with the same letter are not significantly different at 0.05 level.

lower counts for all microbial criteria than other treatments. Furthermore, treatments formulated with 10 % millet with 10 % soy showed lower microbial counts than those formulated with 20 % millet. This may be due to the high content of phenols and tannins compounds in soy flour, which may play an important role in retarding the microbial growth, than in millet varieties. When comparing the bacterial counts in burger treatments containing 20% millet flour, it was found that bacterial counts in burger containing 20% of pearl millet (T4) were less than those in burger containing 20% proso millet (T2). In general, microbial quality criteria of all tested samples were within permissible levels reported by E.O.S. [62] till the third month of storage period, which recommend that the total bacterial and coliform group counts not exceed 10^5 and 10^2cfu/g, respectively. Moreover, at the end of storage period, control sample recorded the lowest TBC (8.50×10^4cfu/g) and coliform bacteria (0.82×10^1) counts. Whereas, burger treatments formulated with millet flour exceeded the maximal permissible limits.

CONCLUSION

Being able to grow under harsh climatic conditions and scarce water needs, millet is strongly candidate to overcome water scarcity periods. The results of this study revealed the wide areasfor using millet in the food industry and to produce new value-added products.Both millet varieties(proso and pearl) contain reasonable levels of phenols, flavonoids and tannins, making them a good source of natural antioxidants. Moreover, the results of this study indicated the possibility of using millet to develop innovative meat products that meetthe consumer demands to provide new types of meat products.

TABLE 8
Microbiological counts (cfu/g) of different beef burger treatments during storage at – 18 °C.

Storage	Control	T1	T2	T3	T4
			Total bacterial count (TBC)		
0	2.31×10^{3}	2.04×10^{3}	1.68×10^{3}	1.99×10^{3}	1.83×10^{3}
1	4.63×10^{3}	8.02×10^{3}	4.74×10^{3}	4.44×10^{3}	6.40×10^{3}
2	9.40×10^{3}	2.29×10^{4}	2.07×10^{4}	1.07×10^{4}	1.85×10^{4}
3	1.75×10^{4}	4.07×10^{4}	5.11×10^{4}	3.48×10^{4}	3.89×10^{4}
4	8.50×10^{4}	2.21×10^{5}	2.53×10^{5}	1.09×10^{5}	1.66×10^{5}
			Psychrophilic bacteria		
0	4.01×10^{2}	3.54×10^{2}	2.91×10^{2}	3.46×10^{2}	3.19×10^{2}
1	1.09×10^{3}	1.88×10^{3}	1.11×10^{3}	1.04×10^{3}	1.50×10^{3}
2	1.75×10^{3}	4.27×10^{3}	3.86×10^{3}	2.00×10^{3}	3.45×10^{3}
3	3.03×10^{3}	7.08×10^{3}	8.88×10^{3}	6.05×10^{3}	6.76×10^{3}
4	2.51×10^{4}	6.53×10^{4}	7.47×10^{4}	3.22×10^{4}	4.91×10^{4}
			Coliform group		
0	4.10×10^{1}	3.27×10^{1}	2.55×10^{1}	2.11×10^{1}	1.80×10^{1}
1	5.60×10^{1}	5.31×10^{1}	4.27×10^{1}	2.62×10^{1}	2.93×10^{1}
2	6.20×10^{1}	6.41×10^{1}	6.83×10^{1}	4.24×10^{1}	4.76×10^{1}
3	7.10×10^{1}	7.76×10^{1}	8.88×10^{1}	6.66×10^{1}	7.20×10^{1}
4	0.82×10^{1}	1.18×10^{2}	1.32×10^{2}	1.03×10^{2}	1.09×10^{2}
			Molds and yeasts		
0	4.22×10^{1}	3.41×10^{1}	2.74×10^{1}	2.25×10^{1}	1.97×10^{1}
1	5.72×10^{1}	5.45×10^{1}	4.46×10^{1}	2.76×10^{1}	3.10×10^{1}
2	6.32×10^{1}	6.55×10^{1}	7.02×10^{1}	4.38×10^{1}	4.93×10^{1}
3	7.22×10^{1}	7.90×10^{1}	9.07×10^{1}	6.80×10^{1}	7.37×10^{1}
4	0.94×10^{2}	1.32×10^{2}	1.51×10^{2}	1.17×10^{2}	1.26×10^{2}

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