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Preparation of Flavoured Yoghurt with Orange Peel- Oil and Evaluation its Potential Antioxidant, Antimicrobial and Antiviral Properties

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Abstract

Baladi orange is an Egyptian type characterized by its acidic taste. It is usually used for juice manufacture, while orange peel as a by-product, especially in the beverage industry, is an economic issue and environmental pollution problem. Orange peel oil (OPO), antimicrobial, antiviral and antioxidant activity was examined with applying in preparation of flavoured yogurt. Results revealed a positive antibacterial activity of the OPO against *B. cereus, S. aureus, L. monocytogenes,* and showed lower effect against *S. typhimurium, Y. enterocolitica* and *E. coli*. The antiviral activities tested by cytopathic effect on MA104 cell lines and cytotoxicity activity demonstrated that the OPO extract had a potent antiviral activity against rotavirus infection**.** The antioxidant activity tested by the DPPH free radical scavenging activity method revealed that the oils presented higher radical scavenging activity. The oil application was carried out through the preparation of plane and flavoured orange yogurt (0.0, 0.5 and 1.0% oil concentrations) which showed a good sensory evaluation in terms of appearance, texture and taste. The microbiological examination of the resulted orange yogurt during cold storage indicated that the addition of OPO extract did not affect the growth of yogurt starter bacteria, *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* while the oil prevented fungus growth during storage. The OPO has pronounced antimicrobial and antiviral activity and could be used successfully for preparation of flavored yoghurt with good sensory, health and microbiological properties.

Key words: Baladi orange – yoghurt – antimicrobial – oil – *E- coli*

1-Introduction

The Egyptian ministry of Agriculture and Land Reclamation declared Egypt ranked first in the world in the export of citrus fruits, with 3.420 million tons, between oranges and other citrus, while orange recorded 2.56 million tons in 2013/2014 with exports of 1.1 million tons. According to Fresh fruit portal.com (26 October 2020), Egypt becomes largest orange exporter by volume. The orange variety Baladi is a fruit abundant in juice and it is a best variety of juice production. On the other hand, orange peel is a secondary product useful for the kitchen in making sweets and baked goods rich in flavor and orange taste and can be used for various other purposes, but considered an industrial waste product. Natural antimicrobial agents have received a high attention in regard to microorganisms control and as a source of pharmaceutical active compounds, with potential benefits over using upon different applications (Amrita *et al*., 2009 and Marjorie, 1999). Essential oils produced from a wide variety of plants by many different plant materials are rich sources of many biologically active compounds with antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties, and have been applied in different area including foods (Shokoh *et al*., 2020). Essential volatile oils (Eos) of the members of the Citrus which belongs to the family *Rutaceae* are characterized by many biologically active secondary metabolites including flavonoids, limonoids, coumarins, sterols, organic acids, alkaloids, phenolic compounds as well as vitamins, minerals, dietary fibers, essential oils and carotenoids (Marwa*et al.*, 2016). However, EOs of peels were a richsource of natural flavonoids with a high concentration of phenolic compounds that have shown highly inhibitory spectrum against pathogenic bacteria and fungi, along with, antiviral, antidiabetic, anti-cancer and antioxidant activities (Azhdarzadeh and Hojjati, 2016 and Aysegul *et al*., 2020). Moreover, as Citrus EOs was mainly presented in oil

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glands located in the peel, their extraction will provide many advantages as to convert this by-product as a waste to economically valuable products (Viviana *et al.,* 2016)). On the other hand, yoghurt as a fermented dairy product is well considered as a functional food because of its important role in therapeutic, nutritional, dietetic functions and sensory properties due to the symbiotic action of starter bacteria, their metabolites and natural additives (Lubbers *et al.,* 2016 and Pereg, *et al.,* 2015).). the aim of this study was to investigate the potential anti-microbial and antiviral activity of Baladi orange peel oil and applying the resultant oil in preparing of a flavored product as yoghurt. Since pulp, seeds and peel form about 50% of the citrus fruit weight, large quantity of wastes and by-products are generated along with industrial process of orange. The utilization of these huge amounts of citrus wastes in production of value added products such as citrus Eos can be profitable from the economical and health point of views.

2-Material and methods.

Sample collection:

Ripe Baladi sweet orange fruits (*Citrus sinensis*) were purchased from local market in residential quarter of Dokki, Cairo, Egypt.

Extraction of crude Baladi orange peel oil EOs: The crude Baladi orange peel oil EOPOs was experimentally obtained by pressing orange peel manually, at room temperature (Ramgopal *et al*., 2016 and Gorinstein, *et al*., 2001). Briefly, after the whole fresh fruits were thoroughly washed with running water (20 °C), the rind portion of the fruit, or the outermost waxy layer of the fruit's peel in particular, flavedo layer (the pigmented region of the pericarp containing numerous oil glands) was gently scraped using a vegetable peeler to avoid cutting off any of the pith not to make bitterness. The rubbed peels were then collected into a plastic sachet which was subsequently pierced and applied to handily pressing and squeezing to force out all the oil-water emulsion. This emulsion was collected into a sealed vial and considered to be a crude extract containing orange peel oil. It was used freshly either to evaluate its biological activity including antibacterial, antifungal, antiviral and antioxidant effects, or to prepare orange peel oil (OPO)- fortified yoghurt.

Bacterial strains and growth conditions:

Yoghurt culteres,*Streptococcus thermophilus* DSMZ 2479 (*Str .thermophilus*) and *Lactobacillus delbrueckii subsp. bulgaricus* DSMZ 20080 *(L.bulgaricus)* were obtained from the Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

The pathogenic bacteria indicator strains including three Gram- positive strains (*Staphylococcus aureus ATCC 2023 (S. aureus), Bacillus cereus ATCC 33018(B. cereus)and Listeria monocytogens (L. monocytogens)* and the same number of Gram-negative bacteria, namely *Escherichia coli 0157:H7 ATCC 6933(E. coli), Salmonella typhimurium ATCC 14028(S. typhimurium) and Yersinia enterocolitica(Y. enterocolitica) , as well as* a fungus strain *of Aspergillus flavus* ATCC 16872(*A. flavus*), were provided from Laboratory of Dairy Microbiology, Dairy Science Department, Food Industries and Nutrition Research Division, National Research Centre, Dokki, Cairo, Egypt.

The yoghurt stater cultures (*Str. thermophilus* and *L. Bulgaricus*) were subcultured weekly in sterile 10% non-fat dried milk and incubated at 37°C for 24 hrs. Between transfers the cultures were stored at 4°C. Before use, the stock was activated by three successive transfers.

All bacterial pathogens were maintained and propagated in Brain Heart Infusion (BHI) broth at 37°C for 24 h, while *A. flavus* was activated in yeast extract peptone dextrose broth (YPD) andincubated at 25°C for 48 hrs, to be activated tillobtaining the concentration of 4 log10 cfu/ml **(Delavenne *et al.,* 2015 and Sourav *et al.,* 2021)**. For antimicrobial assay, the inocula of the test indicators were prepared from overnight old broth cultre, where serial dilutions were done to give a final concentration of approximately 10^6 CFU/ml of each.Different bacterial media were purchased from Oxiod, UK, while the other analytical chemicals were obtained from Sigma, USA.

Antibacterial and antifungal activity of orangpeel oil:

Agar well diffusion method described by **Lyon *et al,* (1993) an Akl *et al,* (2020)**was followed with slight modifications. In brief, The previously prepared BHI agar plates were spreaded with the test indicator strains separately using sterile swab sticks. After absorption, the sterile 6-mm cork borer was used to make wells on the agar film with equal depth. The wells were then filled up with approximately 100 µL of the freshly crude orange peel oil. The plates were incubated at 37 °C for 24 h. In the case of *Aspargillus flavus*, a spore suspension (10^6 spores/ml) was prepared and 100µl of it was spread on YPD agar dishes which were afterwords treated as the same above and incubated for 5 days at 25 °C. The diameter ofinhibition zones was measured in mm around the wells to measure the degree of indicators sensitivity. the control inoculated plates were prepared as the samedescribed but withnb sterilized distilled water. The experiments were carried out in triplicates.

Orange peel oil antiviral activity:

Antiviral via Cytotoxicity evaluation:

Evaluation of Cell morphology by using inverted light microscopy (**Quang *et al.* 2017).** MA104 cell lines (2x105 cells/ml) were seeded in 96-well tissue culture plates (Corning, US). After incubating the seeded

plates for 24 h at 37ºC in a humidified 5% CO2 atmosphere, the culture medium was discarded from each well and replaced with 200 µl of compound dilutions, of orange peel oil, per well prepared in culture medium. For cell controls, 200 µl of culture medium without compounds was added. All culture plates were incubated in a humidified 5% CO_2 atmosphere at 37ºC for 72 h. Cell morphology was checked daily for microscopically detectable morphological changes, such as cell rounding and shrinking, loss of confluences, and cytoplasm granulation and vacuolization. Morphological alteration was scored and the 100% safe dilutions against the cell morphology were selected for the antiviral assay.

Antiviral via cytopathic effect:

For $TCID_{50}$ determination, the 100% safe dilution from each compound (orange peel oil) was selected to be evaluated against RV infection. 10-fold dilution of activated RV SA-11 were prepared in cell culture medium then100 µl of viral dilutions 10^{-4} – 10^{-9} were incubated with 100 µl of each of cell culture containing the compound for one hour at 37oC in CO2 incubator. Virus dilutions either with or without compound were added into four parallel wells. All plates were incubated at 37oC in CO2 incubator for 72 h, then the cytopathic effect was observed under inverted microscope and virus titration was calculated and expressed as 50% tissue culture infection dose ($TCID_{50}$) by using Spearman Kärber method (**Wulff*et al.*, 2012)** ,The reduction in virus titre was calculated as differences between the values of treated and untreated virus.

Antioxidant activity assay oforange peel oil :

The scavenging activity of DPPH free radicals were measured according to **(Zaho *et al.* 2008).** DPPH solution was prepared by weighing 4mgof DPPH 100ml methanol (it has a violet colour). 4ml of DPPH solution and 0.5ml of the extract were mixed. The mixture was shaken vigorously and left in the dark to stand at 30ºC for 30 min. 4ml of methanolic DPPH solution against methanol served as control. Decolorization of the methanolic DPPH solution was determined by measuring the decrease in absorbance at 517 nm using a spectrophotometer model (UV VIS Spectrophotometer PG Instruments United Kingdom) and DPPH was calculated according to the following equation:

Scavenging rate = [1- (A1-A2)] × 100

Where A1 represents the absorption of the sample PC extract A2 represents the absorbance of control.

Preparation of orange peel oil flavoured-yoghurt:

To develop an orange peel oil, OPO,flavoured-yoghurt, whole buffalo's milk obtained from Animal Production Research Institute, Agricultural Research Centre, Ministry of Agriculture, Dokki, Cairo, Egypt, The milk was standardized to 3% fat, then heated at 85^{0}C for 30 min and then cooled to 43- 45^{0}C and inoculated with 3% starter (*Str.thermophilus* and *L.bulgaricus* (1:1, v/v), and incubated at 42±1^{0}C until complete coagulation, cooled at 42 ^{0}C for 18 hr according to **Mohammed *et al.*, (2019)** and devided into three portions. The control (T1) and the experimental yoghurts treatments, T2 and T3, where OPO were added with 0, 0.5 and 1.0% of freshly crude citrus peel oil extract, respectively. They were dispensed into sterilized plastic cups and incubated at 43°C till coagulation (pH 4.6). The yoghurt samples were then cooled, maintained refrigerated at 4°C, and microbiologically, pH values and Sensory evaluation were determined when fresh and after 3, 7, 15 and 21 days of cold storage.

Microbiological analysis:

For microbial counts, 10 g of yoghurt samples were homogenized with 90 mL of 0.1% sterile peptone water for initial dilution. Decimal dilutions were prepared with the same diluents (9 mL) and appropriate dilutions were made prior to pour-plating in duplicate onto agar media as follows:

Enumeration of lactic acid bacteria:

According to **Tharmaraj and Shah (2003),** *Str. thermophilus* was enumerated on M17 agar after being incubated aerobically at 37°C for 24 h, while *L. bulgaricus* was enumerated on MRS agar adjusted to pH 5.2, and incubated at 43°C for 72 h.

Total colony count:

Total colony count (TCC) was determined using plate count agar (**FDA, 2002).** After 48 h of incubation at 35 ±1 ºC, colonies forming units were accounted and calculated per g.

Detection of *Staphylococcus aureus:*

Detection of *S. aureus* in the orange yogurt samples was carried out by spreading 0.l ml of each of appropriate dilutions onto the surface of different selective agar medium according to **(FDA, 2002).** The plates were incubated at 37°C for 24-48 h.

Determination of coliform:

Coliform bacteria were enumerated according to **Leclercq *et al*., (2002)** using violet Red Bile agar (VRBA). The plates were incubated at 37º C for 48 h.

Mold and yeasts count:

Enumeration of mold and yeasts was determined using acidified potato dextrose agar (final pH 5.6). Plates were incubated at 25º C for 5 days **(FDA, 2002).**

Determination of pH:

The pH values were determined using digital pH meter (HANNA instruments, Portugal) with glass electrode.

Antiviral activity of orange peel oil against *Rotavirus, RV*:

Cell culture and the *Rotavirus*:

MA104 monkey kidney cells and simian rotavirus SA-11 stock were obtained from the Department of Virology, National Institute for Cholera and Enteric

Diseases (NICED), Kolkata, India. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of heat inactivated fetal bovine serum(FBS), 100 unit's /ml penicillin, 100µg/ml streptomycin under 5% CO2 humidified incubator (All purchased from Lonza, Belgium). Simian rotavirus SA-11 stock was pre-activated with trypsin 10 mg/ml trypsin for 30 min at 37 ºC. The diluted tenfold of activated RV stock was replicated in MA 104 cells and the cytopathic effect was checked after 72 h of incubation. The 50% tissue culture infectious doses/0.1 ml (TCID50/0.1ml) was estimated as described previously by karber method **(Wulff *et al.*, 2012)**, then stored in small aliquots at – 80 o C until used.

Antiviral activity of orange peel oil against RV SA11 by TCID50:

The cytotoxicity evaluation of OPOs was investigated by MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] method, as described by **Nabil *et al.* (2012).** The MA 104 cell lines were grown in 96-well plate at concentrations of 5 x 10^3 cells /well. After 24 h incubation, the culture medium was removed from all wells and the antiviral assays were performed using $TCID_{50}$assay as described by **Shaheen et al., 2014.** In brief, 10-fold dilutions of pre activated RV (10^6 log10 $TCID_{50}$/ml) were prepared. Each dilution was mixed with equal volume of a non-toxic dose of orange peel oil and incubated for 1 h at 37 ºC. A hundred µl of the above mixture was added to the MA 104 cells in 96-well plates. after a 1h incubation, the mixed solution was removed from wells and the cell culture were rinsed twice using phosphate buffer saline (PBS) and incubated with 200 µl of test medium containing 2 µl of trypsin. Two controls consisting of virus control wells (untreated-infected cells) and cell control wells (untreated non-infected cells) were included in the three experiments. All plates were incubated for 72 h at 37ºC under 5% CO_2 atmosphere. Ten wells were used for each dilution of the virus either with or without oil. The difference between the values of virus with oil and in absence of oil is equal to virus titer reductions.

Sensory evaluation:

The organoleptic properties including flavor 40 points; body and texture 30 points and appearance 30 points was carried out for the orange peel oil-fortified yoghurt according to **Methane *et al*., (2000).** The organoleptic evaluation was done by staff members of Dairy Sciences Department, National Research Centre.

Statistical analysis:

All experiments and analysis were done in triplicate. Data were statistically analyzed using GLM procedure of **SAS**. **2006** software (Version 9.2). Level of significance between treatments was determined by Duncan test. Probability of <0.05 was considered as significantly different.

Results and discussion:

Antimicrobial activities of orange peel oil against food borne microorganisms:

Results of the orange peel oil as shown in **Table (1)** indicated the highest antibacterial activity was against the Gram-positive; *B. cereus*, then *S. aureus and L. monocytogenes*, while it revealed lesser effect on the Gram-negative bacteria; *S. typhimurium*, *Y. enterocolitica* and *E. coli*. Orange peel oil did not show any effect (inhibition zone: 0.0 mm) on the growth of fungus and yeasts, compare with control (without oil). Similar results were obtained by **Anna Gerad *et al.*, 2017** for the antibacterial effect of orange peel oil on the Gram negative bacteria. However **Monia *et al*., 2009** stated that the oils and extracts containing flavonoids are likely to contribute to the antifungal activity**.** In this context, many studies have been conducted on the antimicrobial activity of oil extracts from different plants and their multiple uses, both in health and food applications (Amel *et al*., 2016, Ramgopal *et al*., 2016,Saidat et al., 2018, Verica and Petar2014) and Samy *et al* (2021)

Antiviral activities of orange peel oil against Rotavirus (RV), by measurement of cytopathic effect:

Rotavirus (RV) is one of the major causative pathogens associated with severe diarrhea and can results in death in children below five years of age**.** As shown in Table (2) the current results demonstrated that the orange peel oil has potent antiviral activities against rotavirus infection. The extract showed the higher protection effect for MA 104 cell lines from the harmful effect of virus against these cells, reducing the virus titres by 2.25 $\log_{10}$ $TCID_{50}$. The study suggested that the incubated and activated virus with orange peel oil showed previous cellular infection inhibiting effects of these compounds that may be caused by the oil extract interactions with the viral cap, which prevents the virus from entering the host cell. The obtained results were in line with findings reported by **Mohamed *et al*., (2014),** who **i**nvestigated antiviral properties of orange peel methanolic extract against herpes simplex virus type 1 and 2 (HSV-1 and 2) in vitro by direct plaque assay, where the results showed 100% inhibition against HSV-1 and 2 at 200, 250 and 300 µg/ml. Also similar lines were studied by Roberta *et al.,* (2019) and Damian and Uzochukwu (2020), for the antiviral activity of the oils and natural products against hepatitis A Virus and others. However, more investigations on the effects of these compounds on the oils on the other steps of virus replication are required.

Table 1:
Antimicrobial activities of orange peel oil (OPO) on food borne microorganisms(inhibition zone in mm)

Samples ext.	*B. cereus*	*E. coli O111:H2*	*Salmonella typhy*	*Y. enterocolytica*	*Staph.aureus*	*L. monocytogens*	*A. flavus*
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OPO	12.0	5.0	7.5	3.0	11.0	10.5	0.0

Table 2
Antiviral activity of orange peel oil (OPO) on RV SA11 by $TCID_{50}$/ 0.1 ml measurement

Sample ID	Non-toxic dilution of OPO[≠]	Virus titres without OPO[¥]	Virus titres with OPO[¢]	Reduction value of virus titres [*]
Extract	10^{-4}	10^{6}	$10^{3.75}$	$10^{2.25}$

[≠] 100% safe dilution of each compound; [¥]Titre of Positive control; [¢] Titre of rotavirus when incubated with the nanoparticles prior to infection; [*]Reduction of virus titre was calculated as "virus titre without extract - virus titre with extract ".

Antioxidant activity of orange peel oil by the DPPH free radical scavenging activity:

Results in Table (3) revealed that 10 µL of the prepared extract (orange peel oil) showed a great ability to scavenging the hydrogen radical of the DPPH, with increasing the amount of extract by the ability increase when comparing with the synthetic antioxidant. This is attributed to the antioxidant activity was in pronounced correlation to the level of α-tocopherol which indicates that the oils with higher concentrations of α-tocopherol presented higher radical scavenging activity. The current results agree with those reported by **Malacrida *et al.* (2012),** while evaluating the antioxidant activity of citrus oils, as they found the highest antioxidant activity from the oil, obtained from Pera-rio orange seeds (54.2%). However, in other investigations, antioxidant of citrus oil extracts and oil from oregano leaves showed a higher antioxidant activity with an IC50=2±0.1 mg/L (Monia *et al.*, 2009 and Fatiha *et al.*, 2011). Furthermore, The characteristics of different plant oils and extracts are summarized, with particular attention to their chemical composition, antioxidant , antimicrobial biological activities and different potential applications Verica and Petar ., 2014, Saidat et al (2018) and Aicha Hennia et al., 2018).

Microbiological examination of OPO -flavored yogurt when fresh and during cold storage:

Results in Table (4) show the microbiological quality of yogurt with *Str. thermophilus* and *L. bulgaricus* as the starter culture were not affected either by inhibition or stimulation using orange peel oil additions for treatment 1 (0.5%) or treatment 2 (1.0%) compared to the control group (free from orange oil), during the 21-day cold storage period. While numbers of *Str. thermophilus* and *L. bulgaricus* were between 8.47 - 8.9 log cfu/ml and 7.90 - 8.9 log cfu/ml, respectively, not noticeable affected either by storage period or orange peel oil addition in both of the 2 treatments (T1, 0.5 and T2, 1.0%). However, the addition of orange peel oil to yogurt showed inhibition of fungus growth during the total storage period, while fungi appeared in control samples after 21 days of storage. Microbiological analysis to verify the safety of all yoghurt samples proved to be free from all pathogenic bacteria as well as coliform group bacteria. However, the obtained yoghurt with orange oil showed the higher quality than those in Cairo and Giza markets as reported by **Salwa and Galal (2016)**, and showed similar microbiological quality; in terms of total bacterial count, coli group, yeasts and fungi as well as long shelf life, as reported in many investigations (**Akubor 2016; Salama, et.al., 2019 and Okda et al., 2018).**

Sensory analysis of OPO-flavored yogurt during cold storage:

Sensory evaluation of the arbitrators as shown in Table (5) clarify the sensory quality of yoghurts in orange oil and show the high affinity between the arbitrators which ranged from 96 to 98% for the total scores. The results of the general appearance showed high scores ranging from 28 to 26 out of 30 points, from the first day and then declined slightly to the end of storage (15 days) at the refrigerator temperature. Also, results of body and texture showed high scores among the arbitrators ranging from 30 to 26.5 out of 30 points from the first day and then declined slightly to the end of storage.

Same high quality for taste was given by the arbitrators as the score ranged from 39.5 to 37.0out of 40 points from the first day and then declined slightly to the end of storage. As such, the results obtained for sensory evaluation showed that yogurt with orange oil in the current study outperformed traditional yogurt as well as yogurt with other oils in terms of texture, appearance and taste as reported by **Fatiha et al., (2017), Akubor, (2016) and Natalia *et al*., (2015).**

Table 3

Antioxidant activity orange peel oil (OPO) by the DPPH free radical scavenging activity Method

orange peel oil (the prepared extract /µL)	DPPH scavenging activity %
10	83.5
20	84.9
30	94
40	94
50	94.1

Table 4

Microbiological examination of orange yogurt in treatments when fresh and during cold storage*

Storage time		Microbial counts (log cfu/ml) / treatments			
	Orange concentration	T.C	Mold & yeast	*Str. thermophilus*	*L. bulgaricus*
Fresh	Control (0%)	$7.0^{B}\pm0.17$	0^{B}	$8.90^{A}\pm0.11$	$8.86^{A}\pm0.10$
	Treatment 1 (0.5%)	$7.79^{A}\pm0.25$	0^{B}	$8.70^{B}\pm0.27$	$8.83^{A}\pm0.21$
	Treatment 2 (1%)	$7.8^{A}\pm0.08$	0^{B}	$8.70^{B}\pm0.22$	$8.71^{B}\pm0.19$
3 days	Control (0%)	$7.17^{C}\pm0.11$	0^{B}	$8.78^{A}\pm0.11$	$8.77^{A}\pm0.27$
	Treatment1 (0.5%)	$7.30^{B}\pm0.01$	0^{B}	$8.51^{B}\pm0.31$	$8.61^{B}\pm0.07$
	Treatment2 (1%)	$7.95^{A}\pm0.16$	0^{B}	$8.47^{B}\pm0.07$	$8.66^{B}\pm0.21$
7 days	Control (0%)	$7.0^{C}\pm0.31$	0^{B}	$8.69^{A}\pm0.19$	$8.90^{A}\pm0.09$
	Treatment1 (0.5%)	$7.3^{B}\pm0.21$	0^{B}	$8.60^{B}\pm0.04$	$8.62^{B}\pm0.08$
	Treatment2(1%)	$7.47^{A}\pm0.02$	0^{B}	$8.56^{B}\pm0.11$	$8.61^{B}\pm0.21$
15 days	Control (0%)	$7.04^{C}\pm0.01$	0^{B}	$8.55^{A}\pm0.13$	$8.38^{A}\pm0.52$
	Treatment1 (0.5%)	$7.25^{B}\pm0.19$	0^{B}	$8.30^{B}\pm0.40$	$8.27^{A}\pm0.19$
	Treatment2 (1%)	$7.36^{A}\pm0.14$	0^{B}	$8.43^{B}\pm0.21$	$8.30^{A}\pm0.17$
21 days	Control (0%)	$6.95^{C}\pm0.31$	$1.5^{A}\pm0.12$	$7.96^{A}\pm0.17$	$7.92^{A}\pm0.28$
	Treatment 1(0.5%)	$7.20^{B}\pm0.17$	0^{B}	$7.88^{B}\pm0.29$	$7.90^{A}\pm0.01$
	Treatment 2 (1%)	$7.32^{A}\pm0.41$	0^{B}	$7.86^{B}\pm0.13$	$7.91^{A}\pm0.04$

*all samples were free from pathogenic bacteria and coliform group.

Means with the different capital (A, B, C…,) superscript letters within the same column are significantly ($P\leq0.05$) different between control and treatments for each storage period.

Table 5

Sensory properties of yoghurt containing orange peel oil (0.5%) during storage period at 5 ±2 C° for 15 days

No. of Person	**Appearance 30%**				**Body & texture 30%**				**Flavor 40%**				**Total score**
	1 day	5 day	10 day	15 day	1 day	5 day	10 day	15 day	1 day	5 day	10 day	15 day	
1	28.5 A	27.5 B	27.0 B	27.0 B	30.0 A	29.0 B	28.0 C	27.5 C	39.5 A	38.0 B	37.5 B	37.0 B	98
2	28.0 A	28.0 A	27.5 A B	27.0 B	29.5 A	29.5 A	27.5 B	27.0 B	39.5 A	39.0 A	37.5 B	37.0 B	97
3	29.0 A	29.0 A	28.0 B	28.0 B	28.5 A	28.0 A	27.5 B	27.0 B	39.0 A	38.0 B	37.0 C	37.0 C	96.5
4	29.0 A	28.5 A	28.0 B	26.0 C	29.0 A	28.5 A	28.0 B	28.0 B	38.5 A	37.5 B	37.0 B	36.5 C	96.5
5	29.0 A	28.0 B	28.0 B	27.0 C	29.5 A	28 B	27.0 C	26.5 C	39.5 A	39.0 A	38.5 AB	37.5 B	96

Means with the different capital (A, B, C…,) superscript letters within the same row are significantly ($P\leq0.05$) for each parameter.

Conclusions

The current study confirmed the pronounced antimicrobial, antiviral (RV virus), and antioxidant activities of the orange peel oil. This oil was in synergism with some lactic acid bacteria as *Str. Thermophilus* and *L. bulgaricus*. And by using this oil in preparing orange yogurt, a fermented milk product was obtained that was good for its sensory and health properties. Therefore, it could be recommended for using applications like these for its probiotic synergistic properties.

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