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Physicochemical and microbiological properties of functional Labneh fortified with mandarin peel powder during refrigeration storage

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Abstract
This study was planned to use mandarin peel powder (MPP) as a natural source of bioactive components for the manufacturing of functional Labneh. To achieve this proposal, four batches were inoculated with 2% of *Streptococcus thermophilus* and *Lactobacillus helveticus* CNRZ 32 (1:1). The first batch (control) was manufactured without MPP and the other batches, P1, P2 and P3, were fortified with MPP at the levels of 1.5, 3 and 5%, respectively. The highest components found in mandarin peel oil (MPO) were limonene, beta-pinene, and beta-myrcene, at percentages of 94.56, 1.53, and 1.46%, respectively. The sotal solids, carbohydrate and ash content were significantly increased with increasing the concentration of MPP, while it had no significant ($p<0.05$) influence on protein and fat content. Significant differences were noticed in pH values among all the Lanbeh samples. The P3 fortified with 5% MPP showed a significantly higher level of chemical analysis compared with the other treatments. The counts of *S. thermophilus* and *Lb. helveticus* CNRZ 32 increased in the first week of storage to range between 9.64 and 9.95 log CFU/g respectively, and were still above 108CFU/g until the end of the storage period. Finally, the addition of MPP at 3% resulted in the highly accepted organoleptic properties of functional Labneh without any defects.

Keywords: functional labneh, bioactive components, mandarin peel powder, limonene

Introduction
The consumption of dairy functional foods has increased because these foods have therapeutic effects besides their nutritional values. Consumers are increasingly interested in functional foods that are high in probiotics, natural antioxidants, pigments, organic acids, flavonoids, antimicrobial compounds, and fibres (Mahato *et al*., 2019; Mabrouk *et al*., 2020; Palanivelu *et al*., 2022).Functional foods have different biologically active components that can reduce the risk of diseases and enhance human health [1]. Labneh (concentrated yoghurt) is a popular fermented dairy product consumed as a main dish in the Middle East area and European countries [2, 3]. Citrus peels (mandarin, lemon, orange, and grapefruit) contain valuable substances such as pigments, sugars, organic acids, flavonoid compounds, antioxidants, enzymes, antimicrobial compounds, and fibers. Therefore, they could be used in bioprocessed products with higher health benefits [4]. There has been an intensified demand for functional fermented dairy products fortified with bioactive compounds from citrus peels like phenolics and flavonoids in recent decades because of their health effects and nutritional values [5, 6, 7]. Moreover, fortification of dairy products with polyphenols, antioxidants and anti-inflammatory activities originating from natural sources has increased depending on the consumer's demand [8, 9]. Mandarins are an important group of citrus fruits, classified into different varieties, mandarin hybrid groups, and sub-groups [10]. Mandarin peels are byproducts that represent about 50-65% of the fruit weight that is lost after the juice extraction and is considered a huge load on the environment [11, 12]. The disposal of these by-products leads to a loss of potential revenue and increases the cost of disposal of products [13]. Citrus peels contain high quantities of dietary fibers and flavonoids, and essential oils that exhibit various antifungal, antimicrobial, and antioxidant activities [14, 15, 16]. Additionally, MPP can be used as a source of synthetic antioxidants that are used to extend the shelf life of foods containing fats and oils, imparting health benefits to the consumer [17]. Also, the consumption of citrus fruits and peels with foods containing high antioxidant activities and the stimulating effects of lactic acid bacteria will contribute to the regulation of metabolism by intestinal microflora [18]. The production of processed cheese with essential dietary nutrients from mandarin peel powder was done [19]. Labneh was made from milk retentate with a high total solids content, which was a good vehicle for beneficial bacteria and other functional ingredients because it protects them when added to them [20]. Therefore, The objective of our study was planned to use of MPP as by product for producing high nutritive functional Labneh and study the chemical, microbiological and sensory properties of resultant Labneh under refrigeration storage.

Materials and Methods

Mandarin fruits were purchased from vegetable karkets in El-Fayoum governorate, Egypt. Mandarin peel powder was prepared as follows: Fresh whole peels was washed well with tap water, dried at 50ºC cabinet drier, grinded by electric grinder (BRAUN, MultiQuick5 Vario Type 4191). The powder was saved at 40 mesh size, packed and stored at 4ºC until use [21]. The composition of mandarin peel powder were protein 4.19%, ash 3.31% and moisture 9.5%.

Starter origin and preparation

The starter cultures containing *S. thermophilus* and *Lb. helveticus* CNRZ 32 were obtained from dairy science department (microbiology lab), National Research Centre, Cairo, Egypt. The strains were propagated twice in sterilised reconstituted skimmed milk (12% Total solids) for 24 h for preparing the adding starter.

Functional Labneh manufacture

The concentrated buffalo's milk (Total solids 28.55% and fat 14%) was obtained from dairy processing pilot plant at dairy department, Faculty of Agriculture, El-Fayoum University. Labneh was manufacture as the method mentioned by [22] with some modifications. The milk was divided into four equal portions (1 kg for each), and heated individually to 90º C for 5 min then cooled to 42º C. The first portion used as a control (C) inoculated by 2% of *S. thermophilus* and *Lb. helveticus* CNRZ 32 (1:1) without MPP. The other portions P1, P 2 and P 3 were supplemented with MPP at the level of 1.5, 3 and 5%, respectively then, inoculated by 2% of *S. thermophilus* and *Lb. helveticus* CNRZ 32 (1:1). Afterwards all inoculated treatments were gently mixed and filled in sterilised plastic containers then, incubated at 42º C until complete coagulation. The resultant Labneh were stored at 5 ± 2º C for 28 days for further analysis. Physicochemical, microbiological and sensory evaluation was conducted an overnight after production and each week of refrigeration storage. All analysis of Labneh samples were in three replicate.

Mandarin essential oil extract

The essential oil was extracted from mandarin peel powder according to the method described by [23] with some modifications. About 20 g of MPP mixed with 100 ml of ethanol 98.8 % in sealed glass bottles at room temperature for 24 h with continuous stirring. After that, the extraction was filtered through filter paper (Whatman No 1). Then, the filtrates were concentrated by using rotary evaporator for removing the solvent. The concentrated essential oils of peel samples were transferred to small test tube and the pure oil layer was carefully run out by micropipette and put into a sterile Eppendorf then kept at 5°C until used in the analysis.

Gas chromatography-mass spectrometry analysis (GC-MS)

The extracted essential oil sample was determined according to the method of [24]. Gas chromatography mass spectrometry (GC-MS, Agilent Technologies) using a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The essential oil sample was diluted with hexane (1:19, v/v) then the GC was equipped with HP-5MS column of 30 m length x 0.25 mm internal diameter and 0.25 μm film thickness. Analysis was performed using helium as a carrier gas, flow rate 1.0 mL/min at a split ratio of 1:10, injection volume of 1 µl. The following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50–550 and solvent delay 5 min Identification of different constituents was confirmed by comparing the spectrum fragmentation patterns with those stored in mass spectral library database (National Institute of Standards and Technology and Wiley libraries).

Chemical analysis

The pH values of samples were measured using a digital pH metre (Adwa, AD1000 Romania. Titratable acidity, fiber, ash and moisture contents and total protein as nitrogen content were determined as described in [25].The apparent viscosity was measured using a Brookfield digital viscometer (Middleboro, MA 02346, USA). Viscosity measurements were expressed as centipoise (cP.s) according to [26].

Microbiological analysis

Viability of *Streptococcus* and *Lactobacillus* strains

Ten grams of resultant Labneh samples were homogenized in 90 ml of sterile saline (0.85% NaCl w/v) then; the homogenate was serially diluted up to 10^{-8} [27]. One milliliter from each dilution plated onto sterile Petri dishes in duplicate after that, M17 agar and de Mann Rogosa Sharpe (MRS) agar were poured for *S. thermophilus* and *Lb. helveticus* CNRZ 32 respectively [28]. The plates were incubated anaerobically for *Lactobacillus* and aerobically for *Streptococcus* at 37°C for 48 h. The result was expressed as colony forming units per g (CFU/g).

Mould and yeast counts

Mould and yeast counts in functional Labneh samples were determined using rose bengal chloramphenicol agar medium (Oxoid) according to [29]. The plates were incubated at 25ºC for 3 days. The result was expressed as colony forming units per g (CFU/g).

Total coliform counts
Total coliforms were enumerated on violet red bile agar medium (VRBA) according to [30]. The plates were incubated at 37 ºC for 48 h.

Sensory evaluation
Labneh samples were examined for organoleptic properties by 10 panelists from the staff members at dairy department, faculty of agriculture, Fayoum University. All functional Labneh samples were evaluated when fresh and during the storage period every 10 days according to [31]. All samples were scored organoleptically for flavour (60 points), consistency (30 points) and appearance (10 points). Three replicates for each treatment were selected.

Statistical analysis
Results were expressed as means of at least three replicates. All obtained data were subjected to statistical analysis using General Linear Models (GLM) were performed using [32] for windows, version 19 software packages. Significant differences among treatments, storage period and interaction mean between them were compared at ($p \leq 0.05$) level of significance using Duncan's multiple range test.

Results and discussion
Chemical composition of mandarin peel oil
The chromatogram of mandarin peel oil (MPO) presents in Figure 1. and chemical composition of mandarin peel oil (MPO) is shown in Table 1. The GC-MS results presented that the 10 identified components accounted for 100% of the total amount of oil. Peel oil contented almost exclusively of hydrocarbons with limonene as the major component (94.56%) with beta pinene (1.53%) and beta-myrcene (1.46%). 10-Undecyn-1-ol, trans-.beta.-Ocimene, Cyclopropene, 3,3-diethyl-, Santolina triene, Germacrene D, (E)-.beta.-Famesene, 1,11-Dodecadiyne and (Z, E)-.alpha.-farnesene were identified in the sample at low amounts. The obtained results of HPLC analysis was agreement with those founded by [33, 34]. They found the same composition of essential oil and limonene is the most abundant component in the essential oil extracted from orange peel.

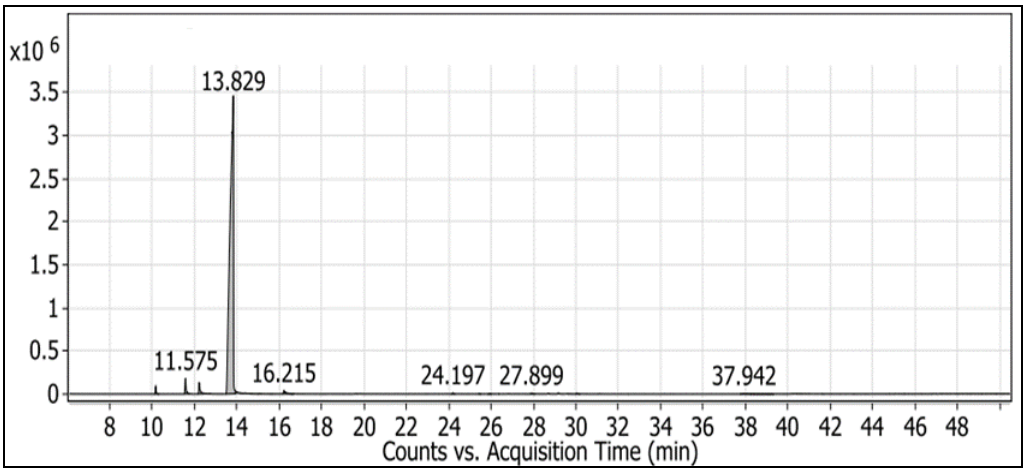

Fig. 1: The GC-MS chromatogram of mandarin peel essential oil.

Table 1: Chemical composition of essential oil of mandarin peel powder.

Compounds	Molecular formula	Retention time (min)	%
trans-.beta.-Ocimene	C10H16	10.167	0.64
beta-Pinene	C10H16	11.575	1.53
beta.-Myrcene	C10H16	12.215	1.46
Limonene	C10H16	13.829	94.56
10-Undecyn-1-ol	C11H20O	16.215	0.74
Santolina triene	C10H16	24.197	0.12
(Z,E)-.alpha.-farnesene	C15H24	25.445	0.06
1,11-Dodecadiyne	C12H18	25.954	0.07
(E)-.beta.-Famesene	C15H24	27.899	0.13
Germacrene D	C15H24	30.045	0.11
Cyclopropene,3,3diethyl	C7H12	37.942	0.57

Physicochemical properties of functional Labneh fortified with mandarin peel powder
Data presented in Table 2. refers to the chemical composition of functional Labneh with different ratios of mandarin peel powder. The total solids, fat, protein, ash, carbohydrate and dietary fiber contents of Labneh

treatments ranged from (28.65 to 31.97%), (13 to 13.07%), (9.21 to 9.23%), (1.77 to 2%), (4.27 to 7.47%) and (0 to 0.46%), respectively. Total solid, carbohydrate and ash content was significantly increased with increasing the concentration of MPP in P1, P2 and P3 compared with the control samples. The addition of MPP had a significant ($p \leq 0.05$) effect on total solids, carbohydrate and ash, probably due to increase the content of carbohydrate (80%), ash, fiber (7.21%) and total solid in MPP relatively increase in dry matter [21], while it had no significant ($p < 0.05$) influence on protein and fat contents of Labneh. As shown in (Tables 2) the viscosity of Labneh was significantly increased ($p \leq 0.05$) by increasing the addition of MPP, which could be attributed to the increase in total solids and high pectin contents of MPP which act as thickenerand gelling agent. The results are agreement with [2, 35, 36].

Table 2: Physicochemical properties of fresh functional Labneh fortified with mandarin peel powder.

	Treatments				
Parameters	**C**	**P1**	**P2**	**P3**	**Std. Error**
TS%	28.65^{d}	29.71^{c}	30.88^{b}	31.97^{a}	0.051
Protein%	9.21^{a}	9.27^{a}	9.39^{a}	9.23^{a}	0.022
Fat%	13.00^{a}	13.02^{a}	13.05^{a}	13.07^{a}	0.018
Ash%	1.77^{d}	1.88^{c}	1.94^{b}	2.00^{a}	0.005
Carbo. %	4.27^{d}	4.95^{c}	6.08^{b}	7.47^{a}	0.065
Fiber%	0	0.12^{c}	0.33^{b}	0.46^{a}	0.004
Viscosity mPa.s	72.51^{d}	84.37^{c}	96.03^{b}	126.00^{a}	15.16

-C: control without MPP; -P1: Labneh with 1.5% MPP; P2: Labneh with 3% MPP; -P3: Labneh with 5% MPP. Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

The data in Table 3. showed that, the acidity of functional Labneh was ranged from 1.47% to 2.97% for treatments (C) and (P3) respectively at fresh storage time, then increased to 2.15% to 4.47 for treatments (C) and (P3) respectively at the end of storage periods. There were significant differences ($p \leq 0.05$) in acidity content among all fresh treatments and during storage periods. pH values were ranged between 4.38 and 4.70 for treatments (C) and (P3), respectively at fresh of manufacturing, at the end of storage period, the pH values were decrease in all treatments, reached to 3.86-4.14 for treatment (C) and (P3) respectively, but there were significant differences ($p \leq 0.05$) among all samples. The effects were noticed in acidity and pH values after the addition of mandarin peel powder compared with the control. The acidity of Labneh was high compared with that reported by [37]. The trend of the pH values of all functional Labneh treatments was opposite to that of acidity. The acidity development in functional Labneh treatments as a result of activity and metabolism of added starters which tearned the residual lactose into lactic acid. Labneh made with mandarin peel powder developed higher acidity than control without MPP. The increase in acidity and decrease in pH of functional Labneh during storage has been reported by [3, 38].

Table 3: Changes in acidity (%) and pH values of functional Labneh fortified with mandarin peel powder during storage.

Treatments	**Acidity%**					
	Storage period (day)					
	fresh	**7**	**15**	**21**	**28**	**Treatment effect**
C	1.47^{p}	1.67^{o}	1.8^{n}	2.1^{m}	2.15l^{m}	1.84^{D}
P1	1.73^{no}	2.20^{i}	2.4^{k}	3.4^{g}	3.65^{e}	2.68^{C}
P2	2.47^{k}	2.82^{j}	3.2^{h}	3.8^{d}	3.92^{c}	3.24^{B}
P3	2.97^{i}	3.15^{h}	3.55^{f}	4.2^{b}	4.47^{a}	3.67^{A}
Storage effect	2.16^{E}	2.46D	2.74^{C}	3.38^{B}	3.55A	
pH values						
C	4.7^{a}	4.53^{b}	4.38^{f}	4.26^{h}	4.14^{k}	4.4^{A}
P1	4.53^{b}	4.45^{d}	4.22^{i}	4.17^{j}	4.07i	4.29^{B}
P2	4.49^{c}	4.41^{e}	4.19^{j}	4.05l^{m}	3.92^{n}	4.21^{C}
P3	4.38^{f}	4.32^{g}	4.03^{m}	3.93^{n}	3.86^{o}	4.12^{D}
Storage effect	4.53^{A}	4.43^{B}	4.21^{C}	4.10^{D}	4.00^{E}	

-C: control without MPP; -P1: Labneh with 1.5% MPP; P2: Labneh with 3% MPP; -P3: Labneh with 5% MPP. -Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

Microbiological analysis

From the obtained results, coliforms group, moulds and yeasts were not detected in all treatments and control of functional Labneh during the storage period. This might be due to the efficient heat treatment of milk and retentate, which inhibits the vegetative cells, also the sanitation, hygienic conditions during the manufacturing process of the Labneh. Peel of mandarin showed antimicrobial activities against most common G-, G+ bacteria and fungi according to [39, 40, 41]. In fact mandarin oil showed antibacterial activity against *Candida albicans,*

Escherichia coli, Listeria innocua, methicillin-resistant S. aureus and Staphylococcus aureus [42]. The oils may be recommended as safe-based antimicrobials for improvement of shelf life of food. Data presented in Table 4. Showed that, the *Lb. helveticus* CNRZ 32 counts in functional Labneh with different ratios of MPP significantly ($p \leq 0.05$) increased in the first week of storage to range between 9.64 and 9.95 log CFU/g and remained in the same log until the end of storage period. Moreover, at the end of the storage time, viable counts were decreased ranged between 8.89 and 9.42 log CFU/g but in the safe level for probiotics to donate a healthy effects to the human. In addition, these data exposed that the used MPP affects the viability of *Lb. helveticus* CNRZ 32 in Labneh during storage period the interactions among probiotic bacteria and citrus fibers in probiotic products. These results agreement with those results recorded by [18] reported that, citrus fruit juices and their peels could be used in the production of functional foods and probiotics, for sustaining and developing vitality of probiotic microorganisms and for enriching products in terms of phenolic constituents. also, [43] found that citrus fibers *enhanced Lb. acidophilus* CECT 903 and *Lb. casei* CECT 475 survival in MRS broth during refrigerated storage. The high viability of added probiotics during storage can be attributed to the high TS of Labneh. The increase in TS offers the protection of growing microorganisms [44], which may explain our finding. In addition**,** [45] reported that storage time plays an important role in the extent of overall proteolytic activity, and consequent increases in the amount of liberated amino acids may cause a higher growth rate of probiotic bacteria even in an acidic environment. Increasing MPP in the Labneh amount significantly ($p \leq 0.05$) affected in *S. thermophilus* counts as Table (4). Initial *S. thermophilus* count in Labneh was about 10.12-9.88 log CFU/g and then continuously reduced to reach 8.06 and 8.77 log CFU/g using 5% of MPP and control, respectively, at the end of storage.

Table 4: Survival of lactic acid bacteria (log CFU/g) in functional Labneh fortified with mandarin peel powder during storage.

Survival of lactic acid bacteria (log CFU/g)						
Treatments	***Lb. helveticus* CNRZ 32**					
	Storage period (day)					
	fresh	**7**	**15**	**21**	**28**	**Treatment effect**
C	9.51^{g}	9.64^{ef}	9.38^{h}	9.16^{j}	8.89^{i}	9.31^{D}
P1	9.67^{ef}	9.76^{cd}	9.59^{fg}	9.42^{h}	9.03^{k}	9.49^{C}
P2	9.87^{ab}	9.85^{bc}	9.72^{de}	9.52^{g}	9.28^{i}	9.65^{B}
P3	9.92^{ab}	9.95^{a}	9.88^{a}b	9.66^{ef}	9.42^{h}	9.77^{A}
Storage effect	9.74^{B}	9.8^{A}	9.64^{C}	9.44^{D}	9.15^{E}	
S. thermophilus						
C	10.01^{b}	9.86c^{de}	9.34^{f}	9.07^{gh}	8.77^{i}	9.41^{A}
P1	9.88^{cd}	9.81^{de}	9.3^{f}	9.02^{h}	8.67^{j}	9.34^{B}
P2	9.89^{c}	9.89^{c}	9.35^{f}	9.11^{g}	8.62^{j}	9.37^{A}
P3	10.12^{a}	9.79^{e}	9.31^{f}	9.04^{gh}	8.06^{j}	9.37^{A}
Storage effect	9.97^{A}	9.84^{B}	9.33^{C}	9.06^{D}	8.67^{E}	

-C: control without MPP; -P1: Labneh with 1.5% MPP; P2: Labneh with 3% MPP; -P3: Labneh with 5% MPP. -Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

Sensory evaluation of functional Labneh during storage period

The addition of MPP and starters can enhance sensory properties of Labneh. The organoleptic scores displayed that the addition of MPP to functional Labneh considerably affected the sensory characteristics Table 5. There is a significant difference in the mean flavour scores between functional Labneh treatments. It confirmed that 1.5% and 3% MPP concentrations of fortified Labneh possessed the best flavour, body & texture, but differed significantly compared with 5% and control evaluation. With respect to the flavour of the tested Labneh, 5% recorded the lowest value of flavour compared to the other Labneh. Appearance 1.5%, 3% and control were the most preferable by the panelists with non-significant differences. As the concentration of MPP was increased in fortified Labneh, the score of flavour, body and texture, appearance and total score was increased, but with the concentration 5%, this parameter decreased. All Labneh made with different levels of MPP had acceptable flavour, body & texture and appearance during the storage period.

Table 5: Sensory evaluation of functional Labneh during storage period.

Treatments	**Storage period (days)**	**Organoleptic properties**			
		Flavour (60)	**Body and texture (30)**	**Appearance (10)**	**Total (100)**
C	Fresh	50.95^{cd}	23.00de	9.05^{a}	83.00^{d}
	15	50.92^{cd}	23.16^{de}	8.92^{a}	83.00^{d}
	28	50.00^{de}	22.00^{e}	8.12^{b}	80.12^{e}
P1	Fresh	52.67^{bc}	25.00^{bc}	9.10^{a}	86.67^{c}
	15	53.00^{b}	25.00^{bc}	8.91^{a}	87.05^{c}
	28	54.13^{ab}	25.00^{bc}	8.77^{a}	88.02^{bc}
P2	Fresh	55.07^{a}	26.00^{ab}	8.93^{a}	89.50^{cd}

	15	55.22^{a}	26.08^{ab}	8.78^{a}	90.08a^{b}
	28	55.75^{ab}	27.21^{a}	8.04^{b}	91.00^{a}
P3	Fresh	47.80^{f}	24.00^{cd}	7.20^{c}	79.25^{e}
	15	48.67^{ef}	24.00^{cd}	7.14^{c}	79.71^{e}
	28	48.00^{f}	23.00^{de}	7.06^{c}	78.06^{e}

-C: control without MPP; -P1: Labneh with 1.5% MPP; P2: Labneh with 3% MPP; -P3: Labneh with 5% MPP. -Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

Conclusion

The present study, concluded that the addition of MPP as a source of bioactive components in Labneh had significant effects on microbiological and physiochemical properties of functional Labneh. The counts of starters *S. thermophilus* and *Lb. helveticus* CNRZ 32 were still above threshold of therapeutic effects (10^{8}CFU/g) during refrigetation storage time. Finally, the treatment containing 3% of MPP had the highest acceptable organoleptic properties, so, we can use MPP in dairy industires for fabricating new functional dairy foods.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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