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Influence of natural food preservatives combined with gamma radiation on certain microorganisms isolated from Egyptian juices

M. Z. El-Fouly*; B. M. Haroun; H.A. Hussein*; M.N. Abu El-Naga***

*National Centre for Radiation Research and Technology, Cairo, Egypt
** Faculty of Science, Al-Azhar University, Cairo, Egypt

ABSTRACT

Twelve strains were isolated from different Egyptian juices. The nine bacteria strains were identified as *Micrococcus agilis, Staphylococcus aureus, S. warneri, , S. epidermidis, S. auricularls, Bacillus sp., Pseudomonas aeruginosa, Citrobacter frundii,* and *Streptococcus pedococcus* while the yeast strains were *Debaryomyces sp., Kluveromyces sp* .and *Pichia sp.* Three of the previous strains were chosen in the present work according to their common contamination in all samples and their characteristics; *S. aureus* represented gram positive bacteria, *P. aeruginosa* represented gram negative bacteria and *Debaryomyces sp.* to represent yeast strains. *S. aureus* has completely annihilated by 250 µg/ml. of nisin, or 0.2% citric acid, or 0.15% lactic acid, or 1.2 % cinnamon or 5 kGy of gamma rays.; *P. aeruginosa* was destroyed by 0.3 % citric acid, or 0.3 % lactic acid, or 4 % cinnamon or 4 kGy of gamma rays, while *Debaryomyces sp*. was eliminated by 4 % citric acid, or 4.5 % lactic acid, or 2 % cinnamon or 7 kGy of gamma rays. Nisin alone has no effect on *P. aeruginosa* or *Debaryomyces sp*.

Combined treatments have decreased both of natural preservatives and irradiation doses needed to eliminate the microorganisms contaminated the juices. *S. aureus* was completely eliminated by 3 kGy combined with only 25 µg/ml. of nisin. The lethal dose decreased to 2 kGy by combination with citric, lactic acid and cinnamon at conc. 0.05%, 0.01% and 0.4 %, respectively.thedose level of gamma rays needed to eliminate *P. aeruginosa* decreased to 3 kGy in combination with citric acid 0.1% or with cinnamon 0.5 % and it decreased to 2 kGy by combination with lactic acid 0.1 %. In case of *Debaryomyces sp* the lethal dose decreased from 7 kGy to 4 kGy by combination with citric acid 1.5 % or cinnamon 1 % and to 3 kGy with lactic acid 1.5 %.

Also, the combination treatment has activated the effect of nisin on both of *P. aeruginosa* and *Debaryomyces sp*. Dose level of 4 kGy combined with 200 µg/ml. nisin completely inhibited their growth. At the same time combined treatment greatly decreased the D_{10} values needed to eliminate 90% of the viable count of the tested microorganisms.

Key words. food preservatives, combination treatment , gamma radiation, juices. .

INTRODUCTION

Foods begin to lose their quality from the moment they are harvested. Microorganisms are the main agents responsible for food spoilage. Food preservation prevents deteriorative reaction, extending the shelf life and assuring its safety. Food antimicrobials are usually chemical compounds added to or present in foods that retard microbial growth or kill microorganisms. The functions of food antimicrobials are to inhibit or inactivate spoilage microorganisms and pathogenic microorganisms.

The latter function has increased in importance in the past 10-15 years as food processors search for more and better tools to improve food safety[1].

The uses of chemical preservatives have harmful effects in long term application. The purpose of this article is to use the natural preservatives instead of chemical preservatives or application of high temperature which cause losses of some essential nutrients of the juice. Interest in natural antimicrobials is also driven by the fact that international regulatory agencies are generally very strict about requirements for toxicological evaluation of novel direct food antimicrobials. In many parts of the world, toxicological testing of new synthetic compounds could take many years and many millions of dollars to obtain approval. For some types of food additives a payback may be possible (e.g., artificial sweeteners), but for food antimicrobials it is less likely that obtaining approval would be profitable [2].

Exposure of cells to gamma rays set off a chain of reaction giving rise to chemical and then to metabolic or physiological changes but the lethal effect of ionizing radiation on the microorganisms is primarily due to DNA damage, which destroys the reproductive capabilities and other functions of the cell [3].

Nisin is a 34 amino acid peptide which contains the unusual amino acids dehydroalanine, dehydrobutyrine, lanthionine, and 0-methyl-lanthionine. Nisin binds to the pyrophosphate moiety of lipid II and removes lipid II from its functional location, thereby inhibiting cell wall synthesis, and it induces the formation of lipid Il-nisin hybrid pores in the cytoplasmic membrane[4]. Recent results show that complexion of lipid II is widespread among lantibiotics; however, pore formation depends on the overall length of the peptide and the lipid composition of the test strain membrane[5].

The antimicrobial activity of cinnamon due to its essential oils, which are lipophilic and could penetrate through the membrane to the interior of the cell and perform the inhibitory activity at the target site.

Lactic acid is a primary end-product of the lactic acid bacteria and serves to assist in preservation of many fermented dairy, vegetables, and meat products. It is used as a food additive primarily for pH control and flavoring. Lactic acid was recently shown to permeabilitize outer membrane of bacteria efficiently and causes lipopolysaccharide release.

In the present work gamma rays, as a physical agent, was used alone or combined by each of four natural preservatives namely nisin, cinnamon, lactic and citric acids to destroy the microorganisms usually contaminated the Egyptian juices.

MATERIALS AND METHODS

Source of Juice Samples

Ten juice samples from each product of apple, guava, mango, orange and cocktail were taken from United Food Industries Company - Bader city and Cairo Agro-Processing Company Al-Obour city. Five samples from the end product and five samples from concentrated juices (raw materials). The raw materials were free from any preservatives while the end product contained sodium benzoate and citric acid with concentrations 0.1 % and 0.3 % (wt/v), respectively. Also, the end product was pasteurized at 90 ^{0}C for five minutes approximately.

Source of preservative

Nisin (Lantibiotic) standard supplied from Biomedical Company U.S.A with activity approximately 1000-units/mg powder, Cinnamon (ground state) supplied from commercial source (Egypt), Citric acid and Lactic acid supplied from El- Naser company (Egypt).

Irradiation source

Irradiation was carried out using cobalt 60 irradiation source (Gamma chamber 4000 India, located at National Center for Radiation Research and Technology) with dose rate 8.33 kGy/min. at the time of experiment). D_{10} values of the tested isolates were calculated from the regression linear equation (Lawerence, 1971).

Isolation media

The media used for bacteria were: Nutrient Agar, Nutrient Broth, de Man Rogosa and Sharpe (M.R.S) Agar, Violet Red Bile Agar, Baired-Parker Agar and Peptone water. The media used for yeast were: Sabauroud's Agar, Sabauroud's broth and Yeast extract-malt extract agar (YM Agar)

Identification

Identification of bacterial isolates was carried out according to Bergey's Manual of Determinative Bacteriology 9th ed[6]. While Yeast isolates were identified according to keys of Barnett *et* al[7] and Kurtzman and Fell[8].

RESULTS AND DISCUSSION

Ten samples of each type of chosen juices were collected represented the raw materials and the end product (table 1): they were taken from United Food Industries Company and Cairo Agro-Processing Company in Egypt. The samples represented apples, guava, mango, orange, and cocktail (mixture of apple pulp, mango and peach juice). The end products of apple juices and guava juices were found to be free from microorganisms. It worth to mention that these end products juices were treated with sodium benzoate and citric acid with the dose permissible by the Egyptian authorities i.e. 0.1% for sodium benzoate and 0.3% for citric acid.

Positive results of contamination in the end product of mango, orange and cocktail were observed in spite of pasteurization and the addition of sodium benzoate and citric acid indicating the inefficient of this treatment. Regarding the pulp of the previous fruits (raw material), all of them, as expected, were contaminated either with bacteria or yeast. End product of cocktail juice samples were contaminated with yeast only. On the other hand, the pH of previous samples was found to be ranged from 3.0 to 4.2. The low pH fruit juice samples, especially apple cider and orange juice, have been associated with food borne diseases[9]. The acidic juices are (pH 3.0 and 3.65) a good condition for the growth of yeasts[10].

Forty two colonies could be obtained from contaminated samples according to their morphological shape, four from apple pulp, twelve from guava pulp, five from mango pulp, one from mango end product, ten from orange pulp, and seven from orange end product and three from cocktail juice end product .

As clearly shown from Table (1), the four colonies obtained from apple pulp were identified as two strains of *Micrococcus agilis.* and two strains of *Staphylococcus*. *aureus.* The twelve colonies

of guava pulp were; seven *S. aureus,* one *S warneri,* three *Debaryomyces sp.,* and one *Pichia sp.* The five colonies of mango pulp were; two *S. auricularis* and three *S, epidermidis* while the only colony isolated from mango end product was identified as *Kluveromyces sp.* The ten colonies of orange pulp were; two *Bacillus sp.,* six *Pseudomonas aeruginosa* and two *Citrobacter frundii.* The seven colonies isolated from orange end product were identified as *Streptococcus pedococcus.* The three colonies of cocktail were identified as; two *Debaryomyces sp.* and one *Kluveromyces sp.*

Table (1): Identification of contaminated microorganisms isolated from certain Egyptian juices

sample	Type	Juice micro flora		
		Total isolates	Identified microorganisms	No. of strains
Apple	Pulp*	4	*Micrococcus agilis*	2
			Staphylococcus aureus	2
Guava	Pulp*	12	*Staphylococcus aureus*	7
			Staphylococcus warneri	1
			Debaryomyces sp.	3
			Pichia sp.	1
Mango	Pulp*	5	*Staphylococcus epidermidis*	3
			Staphylococcus auriculars	2
	End prods. **	1	*Kluveromyces sp.*	1
Orange	Pulp*	10	*Bacillus sp.*	2
			Pseudomonas aeruginosa	6
			Citrobacter frundii	2
	End prods. **	7	*Streptococcus pedococcus*	7
Cocktail	End prods. **	3	*Debaryomyces sp.*	2
			Kluveromyces sp.	1
Total		42		

* Pulp = raw juice without any preservatives.

** End product = juice with sodium benzoate preservative

Saccharomyces cerevisia, Saccharomyces sp., Rhodotorula sp; Bacillus cereus, B Subtilis, E. coli, S. aureus, Streptococcus pyogenes and Micrococcus sp .were isolated from orange juice10. While Candida *tropicalis, Hanseniaspora uvarum, Rhodotorula glutins, Zygosaccharomyces bailii* and *Z. rouxii* were isolated from mango pulp11. Also *Candida tropicalis, Zygosaccharomyces rouxil and Rhodotorüla graminis* were occurred in mango juice12. The thermo acidophlic spore-forming *Alicyclobacillus acidoterrestris* were isolated from commercial pasteurized apple juice in the United Kingdom, Germany, and the United States13.Two strains of gram negative bacteria, rod-shaped, non-spore-forming bacteria; *Gluconacetobacter swingsii* and *Gluconacetobacter rhaeticus* were found in apple fruit juice in the region of the Italian Alps14.

According to Table (1), the 42 identified colonies were divided to 12 different strains belonging to 9 genera ; 15 of genus *Staphylococcus,* 6 of genus *Pseudomonas,* 9 of genus *Streptococcus,* 2 of genus *Micrococcus,* 2 of genus *Citrobacter,* 2 of genus*Bacillus,*5 of genus *Debaryomyces,* 2 of genus *Plchia* and two *Kluveromyces.* For further studies, three different strains of above isolates were chosen. The selection was according to their common contamination in all samples and their characteristics; *S. aureus* represented gram positive bacteria, *P. aeruginosa* represented gram negative bacteria, and *Debaryomyces sp.* chosen to represented yeast strains.

1-Physical treatment

Gamma irradiation

Irradiation increases the shelf-life of foods and ensures their innocuousness because most microorganisms in vegetative form are extremely sensitive to irradiation at low doses[15]. As shown in Fig. (1), it is clear that the viable counts of all the selected isolates decreased by increasing the dose of radiation. The D_{10} values of the tested strains were calculated and found to be 0.75 kGy, 0.69 kGy, and 1.48 kGy, for the *P. aeruginosa*, *S. aureus,* and *Debaryomyces sp* , respectively. Indicating that *P. aeruginosa* was the most sensitive strain followed by *S. aureus,* while *Debaryomyces sp* the most resistant strain to gamma rays

Also the obtained results revealed that dose levels 4 and 5 kGy were completely enough to destroy the viable cells of ***P. aruginosa*** and *S. aureus,* while 7 kGy was needed to obtain the same effecting case of *Debaryomyces* strain. Several investigators have reported that fungi are typically more resistant to radiation than are bacteria[16,17]**.** D_{10} values, i.e., the amount of radiation dose necessary to krill 90 % of the initial microbial count, have been reported for yeasts and molds in the range of 1 to 3 kGy as opposed to D_{10} of 0.3 to 0.7 kGy for pathogenic bacteria on produced juices [18,19].

The lethal dose for yeasts, *S. aureus* and ***P. aeruginosa*** were at 4 to 9, 1.4 to 7.0, and 1.6 to 2.3 kGy, respectively[20]. In general, the gram—negative bacteria, including most of the common food' spoilage organisms (e.g., *Pseudomonas),* are more sensitive to irradiation than are the gram positive organisms (e.g., lactic acid bacteria and *micrococci)* and yeasts are more radiation resistant than typical spoilage bacteria. Generally, *Pseudomonas sp.* lacks the inducible error-prone DNA repair system [21,22] In yeast, enhanced radiation resistance is generally believed to be the result of an increase in the capacity or efficiency of a cell to repair its DNA. DNA being the critical target for ionizing radiation- induced lethality. The recombination DNA repair system is considered to be the major DNA repair pathway that confers resistance to killing by ionizing radiation in yeast cells [23].

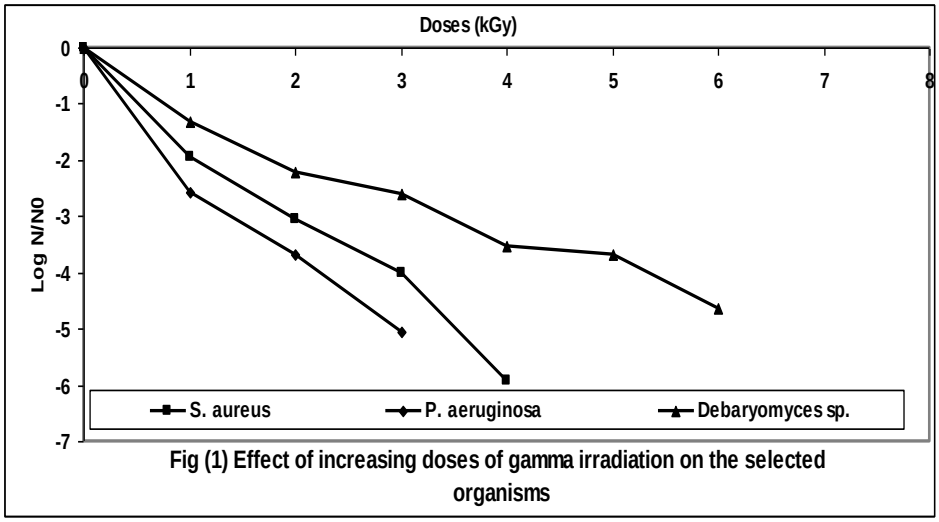

Fig (1) Effect of increasing doses of gamma irradiation on the selected organisms

2-Natural treatments

2.1-Nisin

Nisin is an antimicrobial peptide produced by lactococci and has been used in consumer products for many years. Although this lantibiotic is inhibitory to microorganisms, it is harmless to humans [24].

As shown in Table (2) the effect of nisin on *S. aureus* was examined, when nisin concentration increases the count decreased. At the sub lethal conc. (200 µg/ml) the initially bacteria log count 6.17

was decreased to 1.39, the lethal conc. was at 250 µg/ml. At the same time nisin has no effect on both *Debaryornyces sp* and *P. aeruglnosa.*

The lethality of nisin is due to its effect on the cytoplasmic membrane of vegetative cells and that is the primary site of action of Nisin. The primary mechanism of nisin believed to be the formation of pores on the cytoplasmic membrane which result in depletion of proton motive force (pmf) and loss of cellular ions; amino acids, and ATP[25].Nisin binds to the pyrophosphate moiety of lipid II and removes lipid II from its functional location, thereby inhibiting cell wall synthesis, and it induces the formation of lipid II-nisin hybrid pores in the cytoplasmic membrane resulting in a rapid efflux of cytoplasmic compound[4].

The inability of nisin to attack Gram-negative bacteria such as *P. aeruginosa* is due to the protective outer membrane (OM), which covers the cytoplasmic membrane and peptidoglycan layer of their cells, forming a tight layer endowed with a hydrophilic surface. As a result, OM is a penetration barrier that excludes hydrophobic substances and macromolecule. So nisin, as a hydrophobic macromolecule, is unable to traverse a normal OM and thus cannot reach its target of action[26].

Nisin has no antimicrobial effect on yeasts. These organisms have a rigid cell wall, a complex structure consisting of glucan cross- linked with chitin and cell wall proteins[27].

2.2- Organic acids

Weak acid preservative are generally considered safe antimicrobials, consistent with the long history and widespread use of these compounds for the preservation of foods and beverages. The preservative molecule diffuses into the cell until equilibrium is reached in accordance with the pH gradient across the membrane resulting in the accumulation of anions and protons inside the cell[28].

Therefore, inhibition of growth by weak acid preservative has been proposed to be due to a number of actions including, membrane disruption[29] , inhibition of essential metabolic reaction[30] ,stress on intracellular pH homeostasis [29], and the accumulation of toxic anions [30].

From Table (2) it is clear that 0.2 and 0.3% of citric acid completely destroyed the *S. aureus* and *P. aeruginosa* cells, respectively while *Debaryomyces sp.* needed 4% to reach the same effect .Regarding the lactic acid *S. aureus* and *P. aeruginosa* needed 0.15 and 0.30% to inactivated their cells, respectively while 4.5% was used to eliminate the *Debaryomyces sp* .cells indicating its resistance to organic acids.

2.3- Cinnamon

In recent years, because of negative perception of synthetic additives, consumers are demanding natural and fresh-like food products with guaranteed safety and long shelf- life. For this reason, there is a renewed interest in the use of spices, condiment and plant extracts as alternative food processing method. Several authors reported that cinnamon was one of the inhibitors against pathogens such as *Salmonella sp., Y. enterocolitice* and *S. aureus*[3]. The antimicrobial activity of cinnamon is due to its essential oils, which are lipophilic and could penetrate through the membrane to the interior of the cell and perform the inhibitory activity at the target site.

Table (2) showed that, *p. aeruginosa* was more resistance to cinnamon than S. *aureus* and *Debaryomyces Sp* .cells. It needed 4% of cinnamon for destroyed its cells while 1.2 and 2% were sufficient to reach the same purpose.

3. Combination treatments

Combination with Gamma Irradiation

Attacking various cellular targets will have a synergistic effect by making the organism strain every possible repair mechanism and the activation of stress shock proteins also, becomes more difficult[31]. Gamma irradiation is considered the only known technique capable of ensuring the hygienic quality of raw food in a fresh or frozen state[32] .So, combining this treatment by previous preservatives will give synergistic effects on undesirable microorganisms contaminating food and beverages.

3.1- Combination of Gamma irradiation with nisin

From the previous experiment, application of nisin did not affect either ***P. aeruginosa*** or ***Debaryomyces sp.*** On the other hand, nisin at concentration 25 µg/ml decreased the viable count of *S. aureus* approximately one log cycle in single treatment Table (2). Several investigators have reported about the synergistic effect of combined treatments on microorganisms. The object of the present experiment was to study the effect of gamma radiation combined with nisin on S. *aureus* besides its effect on the cell wall of both of *P. aeruginosa* and *Debaryomyces sp.* which may permit the nisin to enter their cells and hence assist to destroy the cells. In the case of ***S. aureus,*** gamma radiation was combined with 25 µg/ml of nisin. While, 200 µg/ml of nisin was used in case of *P. aeruginosa* and *Debaryomyces sp.*

Data presented in Table (3) clearly showed that, combined treatment of gamma rays and nisin sharply affected the viable count of ***S. aureus*** and dose level of 3 kGy was sufficient to destroy its cells, giving D_{10} value 0.48 kGy, while it was 0.75 kGy in case of single treatment of gamma rays.

As expected from the previous experiment, the results clearly indicated that gamma rays affected the cell wall of both ***P. aeruginosa*** and *Debaryomyces sp.* which permit the nisin to enter their cells giving synergistic effect on destroying the cells of both organisms. In the case of *P. aeruginosa* the data showed that the combined treatment decreased its D_{10} values from 0.69 to 0.45 kGy and hence dose level 3 kGy was quiet sufficient to eliminate its cells instead of 4 kGy in the single treatment.

The same trend was occurred in case of *Debaryomyces sp*.; hence its D_{10} value was decreased from 1.48 kGy in single treatment to 0.68 kGy in combined with nisin. Also, dose level 4 kGy eliminated its cells instead of 7 kGy in the single treatment with gamma rays alone.

It is clear from the previous data that, the highly resistant of *P. aeruginosa* and *Debaryomyces sp.* to nisin become more sensitive to the natural preservative and this may be due to the injuring by irradiation. Irradiated bacteria may be more easily lyses than non-irradiated bacteria[33].

Table (2) :Effect of different conc. of nisin, citric lactic and cinnamon on *S. aureus, P. aeruginosa & Debaryomyces sp*

Microorganism	Nisin		citric acid		lactic acid		Cinnamon	
	conc. ug/ml	Log N*	cond. %	Log N*	Conc. %	Log N*	conc. %	Log N*
S. aureus	0	6.17 A	0	6.92 A	0	6.98 A	0	7 A
	25	5.63 B	0.05	6.02 B	0.001	6.68 A	0.2	6.51 B
	50	4.74 C	0.1	4.11 C	0.01	5.64 B	0.3	-
	100	3.41 D	0.15	2.81 D	0.05	3.06 C	0.4	5.79 C
	200	1.39 E	0.2	** E	0.1	1.59 D	0.6	4.67 D
	250	** F	0.3		0.15	** E	0.8	2.95 E
							1	1.43 F
							1.2	** G
P. aeruginosa	0	6.99 A	0	7.06 A	0	6.99 A	0	6.74 A
	25	6.99 A	0.05	6.64 B	0.001	-	0.5	5.55 B
	50	6.99 A	0.1	5.25 C	0.01	6.46 B	1	4.63 C
	100	6.99 A	0.15	3.72 D	0.05	-	2	3.46 D
	200	6.99 A	0.2	2.44 E	0.1	4.41 D	3	1.93 E
	250	6.99 A	0.3	** F	0.15	-	4	** F
					0.2	2.23 E		
					0.3	** F		
Debaryomyces sp.	0.0	6.61 A	0	6.57 A	0	6.92 A	0	5.84 A
	1.0	6.61 A	1	5.95 B	1.5	5.56 B	0.2	-
	1.5	6.61 A	1.5	4.83 c	2	4.96 D	0.3	5.62 A
	2.0	6.61 A	2	3.96 D	2.5	4.2 E	0.4	-
	2.5	6.61 A	2 5	3.53 E	3	3.63 F	0.6	5.54 B
	3.0	6.61 A	3	2.41 F	4	2.44 G	0.8	-
	3.5		3.5	1.39 G	4.5	** H	1	
	4.0		4	** F			1.2	-
							1.5	3.07 D
							1.7	2.49 E
							2	F **

* The same letters are not significantly different. ** The dash means no distinct growth.

Table (3) Effect of different doze level of gamma rays in combination with nisin on the tested organisms

Irradiation Dose (kGy)	Log viable counts					
	S.aureus		*P. aeruginosa*		*Debaryomyces sp.*	
	Irrad. alone	Irrad + 25 µg/l of nisin	Irrad. alone	Irrad + 200 µg/l of nisin	Irrad. alone	Irrad + 200 µg/l of nisin
0	7.99 A	745 A	7.25 A	6.99 A	7.61 A	6.61 A
0.5	7 B	4.8 C	5.99 B	5.8 B	6.94 B	5.95 B
1	6.06 C	3.81	4.68 C	4.8 C	6.3 C	5.41 C
2	4.92 D	1 .72 E	3.56 D	2.5 D	5.39	3.86 D
3	3.99 E	** F	2.17 E	E	5.0/ D	2.36 E
4	2.07 F		***		4.07 E	** F
5	*** G				3.92 F	
6					2.97 G	
7					***	
D_{10} (KGy)	0.75	0.48	0.69	0.45	1.48	0.68

*The same letters are not significantly different.
** The dash means no distinct growth

Both direct and indirect reactions between ionizing radiation and cellular components occur in direct proportion to the amount of energy that is absorbed. Since 50 to 70% of the cell mass is water, it absorbs much of the radiation. As a result, hydroxyl radicals and hydrated electrons (which are important in irradiation induced cell inactivation) are produced. These radicals damage cell membranes beside protein structures and nucleic acid strands [34]. And hence increase the permeability of the cell wall which permit to the preservative to enter the cell

3.2- Combination of Gamma irradiation with lactic acid

From previous experiment, concentration 0.01, 0.1, 1.5 of lactic acid for *S. aureus, P. aeruginosa,* and *Debaryomyces sp.,* respectively started to give distinct reduction for their growth counts (Table 2).As shown in Table (4), it is clear that, the dose level 2 kGy combined with 0.01 or 0.1 of lactic acid was sufficient to eliminate both S. *aureus* and *P. aeruginosa*, respectively while *Debaryomyces sp.* needed 3 kGy combined with 1.5% lactic acid to reach its lethality. The D_{10} values of the previous organisms were 0.27, 0.40 and 0.47 kGy for S. *aureus, P. aeruginosa* and *Debaryomyces sp.*, in the same sequence comparing by 0.75, 0.69 and 1.48 kGy for the same organisms in the single treatments with gamma rays, respectively. These results clearly indicated the synergistic effect of the combined gamma rays by lactic acid. Also, the dose levels needed for eliminating the tested organisms were reduced from 5 kGy to 2 kGy for *S. aureus* and from 4 kGy to 2 kGy for *P. aeruginosa,* while destroying the cells of *Debaryomyces sp.* needed only 3 kGy instead of 7 kGy in the case of single treatment.

3.3- Combination of Gamma Irradiation with citric acid

It is clear from previous experiment that concentration 0.05, 0.1, 1.5% of citric acid for *S. aureus, P. aeruginosa,* and *Debaryomyces sp.,* respectively started to give distinct reduction for their growth counts (table 2). By combined these concentrations with gamma rays, (Table5) it is obvious that the death value was at 2 kGy for *S. aureus*, 3 kGy for *P. aeruginosa* while *Debaryomyces sp.* requires 4 kGy to destroy its cells. D_{10} was 0.32, 0.58, and 0.75 kGy, respectively for the same organism's combating by single treatments 0.75, 0.69 and 1.48 kGy, respectively.On the other hand, by comparing between lactic acid and citric acid in combination with gamma irradiation, lactic acid was more active against the treated organisms than citric acid and the D_{10} values where distinct decreased from single treatment. This may due to lactic acid has lipophilic character; ability to depress intracellular pH. In addition to membrane diffusion and water activity reduction, specific anion effect, while citric acid lack this character and acting as chelating agent[35].

Table (4): Effect of different dose levels of gamma rays in combination with lactic acid on the tested organisms

Irradiation Dose kGy	Log Number viable counts					
	s. aureus		*P. aeruginosa*		*Debaryomyces sp.*	
	Irrad. Alone	**Irrad + 0.01% lactic acid**	**Irrad. alone**	**Irrad + 0.1% lactic acid**	**Irrad. alone**	**Irrad + 1.5% lactic acid**
0	7.39 A	6.68 B	7.81 A	4.41 B	6.74 A	5.56 B
0.5	6 B	4.04 C	5.74 B	3.41 D	6.94 B	4.11 C
1	5.05 D	1.39 D	4.43 C	2.14 E	6.3 C	2.91 D
2	3.91 D	***E	3.31 D	**F	5.39 D	0.95 E
3	3.99 E		1.92± E		5.01 P	-** F
4	1.06 F		***F		4.00 E	
5	***G				3.91 E	
6					2.96 F	
7					***G	
D_{10} (KGy)	0.75	0.27	0.69	0.40	1.48	0.47

*The same letters are not significantly different.

** The dash means no distinct grow

Table (5): Effect of different dose levels of gamma rays with citric acid acid on tested organisms

Irradiation Dose (kGy)	Log Number viable counts					
	S. aureus		*P. aeruginosa*		*Debaryomyces sp.*	
	Irrad. Alone	Irrad + 0.05% citric acid	Irrad. alone	Irrad + 0.1% citric acid	Irrad. Alone	Irrad + 1.5% citric acid
0.0	7.50 A	6.02 B	7.25 A	5.25 B	7.50 A	5.95 B
0.5	6.6 B	3.25 C	5.74 B	4.91 C	6.83 B	4.99 C
1	5.65 C	1.69 D	4.43 C	4.14 0	6.20 C	4.25 D
2	3.51 D	**E	3.31 D	2.38 E	5.28 D	2.99 E
3	3.59 E		1.92 E	**F	4.9 D	1.65 F
4	1.53 F		*** F		3.91 E	** G
5	***G				3.81 E	
6					2.86 F	
7					***G	
D_{10} (KGy)	0.75	0.32	0.69	0.58	1.48	0.75

*The same letters are not significantly different.
** The dash means no distinct grow

4- Combination of Gamma Irradiation with cinnamon

From the previous experiments, it is clear that dose level 0.75, 0.69 and 1.48 kGy was needed to reduce one log cycle (D_{10} values) of viable cells of *S. aureus, P. aeruginosa,* and *Debaryomyces sp.,* respectively. On the other hand, low concentrations of cinnamon did not affect the viable counts of the tested organisms. Concentration of 0.4% of cinnamon started to decrease the viable counts of *S. aureus* indicating its sensitivity to cinnamon comparing with the *P. aeruginosa* and *Debaryomyces sp.* which started to be affected by cinnamon after concentration 0.5 and 1.0% respectively (Table 2). So, in the present experiment concentration of 0.4, 0.5 and 1.0 % of cinnamon were used combined with gamma rays.

As shown in Table (6), it is clearly obvious that combined treatments reduced the D_{10} values of the tested organisms to 0.31, 0.57 and 1.02 kGy for *S. aureus, P. aeruginosa,* and *Debaryomyces sp.,* respectively. Hence, the dose levels of gamma rays needed to eliminate the previous organisms decreased from 5 to 2 kGy, from 4 to 3kGy and from 7 to 4 kGy for S. *aureus, P. aeruginosa,* and *Debaryomyces sp.,* in the same sequence.

Generally gram-positive bacteria are more sensitive to cinnamon than Gram-negative bacteria. This is probably due to the outer membrane of gram-negative bacteria, which is highly hydrophilic and acts as a strong barrier [36].

CONCLUSION

From the previous experiments it can be reported that the D_{10} of gamma irradiation in single treatment for S. *aureus* was 0.75 kGy which decreased to 0.32, 0.27, 0.31 and 0.48 kGy by combination with citric acid, lactic acid, cinnamon and nisin, respectively.

Also, the D_{10} value of P. *aeruginosa* was 0.69 kGy which reduced to 0.58, 0.40, 0.57 and 0.45 kGy by combination with citric acid, lactic acid, cinnamon and nisin respectively.

The D_{10} of *Debaryomyces sp.* was 1.48 kGy which reduced to 0.75 kGy by combination with citric acid and reduced to 0.47 kGy by combination with lactic acid. Also, this dose was decreased to 1.02 and 0.68 kGy by combination with cinnamon and nisin, respectively.

Table (6) Effect of different dose levels of gamma rays with cinnamon on tested organisms

Irradiation Dose (kGy)	Log Number viable counts					
	S. aureus		*P. aeruginosa*		*Debaryomyces sp.*	
	Irrad. Alone	Irrad + 0.4% cinnamon	Irrad. alone	Irrad + 0.5% cinnamon	Irrad. alone	Irrad + 1% cinnamon
0	7.5 A	5.79 B	7.25 A	5.55 B	6.74 A	4.32 B
0.5	5.39 B	4.44 C	5.99 B	4.77 C	6.07 B	3.99 B
1	4.04 D	2.85 D	4.68 C	3.99 D	544 C	3.57 C
2	.3.91 F	**E	3.65 D	2.17 E	4.52 D	2.72 D
3	3.99 E		2.17 E	**F	4.14 D	1.54 E
4	1.06 F		**F		3.15 E	**F
5					3.05 E	
6					2.7 F	
7					**G	
D10	0.75	0.31	0.69	0.57	1.48	1 02

*The same letters are not significantly different.

** The dash means no distinct grow

GENERAL DISCUSSION

Gamma irradiation enhances the shelf-life of foods and ensures their innocuousness because most microorganisms in vegetative form are extremely sensitive to irradiation at low doses.

The lethal effect of ionizing radiation is primarily due to DNA damage, which destroys the reproductive capabilities and other functions of the cell[37].

On the other hand, 'minimal processing' is a concept describing approaches to food safety and preservation that are designed to retain the natural and as-fresh properties of foods**.** Hence, gamma irradiation can be used in low doses in combination with other preservatives. However, in some circumstances combined treatments are more satisfactory since the dose required for complete sterilization can induce undesirable changes in food flavor or is at higher than permitted level**.** Effective Combinations of two or more preservatives hurdles may be chosen once the modes of action and cellular targets of each treatment are known. As mention previously, nisin, cinnamon, citric acid and lactic acid acts on cell membrane of microbial cell. So, the combination of these preservatives with gamma irradiation it implies more stress will acts on the same target of the microbial cell this refers to hurdles (by placing a number of sub lethal stresses on microbial cell). Hurdles targeting the same elements within the cell have an additive inhibitory effect only, whereas synergistic effects may result from disturbing several functions of the cell [31].

RECOMMENDATION

The obtained result revealed that *Debaryomyces sp*. strain was the most resistant microorganism isolated from Egyptian juices to both of gamma rays, as a physical agent, or nisin, cinnamon, citric and lactic acids, applied as a single treatment. So it is recommended to use a combined treatment of gamma irradiation with any of the previous natural agents according to the kind of the applied juice for its safety and shelf life extension.

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تاثير المعاملات المشتركة للموادالحافظة الطبيعية وأشعة جاما على بعض الميكروبات المعزولة من العصائر المصرية

محيى الدين زهير الفولى*، محمد بكرى هارون، هالة أحمد حسين*، محمد نجاح زايد أبو النجا**

المركز القومى لبحوث وتكنولوجيا الأشعاع – هيئة الطاقة الذرية – القاهرة – مصر

** كلية العلوم – جامعة الأزهر – القاهرة – مصر.*

تم عزل 42 سلالة ميكروبية من خمسة أنواع من العصائر (المنتج النهائى وقبل النهائى) هى التفاح- الجوافة- المانجو- البرتقال والكوكتيل ، ووجد انها تنتمى الى 9 أجناس ، 12 سلالة مختلفة تسع منها تتبع البكتيريا هى استافيلوكوكس أيريوس (9) ، استافيلوكوكس وارنيرى (1)، استافيلوكوكس إبيديرمس (3)،استافيلوكوكس إيريكيولارس (2)، ميكروكوكس أجيلس (2)، استربتوكوكس بيدوكوكس (7)، سيدوموناس إيروجينوزا (6)، باسيلس (2) ، ستروباكتر فرونديای (2) ،بالإضافة الى ثلاث سلالات من الخمائر هى دباروميسيس (5)، بيشيا (2)، كليفيروميسيس (1) .

اختيرت ثلاثة من السلالات السابقة لإجراء التجارب عليها بحيث تمثل الأنواع المختلفة وعلى أساس أعدادها الملوثة للعصائر وهى استافيلوكوكس أيريوس ممثلة للبكتيريا الموجبة لجرام ، سيدوموناس إيروجينوزا ممثلة للبكتيريا السالبة لجرام ، وسلالة دباروميسيس ممثلة للخمائر.

سلالة استافيلوكوكس أيريوس تم القضاء عليها بإستخدام 250 ميكروجرام/لتر من النيسين أو 0.2 % من حمض الستريك أو 1.5 % من حمض اللاكتيك أو 1.2 من القرفة أو بإستخدام 5 كيلوجراى من أشعة جاما.

سلالة سيدوموناس إيروجينوزا تم القضاء عليها بإستخدام 0.3 % من حمض الستريك أواللاكتيك أو 1.2 % من القرفة أو بإستخدام 4 كيلوجراى من أشعة جاما.

أما سلالة الخميرة دباروميسيس فكانت أكثر السلالات مقاومة لفعل المواد الحافظة الطبيعية والإشعاع تم إزالتها من البيئة السائلة بإضافة 4% من حمض الستريك أو 4.5 % من حمض اللاكتيك أو 2 % من القرفة أو تعريضها الى 7 كيلوجراى من أشعة جاما. ولوحظ أن أستخدام النيسين بمفرده لم يكن له أى تأثير مثبط على أى من سلالتى سيدوموناس إيروجينوزا، دباروميسيس.

استخدام المعاملات المشتركة لكل من الإشعاع والمواد الحافظة الطبيعية أدى الى تقليل الجرعات المطلوبة للقضاء على الميكروبات الملوثة للعصائر بشكل ملحوظ. فالقضاء على سلالة استافيلوكوكس أيريوس إحتاج الأمر فقط الى تعريضها الى 3 كيلو جراى من أشعة جاما مع 25 ميكرو جرام من النيسين ، أو التعريض لجرعة 2 كيلو جراى مع حمض الستريك أو اللاكتيك أو القرفة بتركيزات 0.05 ،0.01 ، 0.4 % على التوالى.

وسلالة سيدوموناس إيروجينوزا احتاج التخلص من أعدادها الى استخدام 3 كيلو جراى من أشعة جاما بالإقتران مع 0.1 % من حمض الستريك أو 0.5 % من القرفة وإنخفضت الجرعة الإشعاعية الى 2 كيلو جراى بالإقتران مع 0.1 % من حمض اللاكتيك.

فى حالة سلالة دباروميسيس إنخفضت الجرعة المميتة لها من 7 إلى 4 كيلوجراى بالإقتران مع 1.5 % من حمض الستريك أو 1 % من القرفة ، وانخفضت الجرعة إلى 3 كيلوجراى بالإقتران مع 1.5 % حمض اللاكتيك.

أيضا المعاملة المشتركة مع الإشعاع أدت الى تفعيل تأثير النيسين على كل من سلالتى سيدوموناس إيروجينوزا، دباروميسيس ، فجرعة 4 كيلو جراى بالإقتران مع 200 ميكروجرام من النيسين أدت الى القضاء تماما على الخلايا الحية من كل منهما. ووجد أيضا ان المعاملات المشتركة أدت الى إنخفاض ملحوظ فى القيمة العشرية (د 10) للسلالات الثلاث أى الجرعة الإشعاعية التى تؤدى الى خفض 90 % من اعداد الخلايا الميكروبية.