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Evaluation of the effectiveness of nanomagnesium oxide combined with a statin compound on the microbial content and sensory characteristics of .cold-preserved beetroot juice

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KEYWORDS
,beetroot juice nanomagnesium oxide, statin ,compound microbial ,contamination sensory characteristics

Abstract

The study aimed to try to determine the effect of adding Mg-NPs, statins, or their nanoconjugate on the pH level and microbial contamination by determining the total numbers of microbes and diagnosing the types of microbes contaminating beetroot juice, as well as the sensory characteristics of natural beetroot juice stored for 10 years. days compared to their condition in the control group without supplementation. The results showed that the addition of the above treatments maintained a significant reduction in the numbers of contaminated microbes from the total numbers, the numbers of lactic acid bacteria, and the numbers of cold-loving bacteria, and that they remained within the acceptable limits for human use compared to the juices of the control group, which were unfit for human use at the end of the storage period. . The types of isolates identified in beet juice are Lb type lactic acid bacteria. plantarum and the Gram-positive species Streptococcus thoraltensis. As for the Gram-negative species, the juice was contaminated with Citrobacter freundii, Cronobacter sakazakii, Enterobacter cloaca, as well as the yeast Candida utlius. The diagnosis was confirmed for all species using the VITEK2 Compact device. The above additives also preserved the sensory characteristics of natural beetroot juice until the end of the storage period at 10 days, compared to their condition in the control group without the addition, whose values of the above characteristics were unacceptable for human consumption.

introduction:

Juice represents an important part of the human diet, and natural juice is considered to be the watery liquid, puree of edible parts, or any concentrates of this liquid or puree from one or more fruits or vegetables. Fruit juices are used because they have high nutritional value and medicinal importance, as well as their refreshing nature. It is produced by squeezing fresh fruits or vegetables without using heat, so it is one of the ingredients that participate in providing a person's daily water need (Kaczmarek et al., 2019).

The nutrients available in juices are characterized by their content of vitamins, fibers, minerals, some amino acids, sugars, antioxidants and organic acids, as well as some active compounds depending on the type of juice source, which are of great importance to the cells of the human body (Balkhair and Ashraf (2016).

Beetroot is considered the most famous fruit of the Chenopodiaceae family, which is characterized as a rich source of nutrients, including vitamins B and C, minerals, fibers, proteins, and a variety of biologically active phenolic substances, as well as enzymes, fibers, and antioxidants, including Cumarins, Carotins, Terpins, and Flavonoids. .

Natural beet juice is also considered an important beverage in the food industry, as it is important in the human diet due to its physical and chemical properties of high health importance, as well as the low calories of its components Sentkowska and Pyrzynska, (2022).

The nutrients contained in beetroot juice make it a functional food that can contribute to getting rid of many

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diseases, as it can enhance the maintenance of blood hemoglobin levels and the numbers and shapes of red blood cells, in addition to the body's immune parameters (Wulandary and Hasibuan, 2022). .

The problem of contamination of juices, including beetroot juice, is of great importance due to the multiplicity of microbial species that can use the components of the juice and produce toxins in it, thus causing food poisoning. The problem of contamination increases if ideal health methods are not followed in production or storage, so it can be a source of transmitted diseases. For its consumers. The most important causes of contamination of natural juices with microbes are unclean or contaminated raw materials, as fruits and vegetables can be exposed to contamination with microbes through the soil or water used in their cultivation, in addition to their contamination during the harvesting, transportation and storage processes. Juice extraction processes can also increase the contamination of natural juices with microbes. Cases of contamination through the equipment used, and the most important microbial contaminants could be of the species S. aureus, E. coli, Kelbsiella, Vibrio cholera, Streptococcus spp. and Candida albicans Nicolas et al., (2007).

Nanoparticles, especially MgO-NPs, are small particles with dimensions between 1 and 100 nanometers, with properties and compositions that differ from those of larger particles. It has been used in multiple fields, including medicine and health, and some studies have shown the properties of these substances as antimicrobials for most types of microorganisms that cause diseases and food poisoning, making them effective in preventing infection and the spread of infectious diseases (Ibrahem et al., 2017). From the above, the aim of the study was to determine the effectiveness of biosynthetic magnesium oxide nanoparticles (MgO-NPs) on the microbial content and sensory characteristics of refrigerated beetroot juice

Materials and methods

Sample collection and juice preparation: Beetroot samples were collected from local markets in the city of Tikrit, which were washed directly using soap with distilled water, then dried to remove water residue on the outer surface. The fruits were then mashed using an electric blender, then filtered through several layers of clean, sterilized milling cloth. Collect the juice filtrate into clean, sterile, opaque glass bottles of sufficient size to conduct tests and store in the refrigerator at 4°C until use.

Preparing juices for tests

Fresh beetroot juice was distributed in quantities of 50 ml per bottle, which had a capacity of 100 ml, in sufficient numbers for testing. The experiment was divided into multiple treatments that included: B1: standard beet juice (fresh without any addition), B2: beet juice treatment with 0.02 mg/ml Mg-NPs added, B3: beet juice treatment with 0.025 mg/ml Statins added, B4: juice treatment. Beetroot added 0.02 mg/ml Mg-NPs with 0.025 mg/ml Statins.

Studied tests of juices

Measurement of the pH of juices: The pH value of each of the juice treatments was estimated using a pH-meter in three replicates for each sample. Then record the average of the three readings for each.

Determining the numbers of contaminated microbes and diagnosing their types:

25ml of each juice sample was withdrawn and added to 225 ml of sterile physiological solution in a sterile, tightly closed vial. The dilutions were completed to the fourth dilution of 10-4, after which one ml was withdrawn from each of the last two dilutions and transferred to sterile Petri dishes, to each of which 15 ml of media was added. Prepared and pre-sterilized MacConkey agar, MRS-CaCo3, Nutrient Agar and PDA Agar. The plates were moved in a circular manner to ensure homogeneity of the medium with the juice samples. The plates were planted in three replicates for each dilution of the samples. The plates were then incubated at 35 °C for 24 hours for the bacterial growth medium and at 25 °C for 4-6 days for the fungal growth medium. After the end of the incubation period, the colonies on the plates were counted and the rate of colonies in the replicates for each treatment was calculated. This was followed by purification of the isolates on the same media in which they were isolated to reach the diagnosis of the bacterial isolates by determining the phenotypic, cultural,

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microscopic and biochemical characteristics of each of them (Roberts, D. and Greenwood, 2003).

Evaluation of sensory evaluation of drinks

Sensory evaluation of beetroot juice was conducted immediately after its preparation and after storage at room temperature by nutritional specialists from the Department of Food Sciences at the College of Agriculture - Tikrit University. The evaluation form listed below was used to monitor the values of the juices' sensory attributes, which included color, odor, and taste. Texture and overall acceptance. The weight of each attribute was 20 degrees, as in (Choonhahirun, 2006).

statistical analysis

The data were analyzed using a complete randomized design to study the effect of parameters on some of the studied characteristics, and the significant differences between the means were compared using the Duncan test (18), at a probability level of 0.05, and the statistical program (19) was used in the statistical analysis

Results and discussion

pH level of juices fortified with Mg-NPs alone or combined with statins.

The results in Table (1) show the effect of fortifying beetroot juices with Mg-NPs, statins, or their conjugates after storing them for 10 days at 4 °C. The results showed that the pH values of beetroot juice did not change significantly ($p<0.05$) in the case of fortified juices of all types compared to standard O1 juices without additives, in which the pH level decreased significantly during the preservation period at 0, 5, and 10 days. They were at 5.9, 5.6, and 5.3, respectively. The results converged with what was reported by researchers Domínguez et al., (2017).

This is also the case with beetroot juices, as they also did not differ significantly except in the case of treatment with statins alone, which were at 6.0, 6.2, and 6.5, respectively, compared to the juices without addition in the control group, which were at 6.5, 6.0, and 5.6, respectively. .

The decrease in the pH of the juices could be due to the decomposition of some components of the juices to release organic acids resulting from the increased presence of enzymes decomposing those components, which increases with the increase in contamination with microbial species in the juices. The case of no change in the pH of the juice treatments could be related to the lack of Contamination occurs in those juices that result in a decrease in the state of decomposition of the juice components, which is evidenced by the lack of change in their pH values.

Table 1. pH values for beetroot juice fortified withMg-NPs alone or combined withstatins after 10 days of storage at 4° .C

pH values in juice samples after 10 days of refrigeration storage			Type of transaction	Type of juices
10	5	0		
c ±5.60.52	b 0.75±6.0	a 0.94±6.5	B1	Beetroot juice
a ±6.30.54	a ±6.30.73	a 0.74±6.4	B2	
c ±6.00.71	b ±6.20.64	a 0.81±6.5	B3	
a ±6.30.83	a ±6.40.72	a 0.90±6.4	B4	

Total numbers of microbes contaminating natural beetroot juice

Table (2) shows the total numbers of microbes contaminating natural beet juice fortified with each of the Mg-NPs particles.) B2 or (Statins compounds) B3 or the ( ) one associated with themB4 The results showed that .(the total numbers of microbes, the numbers of lactic acid bacteria, and the numbers of cold-loving bacteria in the) above treatments decreased significantlyp<0.05 as in ,(

ad Different letters on the rates in one line indicate the : presence of significant differences at the probability level .of 0.05

.standard error values =±

B1 ,Standard beet juice treatment :B2 Beet juice : treatment with 0.02 mg/mlMg-NPs added ,B3 Beet juice : treatment with 0.025 mg/mlStatins added. B4 Beet juice : treatment adding 0.02 mg/mlMg-NPs with 0.025 mg/ml Statins .

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numbers were at the highest. At 1.11, 0.96 and 1.04 (log W.T.M./gm), respectively. The numbers above are ,compared to the microbial numbers in the control group whose numbers were 6.78, 3.79, and 3.41 (log .W.T.M./gm), respectively

the case of treatmentB2 at a storage period of 10 days, the microbial numbers were at 1.54, 0.83, and 0.93 (logarithm and. t.m./gm) respectively. In the case of juice treatmentB3 the total numbers of each type of microbes were at the , ,highest, at 2.80, 1.68, and 1.36 (log W.T.M./gm) respectively. In the case of treatmentB4 the microbial ,

Table 2. Total numbers of microbes contaminated with beetroot juice fortified withMg-NPs andStatins after 10 days of refrigerated storage at 4° .C

Total numbers (log W/g) of bacteria contaminating beetroot juice fortified withMg-NPs andStatins after10 days of cold storage			Type of bacteria	Type of juice treatment
Day10	Day5	Day1		
6.78a	4.65b	0.45c	Total numbers of bacteria	B1
3.79a	2.51b	0.04c	Numbers of lactic acid bacteria	
3.41a	3.36a	0.07b	Numbers of cold-loving bacteria	
1.72a	1.22a	0.52b	Total numbers of bacteria	B2
0.95a	0.67a	0.04b	Numbers of lactic acid bacteria	
1.08a	0.47b	0.06c	Numbers of cold-loving bacteria	
2.80a	1.81b	0.58c	Total numbers of bacteria	B3
1.68a	1.32a	0.03b	Numbers of lactic acid bacteria	
1.36a	1.00a	0.06b	Numbers of cold-loving bacteria	
1.11a	0.96a	0.62b	Total numbers of bacteria	B4
0.96a	0.60a	0.03b	Numbers of lactic acid bacteria	
1.04a	0.51b	0.05c	Numbers of cold-loving bacteria	

.Different letters on the rates in one line indicate the presence of significant differences at the 0.05 probability level

it For the number Total For bacteria in Natural juices do not exceed one millionCFU .g/

,The results also showed that added nanoparticlesstatins , or their conjugates caused the juices to be safe for human consumption compared to juices without additives in the control treatment, which were not suitable for human consumption according to the Iraqi standard for juices. This could be due to the ability of nanoparticles to destroy contaminated microbes and reduce the increase in their numbers, as well as increasing this inhibitory ability in the case of treatment with conjugates for both nanoparticles andstatins . This is in addition to the ability of nanoparticles to cellularly destroy the walls of microbes because of Interaction Electrostatic And hacking Direct for

B1 ,Standard beet juice treatment :B2 Beet juice : treatment with 0.02 mg/mlMg-NPs added ,B3 Beet juice : treatment with 0.025 mg/mlStatins added. B4 Beet juice : treatment adding 0.02 mg/mlMg-NPs with 0.025 mg/ml Statins .

By observing the results of the total numbers of types of ,contaminated microbes for the two types of natural juices it was found that the total numbers in the treatments with ,nanoparticlesstatins or their conjugates did not exceed , the limits permitted in the Iraqi standard to be safe for .human consumption according to What she indicated Specification standard Iraqi For standardization And control Quality Which I mentioned Permissible limits With

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.phenotypic, microscopic, and biochemical tests (Table 3) The numbers of isolated microbes were 21 isolates. The bacterial isolates were initially identified based on the phenotypic characteristics of the colonies growing on Mannitol salt agar , MRS Agar and MacConky ,agar , .which are considered differentiation mediaMSA distinguishes Staphylococcus species from each other by distinguishing their ability to ferment mannitol. As forMRS medium , it is optional for types of lactic acid bacteria, as .it inhibits the growth of other types of bacteria

those particles in (microbial cell Mengistu ,.et al2022 All (studies related to the effectiveness of microbial inhibition of nanoparticles in food have proven this effect by .extending their shelf life For the food productFauss 2008 *)*) .

Isolation and identification of bacterial contaminants in juices

The distribution of contaminated microbial isolates for each of the beetroot juices It is diagnosed through

Table 3. Results of microscopic and biochemical tests used to diagnose bacterial isolates contaminating juices

Biochemical examinations													Bacterial species
Hemolysin	H2S _ _	Kligler	Urease	Citrate Utilization	VP	Methyl red	Indole	Catalase	Coagulase	Oxidase	Shape	Gram stain	
N W	N W	NW	N W	N W	N W	NW	N W	-	-	-	Spheric al	-v	*Pediococcus pentosaceus*
N W	N W	NW	N W	N W	N W	NW	N W	-	+	-	Spheric al	+v	*Staph.lentus*
N W	N W	NW	N W	N W	N W	NW	N W	-	-	-	Bac illu s	+v	*Strept. Thoraltensis*
Γ	-	A/A	+	+	+	-	+	+	+	-	Bac illu s	-v	*K. pneumoniae*
Γ	-	A/A	+	+	+	-	-	+	+	-	Bac illu s	-v	*K. oxytoca,*
Γ	+	A/A gas	-	+	-	+	-	+	+	-	Bac illu s	-v	*C. freundii*
Γ	+	K/A	-	+	+	-	-	+	+	-	Bac illu s	-v	*Cronobacter. Sakazakii*
Γ	-	A/A	-	+	-	-	-	+	+	+	Bac illu s	-v	*Sphingomonas paucimobilis*
β/γ	-	A/A gas	-	+	+	-	-	+	+	-	Bac illu s	-v	*Ent. Cloaca*
Use phenotypic and biochemical diagnosis											Ov al	-	*Cand. lusitaniae*

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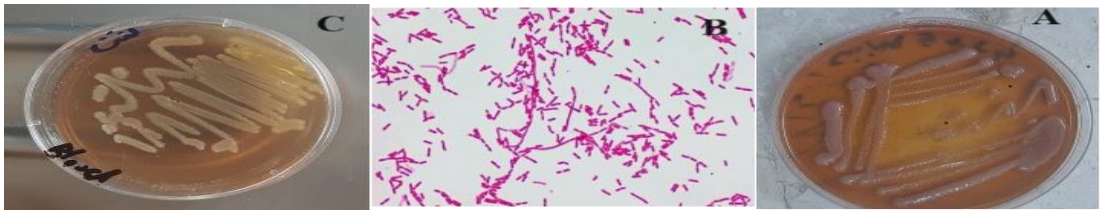
the microscopic characteristics through the shape and ,arrangement of the cells and their receptivity to Gram stain in addition to the results of a group of biochemical tests such as the(IMViC) group of tests and the oxidase and catalase tests, as shown in Table (3), and then confirmatory examination of all isolates using theVitek-2 Compact device was used .

MacConkey Agar medium , as it contains bile salts and Crystal violet dye, which facilitates the growth of negative bacteria without Gram-positive bacteria, in addition to some optional media that distinguish bacterial species from others according to their ability to grow or the formation of a specific color characteristic as an indicative characteristic of that bacterial species. They were classified according to



appearance .1A :*Klebsiella* bacteria Isolated onMacConkey agar medium B :*Sphingomonas paucimabilis* 40X bacteria at magnification. C :*Enterobacter* bacteria *cloacae on*MacConkey agar medium .

contaminated with bacteria of the type*Lb. plantarum* It is a type that is positive for gram stain*Streptococcus thoraltensis.* Gram-negative species may contaminate the .juice of the species*Citrobacter freundii* And *Cronobacter sakazakii* And the type *Enterobacter cloaca* In addition to the type of yeast*Candida utlius .*

Distribution of contaminating microbes in juice samples

Table (4) shows the results of tests for microbes contaminating beet juice samples. The results of the diagnosis of the types of bacteria conducted with the Viteck-2 Compact device showed that beetroot juice was

.Table 4. Types of microbial isolates contaminating beetroot juice samples

Fungi	Gram-negative bacteria	Cocci bacteria	Lactic acid bacteria	Types of transactions
Candida utlius	*Citrobacter freundii, Cronobacter sakazakii, Enterobacter cloaca*	*Streptococcus thoraltensis*	*Lb. plantarum*	Beetroot juice samples

The above results converged with the findings of As explained) Abdel-Azeem .et al , (2019) regarding the ,possibility of contamination of juices, especially beets with the bacterial species*Klebsiella oxytoca* which agreed , .with the results obtained

The results were also similar regarding contamination of beetroot samples with the bacterial species*Enterbater cloacae* With what Aquino-Andrade found),And others 2018(in Mexico. The difference in the presence of causes ,of microbial contamination could be due to various reasons including agricultural practices and the morphological nature of the plant, and the absence of pathogenic microbes on it may be due to it being far from soil and water contamination compared to beets, which are adjacent to the soil, which was confirmed by Ismail,. 2013 . (

ChemotherapyKhan . ,.et al2022 (,In southern Taiwan with the bacterium of beets who reported the contamination *Sphingomonas paucimabili* On the contamination of beets . .with this bacterial speciesFranke et al. , 2005) .

The results agreed with what he found) Ghorbani ,.et al Contamination of vegetables and fruits with (2020*Candida yeast lusitaniae* in beetroot contamination in Japan with , this yeast. The cause of this pollution is irrigation with .contaminated water

As for the contamination of juices with bacteria *Staphylococcus*) it agreed with the results of the study , Askari et al., 2012) .on beetroot contamination Dorotíková et al. , 2022(

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The end of the preservation period is 10 days compared to the juice preservation treatment withoutB1 treatment in , which the sensory characteristics were significantly .reduced

The differences occurring in the values and for all the studied characteristics may be explained by the nature of and the variation in their concentrations their components and sources of production. The results agreed with what . was mentioned above González) ,and others 2023) that there are several factors that may explain the differences and changes occurring in the characteristics and compositions of fermented products among them and their effect on the physiological, sensory, and functional characteristics in general, including the type and composition of the raw materials, the proportions of their components, the bacterial strain present, and the conditions . of the production process

of beetroot juice samples Sensory evaluation .Find out 5 after a storage period of 15 days at 4° .C

The results of the study showed that beetroot juice was contaminated with the bacterial species*Cronobacter sakazakii* .Which was not present in beetroot juice samples The results agreed with those ofLi et al., 2018(who found the spread of the bacterium*Cronobacter sakazakii* on fruits and vegetables. Likewise with what was found in the) Kingdom of Saudi ArabiaHölzel et al., 2018) in the contamination of beets with a high frequency by this .bacterial species

Influence on sensory qualities: The results in Table)5(show the effect of beetroot juice treatments withMg-NPs, statins , or their conjugates on the values of the characteristics chosen for the sensory evaluation of the juice samples. The results showed that the values of the sensory characteristics of natural beet juice, which included the characteristics of appearance, color, texture, flavor, and general acceptance, were the best in their values with the treatments that were supported by both Mg-NPs particles or Statins compounds or their conjugates, which preserved the sensory characteristics of the juicein themeven with

Public acceptance degrees 20	Flavor degrees 20	Texture degrees 20	the color degrees 20	The appearance degrees 20	Storage period (day)	Transactions
14.38d	17.73a	12.85d	14.28c	16.56 c	0	
14.52d	17.65a	11.39e	14.47c	16.29c	5	B1
14.45d	17.60a	11.41e	14.68c	15.71c	10	
16.24b	17.85a	13.88c	16.07b	17.92b	0	
16.92b	17.93a	14.25b	17.11a	18.55a	5	B2
17.34a	17.97a	14.93a	17.38a	18.70a	10	
15.08c	17.45a	14.02b	16.68b	17.11b	0	
15.28c	17.49a	14.57b	17.12a	17.30b	5	B3
15.87c	17.53a	15.00a	17.47a	17.35b	10	
16.24b	17.85a	13.88c	16.07b	17.92b	0	
16.92b	17.93a	14.25b	17.11a	18.55a	5	B4
17.34a	17.97a	14.93a	17.38a	18.70a	10	

B1 ,Standard beet juice treatment :B2 Beet juice : treatment with 0.02 mg/mlMg-NPs added ,B3 Beet juice : treatment with 0.025 mg/mlStatins added. B4 Beet juice : treatment adding 0.02 mg/mlMg-NPs with 0.025 mg/ml Statins .

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