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Antioxidant properties of synbiotic orange juice with free and encapsulatedprobiotic bacteria

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Abstract

The effect of free and microencapsulated probiotics in orange juice containing 4 % inulin as prebiotic substance was investigated for their effects on phytochemical of juice (total phenolic content, total anthocyanin, ascorbic acid and total carotenoids) and their antioxidant activity of fermented juices: Fermentation seemed to have a negative effect on the polyphenols content, the reduction in total phenolic content in synbiotic orange juice after 5 weeks of storage was 27.05 and 26.07 % for orange juice with free *L. acidophilus* and orange juice with free *L. plantarum*, respectively. While, the reduction in total phenolic content in synbiotic orange juice after 5 weeks of storage was 21.66 and 20.79 % for immobilizedL. acidophilus and immobilized *L. plantarum*, respectively. The reduction in ascorbic acid was less in fermented juices compared to un-inoculated samples. The reduction ranged from (44.70 and 44.30) and (45.11 and 44.70) mg/ 100 ml for synbiotic orange juice with free (*L. acidophilus* and *L. plantarum*) and immobilized (*L. acidophilus* and *L. plantarum*), respectively, after one day of cold storage to (38.94, 38.49, 39.17 and 39.17mg/ 100 ml synbiotic orange juice with free *(L. acidophilus* and *L. plantarum*) and immobilized *(L. acidophilus* and *L. plantarum*), respectively, after 5 weeks of cold storage. Fermentation did not effect on total carotenoids in orange juice during storage. The results reveal thattheantioxidant activity was increased with increasing concentration of orange juice which indicates reducing potential of juices. Also, the antioxidant activity was found to increase with orange juice was inoculated by free and immobilized *L. acidophilus* and *L. plantarum* compared with un-inoculated orange juice. Key words: Lactobacilli, probiotic, prebiotic fermented juice, total phenolic content, ascorbic acid, total carotenoids and Antioxidant activity.

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1. Introduction

Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism [17]. The combination of probiotic and prebiotic in a food product is interesting because in addition to directly introducing live beneficial bacteria to the colon, there is an increase in the number of beneficial Bifidobacteriumand *Lactobacillus* species in the intestinal microbiota with the use of a prebiotic [9,15]. Probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [13]. A prebiotics is defined as a non-digestible food ingredient that, beneficially affects the host by selectively stimulating the growth and/ or activity of one or a limited number of bacterial species already resident in the colon) [14].

Commercial probiotic products include primarily dairy products (fermented milk, yoghurts, ice cream, cheeses), and to a lesser extent non-dairy products (beverages, breakfast cereals, fermented meats, dry-foods) and dietary supplements. Novel products containing probiotics have been launched recently, mainly beverages based on fruits and

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cereals. However, fruit juices are more adverse environments than fermented milks for probiotics as they have high acidity and high levels of phenolic compounds. Consumers, on the other hand demand that the product they purchase contains the concentration of probiotic cells stated on the package at the time of consumption. Therefore, identifying the factors influencing probiotic survival in juices and developing ways to enhance probiotic survival during storage is an important area of research with considerable impact for the food industry [31].

Orange juice (*Citrus sinensis*), which is the most popular fruit beverage worldwide due largely to its widely appealing flavour and nutritional properties [34], is considered a rich source of vitamin C and natural folate. It also contains phytochemicals (e.g. polyphenols and carotenoids) [16, 32].

Soliman et al. (2015) [36] mentioned that Lactobacillus acidophilus ATCC 20552 and Lactobacillus plantarum ATCC 14917 had a good probiotic characteristicsin terms of acid tolerance, bile tolerance, antibiotic sensitivity and antibacterial activity against differentpathogens and could be used as potential functional probiotics in food industry for commercial use.

Encapsulation is a process in which the cells are entrapped within an encapsulating polymer with the aim to reduce cell injury or cell loss and thus improve cell survival [21]. Encapsulation can be used to protect probiotic cells from adverse environments, such as mild heat treatment during processing, or high acidic conditions in the food product [27] and thus it reduces the likelihood of cell injuries and cell death during processing and storage.

Among probiotics beneficial effects, some authors have reported the protection against oxidative stress and the capability to decrease the risk of accumulation of reactive oxygen species [25]. The antioxidant mechanisms of probiotics could be assigned to ROS scavenging, metal ion chelation, enzyme inhibition, and to the reduction activity and inhibition of ascorbate autoxidation) [23, 37]. Probiotic metabolic activities may have an antioxidant effect via the scavenging of oxidant compounds or the prevention of their generation in the intestine [6].

Also, *Ankolekar et al. (2012)* [3] found that the total phenolics in pear juice fermented with *L. acidophilus* was decreased with fermentation and DPPH linked antioxidant activity increased; α-glucosidase inhibitory activity significantly increased for fermented acidic samples.

Pereira et al. (2013) [33] reported that the fermentation provided a good preservative effect on the antioxidant activity and ascorbic acid content of cashew apple juice when compared to the nonfermented juice (control), which confers nutritional benefits to this functional food. *Mousavi et al. (2013)* [28] indicated that the DPPH Radical scavenging studies showed that fermentation of pomegranate juice using selected probiotic starters increased the antioxidant activity significantly.The objective of the present study was investigated for their effects of adding probiotic bacteria in free and encapsulated form in orange juice on phytochemical of juice (total phenolic content, total anthocyanin, ascorbic acid and total carotenoids) and their antioxidant activity of fermented juices by free and microencapsulated probiotics.

2. Materials and Methods

2.1. Preparation of cultures for inoculation into the juice: Free cell pellets preparation:

Lactobacillus acidophilus ATCC 20552 and *Lactobacillus plantarum* ATCC 14917 were obtained from Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

The *Lactobacillus* strains were cultured overnight (17 h) in 10 ml MRS broth from a 1% (v/v) stock inoculum, and incubated at 37 C. This was followed by a subculture step where 100 ml of MRS was inoculated with a 1% inoculum and incubated under similar conditions. The cells were harvested by centrifugation (5000 rpm/15 min), and washed twice with sterile water, then resuspended in 1/10 volume of pasteurized juice [24].

2.2. Microencapsulated cultures preparation:

The washed cells were suspended in 5 ml sterile water. It was mixed with 20 ml of sterile sodium alginate solution (2% w/v). The suspension was placed into a sterile syringe and injected through 0.11 mm needle into sterile 0.05 M CaCl2 solution containing Tween 80. After 30 minutes of gelification, the beads were rinsed twice with sterile water.

Coating with chitosan low-molecular-weight. Chitosan (0.4 g) was dissolved in 90 ml distilled

water and acidified with 0.4 ml glacial acetic acid. The pH was adjusted to between 5.7-6.0 with 1 M NaOH. The mixture was filter through Whatman No. 4. The volume was adjusted to 100 ml and sterilized at 121 °C for 15 min. After that 15 g of washed beads were suspended into chitosan solution and shaken at 100 rpm for 40 min. The coated beads were washed twice with sterile water and kept in sterile water at 4 °C [20].

2.3. Juices preparation:

Valencia orange (*Citrus sinensis*), called summer orange was purchased from local market. Delta Valencia' orange was washed thoroughly in water. The juices were extracted by cutting the fruits into two halfs and carefully squeezing to extract juices by orange extractor (Sonai SH- 256, China). The collected juice was filtered through 4-folds muslin cloth and the juice was collected in clean containers and then filtered toget clear juice to be used freshly.The orange juicewas filled into glass bottles. The bottles were sealed, after a heat treatment (850 C) for held time (10 min.), and the bottles were suddenly cooled using tap water of 24 ° C.

2.4. Fermentation of fruit juice:

Fermentation experiments were conducted in sterile glass bottles, each of them containing 50 ml of pasteurized fruit juice, with 4 % inulin as prebiotic component. In case of adding probiotic bacteria in free form, juices were inoculated with the 18 h cultures (109- 1010 log/ml) and incubated at 37 ◦C. After 48 h of incubation, the fermented samples were stored at 5±2 ºC for 5 weeks. In case of adding probiotic bacteria in microencapsulated form, three grams of microencapsulated beads of each probiotic bacteria were added aseptically into 100 ml of pasteurized juices and stored at 5±2 ºC for 5weeks.

2.5. Enumeration of probiotic microorganisms:

Enumeration of free cell: Viability of probiotic cultures in fermented juice was determined and expressed as colony forming units (cfu/ml) on MRS agar (Oxoid, Milan, Italy). Serial dilutions were prepared in sterile physiological solution before plating onto MRS agar. Plates were incubated at 37 0C for 48 h [11].

Enurmation of immobilized cells: For immobilized cells, Ca-alginate gel beads containing cells were depolymerized in sterile 1% (w/v) sodium citrate solution with gentle shaking for 20 min at room temperature to produce a cell suspension. The cells were then serially diluted and cultured as free cells for colony counting.

2.6.Determination of total phenolic content:

Total phenolic content of all treatments was analyzed according to the Folin Ciocalteu method according to [39]. The results were expressed as milligram per 100 ml gallic acid equivalent (GAE). Calibration curve was carried out with gallic acid aqueous solutions (8-80 µg / ml).

The percent of degraded phenolics during storage of each sample was calculated as follows:

Degraded phenolic (%) = phenolicI – phenolicR /phenolicI X100

Where phenolicI (initial) and phenolicR corresponds to (residual) phenolics (mg/100 ml GAE) before and after storage, respectively.

2.7. Determination of ascorbic acid:

Ascorbic acid content was determined according to the titration method using 2, 6 di-chlorophenol-indophenol as reported by (A.O.A.C., 2012) [1].

2.8.Total carotenoids content:

The total carotenoids were determined according to *Askar and Treptow (1993)* [5] as follows: Ten ml of the juice was mixed with 30 ml of 85% acetone solution in a dark bottle, and left 15 hours before filtering on glass wool into a 100 ml volumetric flask and made up to volume by 85% acetone solution. The absorbance of the acetone extract was measured at 440, 644 and 662 nm against 85% acetone as a blank using (UV-Vis Jenway 6705 Series Spectrophotometer). The amounts of the total carotenoids were calculated according to the following equations:

Chlorophyll (A) = (9.784 x A662) – (0.99 x A644) = mg/l
Chlorophyll (B) = (21.426 x A644) – (4.65 x A662) = mg/l
Total carotenoids = (4.695 x A440) – 0.268 (Chl. (A) + Chl. (B) = mg/l
Where A= Absorbance of the sample at the indicated wavelength

Results were expressed as mg/100 ml sample as such.

2.9. Evaluation of antioxidant activity:

Antioxidant activity of juices by DPPH free radical scavenging assay: The radical scavenging ability of orange juices was tested on the basis of

the radical scavenging effect on the DPPH free radical. The fruit juices (12.5 to 100 µl/ml) were prepared in methanol according to [19]. In clean and labeled test tubes, 2 ml of DPPH solution (0.002% in methanol) was mixed with 2 ml of different concentrations of juices separately. The tubes were incubated at room temperature in the dark for 30 minutes and measured at 517 nm (UV-Vis Jenway 6705 Series Spectrophotometer). The absorbance of the control DPPH was also noted. The scavenging activity of the juices was calculated using the formula:

Scavenging activity (%) = [(A – B) / A] x 100,

where A is absorbance of DPPH and B is absorbance of DPPH and fruit juice combination.

Antioxidant activity of juices by ferric reducing assay: Probiotic juices (12.5 to 100 µl/ml) were prepared in methanol in 1 ml of different concentrations of orange juices separately were mixed in separate tubes with 2.5 ml of phosphate buffer (200 mm, pH 6.6) and 2.5 ml of 1% potassium ferricyanide according to [19]. The tubes were placed in boiling water bath for 20 minutes at 50oC, cooled rapidly and mixed with 2.5 ml of 10% trichloroacetic acid and 0.5 ml of 0.1% ferric chloride. The amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 700 nm after 10 minutes. The increase in absorbance of the reaction mixtures indicates increased reducing power.

2.10. Statistical analysis:

The obtained data of the sensory evaluation were exposed to analysis of variance. Least significant difference (L.S.D) at 5 % level was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System [35].

3. Results and Discussions

3.1. Effect of cold storage on total phenolic content in synbiotic orange juice during storage:

Effect of inoculated of orange juices with 4 % inulin separately by *L. acidophilus* and *L. plantarum* in two forms (free form and immobilized form) on contains orange juice from total phenol content during cold storage at 5 ± 2 ºC for 5 weeks.

Data in Table (1)shows that the initial total phenolic content of un-inoculated orange juice ranged from 38.81 to 35.38 mg GAE/ 100 ml juice after five weeks of cold storage. Fermentation seemed to have a negative effect on total phenol content. In the case of free form of *L. acidophilus* and *L.plantarum*, after 24 h of fermentation the reduction in total phenolic in orange juice was 8.47 and 9.06 % in OAfr4 and OPfr4 samples, respectively, compared with controlsample.

While, in the case of the immobilized form, there was approx. 3.5 % reduction in total phenolic content in orange juice. The reduction in total phenolic content in orange juice after 5 weeks of storage was 27.05 and 26.07 % for OAfr4 and OPfr4 samples, respectively. While, the reduction in total phenolic content in orange juice with immobilized probiotic was less than free probiotic, the reduction in total phenolic content in orange juice after 5 weeks of cold storage was 21.66 and 20.79 % for OAim4 and OPim4 samples, respectively compared with control sample.

Decrease in total phenolic during fermentation was further supported by the work of *Apostolidis et al. (2007)* [4]. The decrease in soluble phenolic content could result from the polymerization of phenolic compounds. Towo et al. (2006) [38] reported that LAB fermentation of cereal products leads to the reduction of phenolic compounds. LAB has a range of enzymes such as β-glucosidase, p-coumaric acid decarboxylase, decarboxylase, which may help in degrading certain phenolic compounds. Also, *Ankolekar et al. (2012)* [3] found that total phenolics in pear juice Fermented with *L. acidophilus* was decreased with fermentation and DPPH linked antioxidant activity increased; α-Glucosidase inhibitory activity significantly increased for fermented acidic samples.

3.2. Effect of cold storage on ascorbic acid content in synbiotic orange juice during storage:

The influence of inoculated orange juice with 4 % inulin by free and immobilized of *L. acidophilus* and *L. plantarum* on ascorbic acid content of orange juice. The data were statistically analyzed ($p \leq 0.05$). Data in Table (2) indicate that the ascorbic acid content was reduced during storage. This reduction was less in fermented juices compared to un-inoculated samples. Degradation in ascorbic acid content ranged in control juice from 44.70 mg/100 ml after one day to 38.72 mg/100 ml after 5 weeks of cold storage.

4	37.36	30.02	29.71	33.83	34.21	33.03[d]
5	35.38	28.31	28.69	30.40	30.74	30.70[e]
Average	37.48[a]	31.44[c]	31.03[c]	34.51[b]	34.58[b]	

LSD Storage period=0.7897 LSD Treatments= 0.7209

LSD(Storage period* Treatments)= 1.147

Type of experiment	Solvent type	Ratio (sample/solvent)	Time (min)	Temperature (°C)	Betalain yield (mg/g sample)
Solvent					
	Water	1:5	10	25	71.4 ± 0.2
	1% CA	1:5	10	25	88.6 ± 0.7
	0.5% CA	1:5	10	25	92.3 ± 0.4
	0.2% CA	1:5	10	25	95.1 ± 0.6
	0.1% AsA	1:5	10	25	63.9 ± 0.6
	50% EtOH	1:5	10	25	72.7 ± 0.3
	20% EtOH	1:5	10	25	75.4 ± 0.6
	0.5% CA + 0.1% AsA	1:5	10	25	98.2 ± 0.5
	0.2% CA + 0.1% AsA	1:5	10	25	101.0 ± 0.4
	20% EtOH + 0.1% CA	1:5	10	25	91.7 ± 0.4
	20% EtOH + 0.5% CA	1:5	10	25	106.3 ± 0.3
Sample/solvent ratio					
	20% EtOH + 0.5 %CA	1:10	10	25	110.7 ± 0.3
	20% EtOH + 0.5% CA	1:15	10	25	112.4 ± 0.3
	20% EtOH + 0.5% CA	1:20	10	25	114.0 ± 0.5
Extraction time					
	20% EtOH + 0.5% CA	1:20	15	25	116.3 ± 0.3
	20% EtOH + 0.5% CA	1:20	20	25	117.5 ± 0.4
	20% EtOH + 0.5% CA	1:20	25	25	118.9 ± 0.4
	20% EtOH + 0.5% CA	1:20	30	25	119.0 ± 0.3
Temperature					
	20% EtOH + 0.5% CA	1:20	30	30	121.2 ± 0.3
	20% EtOH + 0.5% CA	1:20	30	35	124.5 ± 0.2
	20% EtOH + 0.5% CA	1:20	30	40	129.9 ± 0.3

Citric acid (CA), ascorbic acid (AsA), and ethanol (EtOH) was dissolved in distilled water.

Means within a column showing the same letters are not significantly different (P≥ 0.05). OAfr4: Orange juice with free L. acidophilus + 4% inulin
OAim4: Orange juice with immobolized L. acidophilus + 4% inulin
OPfr4: Orange juice with free L. plantarum + 4% inulin
OPim4: Orange juice with immobolized L. plantarum + 4% inulin
O: Orange juice

Table 2: Effect of cold storage on ascorbic acid mg/100 ml in synbiotic orange juice.

Storage period (Weeks)	O	Free cell		Immobilized cell		Average
		OAfr4	OPfr4	OAim4	OPim4	
0	44.70	44.70	44.30	45.11	44.70	44.70[a]
1	44.20	44.40	44.10	45.01	44.60	44.46[ab]
2	44.00	44.20	44.00	44.80	44.40	44.28[b]
3	43.78	43.98	43.57	44.40	43.16	43.78[c]
4	41.08	40.51	40.74	41.19	41.41	40.99[d]
5	38.72	38.94	38.49	39.17	39.17	38.90[e]
Average	42.75[bc]	42.79[bc]	42.54[c]	43.28[a]	42.91[b]	

LSD Storage period=0.386 LSD Treatments=0.353

LSD (Storage period* Treatments) = 0.977

Means within a column showing the same letters are not significantly different (P≥ 0.05). OAfr4: Orange juice with free L. acidophilus + 4% inulin
OAim4: Orange juice with immobolized L. acidophilus + 4% inulin
OPfr4: Orange juice with free L. plantarum + 4% inulin
OPim4: Orange juice with immobolized L. plantarum + 4% inulin
O: Orange juice

While, the reduction in ascorbic acid content was less in orange juice with free and immobilized probiotic bacteria during cold storage. The reduction ranged from (44.70, 44.30, 45.11 and 44.70 mg/ 100 ml for OAfr4, OPfr4, OAim4 and OPim4 samples, respectively), after one day of cold storage to (38.94, 38.49, 39.17 and 39.17mg/ 100 ml for OAfr4, OPfr4, OAim4 and OPim4 samples, respectively) after 5 weeks of cold storage.

The presence of ascorbic acid into vegetable juices submitted to fermentation by probiotic bacteria, especially by *Lactobacillus acidophilus*, is desired not only from the nutritional point of view but also due to the fact that it could promote anaerobic conditions, acting as an oxygen scavenger [18]. Carbon dioxide can directly be produced an anaerobic environment and is toxic to some aerobic food microorganisms through its action on cell membranes and its ability to reduce internal and external pH [8]. Produced carbon dioxide replaces the air and provides anaerobic conditions favorable for the stability of ascorbic acid and the natural color of vegetables [22]. Also, *Pereira et al. (2013)* [33] mentioned that the fermentation provided a good preservative effect on the antioxidant activity and ascorbic acid content of cashew apple juice when compared to the nonfermented juice (control), which confers nutritional benefits of this functional food.

3.3. Effect of cold storage on total carotenoids in synbiotic orange juice:

The influence of cold storage of inoculated orange juice with 4 % inulin by free and immobilized *L. acidophilus* and *L. plantarum* on total carotenoids content of orange juice.

The data were statistically analyzed ($p \leq 0.05$). Data in Table (3) indicate that there were no significant differences between orange juice was inoculated by free and immobilized probiotic bacteria. Also, during cold storage at 5±2ºC, the total carotenoids of orange juice were no significant ($P \leq 0.05$) between all samples at zero time and after five weeks of cold storage. These results are in harmony with *Nazzaro et al. (2008)* [29] mentioned that the presence of probiotic and prebiotic components in carrot juice and found that some biochemical characteristics of the fermented juice, such as β-carotene content and antioxidant activity, were also preserved, indicating that the metabolism of the *Lactobacillus* sp. did not degrade these nutritional components after 4 weeks of storage at 4 ◦C. Also, *Bartkiene et al. (2013)* [7] reported that fermentation of tomato pulp by the bacteriocin-producing lactic acid bacteria (*Lactobacillus sakei* KTU05-6, Pediococcus acidilactici KTU05-7 and Pediococcus pentosaceus KTU05-8)was applied. They found that treatment with LAB breaks down the tomato cell matrix and makes the carotenoids more available, which resulted in higher levels of total carotenoids.

Table 3. Effect of cold storage on total carotenoids (mg/100 ml) in synbiotic orange juice.

Storage period (Weeks)	O	Free cell		Immobilized cell		Average
		OAfr4	OPfr4	OAim4	OPim4	
0	1.83	1.82	1.82	1.84	1.82	1.83a
1	1.84	1.81	1.81	1.87	1.84	1.83a
2	1.82	1.85	1.87	1.84	1.85	1.85a
3	1.83	1.84	1.88	1.85	1.84	1.85a
4	1.80	1.87	1.86	1.87	1.86	1.85a
5	1.81	1.82	1.81	1.87	1.89	1.84a
Average	1.82^{b}	1.83^{ab}	1.84^{ab}	1.86^{a}	1.85^{a}	
LSD Storage period=0.023				LSD Treatments=0.027		
LSD (Storage period* Treatments)= 0.069						

Means within a column showing the same letters are not significantly different ($P \geq 0.05$).
OAfr4: Orange juice with free L. acidophilus + 4% inulin
OAim4: Orange juice with immobolized L. acidophilus + 4% inulin
OPfr4: Orange juice with free L. plantarum + 4% inulin
OPim4: Orange juice with immobolized L. plantarum + 4% inulin; O: Orange juice

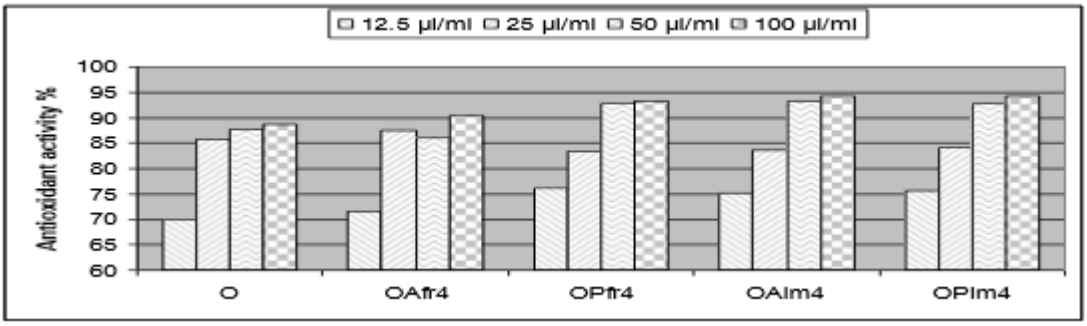

Figure 1. DPPH radical scavenging activity of synbiotic orange juice

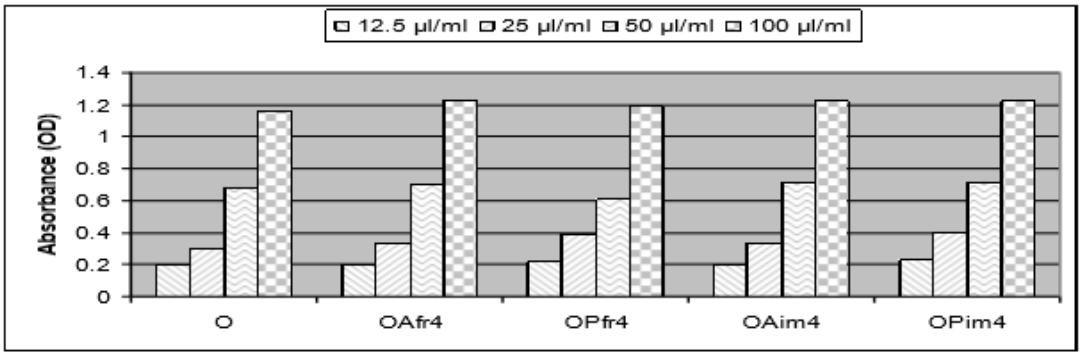

Figure 2. Absorbance (OD) of ferric reducing power (FRAP) of synbiotic orange juice.

3.4. Antioxidant activity of the juices inoculated by synbiotic orange juice in free and immobilized forms:

DPPH radical-scavenging activity: The DPPH• is considered to be a model of stable lipophilic radical. A chain reaction in lipophilic radicals was inhibited by the lipid autoxidation. Antioxidants react with DPPH•, reducing a number of their available hydroxyl groups [41]. The method is based on the reduction of alcoholic DPPH solution shows a strong absorption band at 517 nm appearing a deep violet color. Molecules can quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably by free radical attack) and convert them to a pale yellow or bleached product (i.e. 2, 2diphenyl-1hydrazine) or substituted analogous of hydrazine, resulting in a decrease in absorbance at 517 nm [42]. Hence, the more potent in the antioxidant activity of the extract, in terms of hydrogen – atom – donating capacity. Free radical–scavenging capacities of extracts of orange juice compare with their juices inoculated by free and immobilized *L. acidophilus* and *L. plantarum* were measured by DPPH assay at different concentrations are illustrated in Fig. (1).

DPPH radical scavenging activities (%) were increased with increasing concentration of juice in methanol from 12.5 to 100 µl/ml. In the same Table, antioxidant activity for juices inoculated by free and immobilized *L. acidophilus* and *L. plantarum* were higher than the un-inoculated juices. A high antioxidant activity was observed for orange juice was inoculated separately immobilized *L. acidophilus* (OAim4) and *L. plantarum* (OPim4) was 94.25 % at 100 µl/ml followed by orange juice was inoculated separately free L. plantarum (OPifr4) was 93.25 % at 100 µl/ml

3.5. Ferric reducing power (FRAP) of synbiotic orange juice:

The presence of reluctant such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+} ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [10]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [26]. Data in Fig. (2) show that a marked increase in absorbance with increasing the concentration of fruit juices which indicates reducing potential of juices in methanol from 12.5

to 100 µl/ml in Ferric reducing assay. Also, the absorbance at 700 nm was found to increase with orange juice was inoculated by free and immobilized *L acidophilus* and *L. plantarum* compared with un-inoculated orange juice.

The increase of antioxidant activity of fermented juice are in agreement with those presented by *Wang et al. (2006)* [40] who cited an increase in reducing activity of *Lactobacillus acidophilus* and *Bifidobacterium longam* involved in soybean milk fermentation. Also, *Di Cagno et al. (2011)* [12] who evaluated the effect of lactic acid fermentation on antioxidant properties of red and green smoothies. These authors reported an increased antioxidant activity in the smoothies after fermentation. The increase in antioxidant activity after fermentation by Lactobacillus acidophilus, L. casei, and B. longum was reported by *Ng et al. (2011)* [30], who evaluated the traditional Asian herb Anoectochilus formosanus as substrate for lactic acid fermentation. Also, *Ankolekar et al. (2012)* [3] found that Total phenolics in pear juice Fermented with *L. acidophilus* was decreased with fermentation and DPPH linked antioxidant activity increased; αGlucosidase inhibitory activity significantly increased for fermented acidic samples. Also, *Afify et al. (2012)* [2] demonstrated that antioxidant activity of cell-free extracts of (*Lactobacillus rhamnosus* GG, *Lactobacillus retueria*, *Bifidobacterium breve* and *Probionebacterium freudenreichii* ssp. Shermanii) were detected by DPPH method. They found that all cell-free extracts showed highly scavenging potential against DPPH radical. Also, *Pereira et al. (2013)* [33] reported that the fermentation provided a good preservative effect on the antioxidant activity and ascorbic acid content of cashew apple juice when compared to the nonfermented juice (control), which confers nutritional benefits to this functional food.

4. Conclusion

Fermentation seemed to have a negative effect on the polyphenols content. While, the reduction in total phenolic content in juices when used immobilized probiotic was less than a free probiotic. The reduction in ascorbic acid was less in fermented juices compared to un-inoculated samples. Fermentation did not effect on total carotenoids in orange juice during storage. Antioxidant activity of juices was increased in fermented orange juices compare with unfermented juice.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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