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Innovative formulation of oleogels using bioactive compounds and sal starch and characterization of their products

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There is a focus on oleogels nowadays with low saturated fatty acids and zero trans fatty acids to substitute solid fats in various food formulations. The other advantage of using oleogels is that it can retain the individual oils' health benefits, especially the micronutrients as the processing does not involve high temperature. The present study used a blend of rice bran oil and sesame oil. Both the oils are rich in micronutrients, together with starch obtained from sal meal and lecithin as a gelling agent to produce oleogels. The novelty of the study includes using naturally derived starch moiety from sal meal as a gelling agent, which remains unutilized instead of commercially available starch. The gelator concentration was varied from 3-12%, and the six oleogels thus produced were characterized for their suitability as solid fats. Microscopic characteristics test for gelation, degree of hardness, XRD analysis, and storage stability studies were carried out. Results showed that oleogels prepared with 8 and 10% gelator displayed the most desirable properties compared to other oleogels. The gels were kept for three months to study the physical stability also. The retention of micronutrients was also studied. Therefore, it could be concluded that a novel oleogel can be formulated with two micronutrient-rich edible oils and gelling agents like sal starch and lecithin.

1. Introduction

In view of improving the nutritional and other quality characteristics of food products, fats and oils are widely used in food formulations. Fats and oils are indispensable parts of our diet for their energy source, bioactive components, vitamins, precursors, and flavour carriers. Scientists have been busy modifying the physical properties of oils to resemble those of fats (Vaclavik and Christian, 2014). Hydrogenation, interesterification, and fractionation processes lead to the formation of solid fats, which are mainly used in food formulations (Mills et al., 2017). Solid fats offer the disadvantage of carrying higher amounts of saturated fatty acids and trans fatty acids. Associated with these fatty acids is one of the biggest societal challenges in the twenty-first century that is obesity and its related impaired health conditions (Wang et al., 2011).

This problem is increasing in India. To omit this huge economic and health concern of saturated fatty acids novel technologies need to be implemented to curb the associated epidemics and address the resulting gigantic food manufacturing problem. To fulfil these requirements, the food industry is trying to structure the triacylglycerol and reformulate food using these structured triacylglycerols. These fabricated solid fats are typically used in bakeries, breakfast spreads, mar-

garines, chocolates, and chocolate-derived products generally related to high saturated fat content.

Oleogels have been proposed to structure oil in food formulations (Marangoni and Garti, 2011). This can be achieved due to the addition of a gelator, a structuring agent, to the liquid oil phase. Literature review suggests that the quality characteristics of already prepared oleogels have been found at acceptable limits. Oleogel is required to eliminate the quality defects such as preventing or decreasing fat migration substantial for fat bloom and reducing saturated fatty acid content of the corresponding products (Hughes et al., 2009). The phase behaviour of oleogels depends on the type of oil used in the formation of gels indicating the molecular interaction and crystallization property. Oil type is an important factor affecting the rheological, texture property, thermal property of the oleogels, and oleogelator type and concentration.

A blend of rice bran oil and sesame oil is well known for their unsaturated fatty acids and high antioxidant content. The blend has also been reported to depict significant anti-hypertensive and lipid-lowering action and produce an additive effect along with anti-hypertensive medication (Devarajan et al., 2016).

In our study, the blend of rice bran oil and sesame oil was used as the liquid oil in oleogelator preparation. The liquid oil was structured to form 3-D networks by dispersing the oleogelator molecules into the oil phase by using appropriate processing methods like heating, stirring, and cooling (Almdal et al. 1993; Co and Marangoni, 2012; Flory 1953). Oleogelators are chosen because they are soluble in the oil and form a network during gelation. Starch derivatives like ethylcellulose are a common oleogelator used in today's world. Sal fat is well known as a minor oil-bearing material of tree origins commercially used as a cocoa butter substitute. After oil extraction, the deoiled cake of sal has limited commercial value and is either thrown away or stacked for years. Thus, starch extracted from the deoiled cake can serve as a low-cost oleogelator. Lecithin is also a well-known food-grade oleogelator which itself has a role in cognition, regulation of blood lipid levels, and essential component of the cell membrane (Perez-Monterroza et al. 2014).

Oleogels and its emerging technology are the main focus of many research areas geared towards catalyzing the decrease in occurrence of obesity and cardiovascular disease. In this connection it is necessary to discover a food-grade gelator system possessing the functional properties required by most food items. By using food-grade gelators, many food components can be made available together in the form of gel. The aim of my present study is to formulate oleogel using a mixture of rice bran oil and sesame oil as the liquid oil and a combination of sal starch and lecithin as the oleogelator. Polyphenol was used in the formulation to increase the gel's antioxidative property and increase its stability and shelf life. The oleogel thus produced was characterized to evaluate its physicochemical properties.

2. Materials and Methods

2.1. Preparation of blended oil

A blend of rice bran oil and sesame oil was obtained by mixing both the oils in the ratio of 1:1 and then determining the fatty acid composition of the blended oil.

2.2. Determination of the fatty acid composition of blended oil

Fatty acid composition of the blended oil was analyzed by GC. Fatty acid ethyl esters (FAME) were prepared by the method described by Metcalfe and the compositions were determined by GC analysis. The GC (make: Agilent, model: 6890 N) instrument used was equipped with an FID detector and capillary DB-Wax column (30mL, 0.32mm I.D, 0.25µm FT). N2, H2 and airflow rate were maintained at 1ml/min, 30 ml/min, and 300ml/min, respectively. Inlet & detector temperature was kept at 250˚C, and the oven temperature was programmed as 150-190-230˚C with an increased rate of 15˚C/min and 5 min hold up to 150 ˚C and 4˚C/min with 10 min hold up to 230˚C. The percentage proportions of fatty acids were calculated.

2.3. Extraction of Sal Starch from Sal De-oiled Cake

Sal Deoiled Cake (Sal DOC) was steeped with four volumes of 1.25% sodium hydroxide (NaOH) solution at room temperature for 90 minutes. After that, the steeped solution was centrifuged at 5000rpm for 15 minutes. The solid matter thus obtained consisting of mainly starch and fibre was washed further with water and centrifuged. The solid matter was steeped in water, wet ground, and filtered after water washing

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(at least 4 washings) to obtain an aqueous suspension. This suspension was centrifuged, and starch was obtained as residue. The crude starch thus obtained was washed successively with 0.1N HCl and 5% NaOH solution and water repeatedly. The starch obtained thereafter was bleached with sodium hypochlorite solution till there was a satisfactory decolourization. The bleached starch was washed with water till the chlorine odour was removed. Starch was finally dried at about 50-60°C under vacuum over a tray drier.

2.4. Extraction of tea polyphenol by microwave-assisted method

Polyphenol extraction from tea leaves was performed using a microwave-assisted extraction (MAE) process using a domestic microwave (Electrolux EM17MS80WH) i.e., the experimental setup was done using a closed vessel system. It was employed at a power supply of 200W. A total of 10 grams of tea leaves were weighed and crushed and then placed in a microwave-proof glass container of a capacity of 500 mL. Then solvent (methanol) was added where the solvent-to-material ratio was 1:40, and polyphenol was extracted under MAE conditions (Ghasemzadeh-mohammadi et al., 2017). Total polyphenol content was assessed thereafter using Folin–Ciocalteu's reagent (Ghasemzadeh-mohammadi et al., 2017).

2.5. Oleogel Preparation

Oleogels with two organogelators (Starch and Lecithin) were prepared at different addition levels (3-12% w/w). The decision of the selected levels was based on pre-experiments to determine the most suitable oleogels as spreadable products. Two types of oleogels were prepared by blending the blended oil with food-grade gelators like starch and lecithin. Polyphenol was added (1% w/w) to the prepared oleogel. Total 6 sets of oleogels (Sample 1: 3%; Sample 2:5%; Sample 3:6%; Sample 4:8%; Sample 5:10%; Sample 6: 12%) were prepared in the study. Different sets of gels were prepared using different concentrations of gelators and blended oil. All samples were prepared with continuous stirring at 300rpm and cooled immediately at -20°C, and stored at 4°C. After overnight setting at room temperature, the oleogels were completely formed and then analyzed.

2.6. Characterization of Oleogels

2.6.1. Test for gelation:

The completely formed oleogels are taken in 10ml vials, and pictures of the overturned vials are taken to observe the efficacy of gel formation (Maroto-Conteno et al., 2015).

2.6.2. Hardness Evaluation:

Hardness or consistency of the samples was determined in terms of penetration yield value (g/cm2) using a penetrometer with a 40° angle. The penetrometer is a fast and empirical method that is used in the determination of texture. The cone used for penetration was placed just above the sample before the cone was released. The penetration time was 5s, and depth was read directly from the instrument in 0.1mm units. Yield values were calculated with the help of the equation given below:

a. Yield value (g/cm^2)= $KW/P^{1.6}$
b. Where K=constant (5840 for 40 cone angle); W=-weight of the cone assembly (79.03); P=mean of penetration depth from three replicates (cm) (Kouzounis et al., 2017).

2.6.3. Microstructural Analysis:

Microstructural observation of lipid samples was conducted with a polarized light microscope attached to a digital camera. The oleogel samples (10µl) were placed on a micro slide after melting the sample at 80°C, and then the samples were covered with coverslips. The microstructure analysis was performed of the samples, which were stored at 4°C (Patel and Dewettinck, 2015).

2.6.4. XRD analysis:

The polymorphic crystal forms were determined using an X-Ray Diffractometer. X-ray diffraction (XRD) patterns of the oleogels were taken with a P.W 3040/60 model X-Ray Diffractometer (PA Nalytical, Netherland). Angular scans from 2.0 to 70° (2θ) were performed at a 2°/min scan rate with a Cu source X-ray tube (Jendrzejewska et al., 2020).

2.6.5. Physical stability of oleogel formulations:

Each oleogel formulation was kept in glass and plas-

tic containers with tight lids. Then, all of them were stored in different storage conditions: 4°C and 25°C and observed for 0, 1, 2, and 3 months. For accelerated stability studies using centrifugation test, 5g of each oleogel was freshly prepared, and during the cool-down process, each oleogel was dispensed to 15ml tubes. These tubes were kept at room temperature for a week to maintain their gelation properties before they were centrifuged at 2500 rpm for 30 minutes with REMI centrifuge (Goupale and Rajkapoor, 2011).

2.6.6. Storage stability of the oleogels:

To evaluate the storage stability of oleogels, peroxide value was determined using AOCS official methods (AOCS Official Method Cd8b-90). The peroxide value determines the milli-equivalent of peroxide per kg of a sample that oxidizes potassium iodide under the test conditions.

2.7. Statistical Analysis

All the data were expressed as mean ± S.E.M. Two ways ANNOVA was used to test the differences between control and experimental subjects.

3. Results and Discussion

3.1. Fatty acid composition of blended oil

Analysis of the lipids in the diet showed that blended oil contained 43.65% linoleic acid ($C_{18:1}$) and 32.75% linolenic acid ($C_{18:2}$). The fatty acid composition of the blended oil is given in Table 1.

3.2. Polyphenol Content of tea leaves

The polyphenol content of the tea leaves used as an antioxidant in the study was 18.20 ± 0.32 of Gallic acid equivalent.

3.3. Oleogel Characterization

The oleogel prepared in this study has a smooth structure and good spreadability. Even though all the oleogels have a homogeneous texture, the oleogels produced by low concentrations of oleogelators, i.e., 3% and 5%, do not exhibit significant stiffness, and the flow property is more or less like that of a liquid. On the other hand, by increasing the concentration of oleogelators, the oleogel formed exhibited considerable amount of stiffness. Fig. 1a shows the pictures of the oleogels produced, which exhibited the physicochemical properties of oleogels. It was also shown from the study that the oleogels formed with 12% oleogelators exhibited a considerable amount of stiffness and greasiness. Undesirable amount of greasiness, oiliness, grittiness, tackiness, stiffness, or stickiness attributes an uneasy feeling and limits the oleogels to be released inside the body. The study depicts that C, D, E have a good appearance compared to A, B, and F oleogels. Moreover, the A and B oleogels showed liquid behaviour indicating that these formulations were not very stable.

3.4. Microstructure characteristic of oleogel formulation

Aggregation processes of oleogel forming materials through self-assembly and crystallization are the important properties of oleogel formation. Fig. 1b depicts the microstructures of the oleogels formed. From the figure, it is evident that all the oleogels showed the formation of crystal aggregates i.e. a network of fat crystals as shown by their microstructures

Table 1. Fatty acid composition of blended oil

Fatty acid / Sample	**Fatty Acid (% w/w)**			
	$C_{16:0}$	**$C_{18:0}$**	**$C_{18:1}$**	**$C_{18:2}$**
Blended Oil	19.48±0.11	4.12±0.09	43.65±0.19	32.75±0.22

Values are Mean±SEM

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where the crystal appears as dark spots. All the figures showed the formation of small crystal aggregates in the starch oleogel network except Fig 1b. All the figures do not show a self-assembly network. Only the oleogels formed with a high concentration of starch as oleogelators showed the presence of self-assembly structures in their crystal networks. These aggregates are identified as sphere-shaped structures called spherulites.

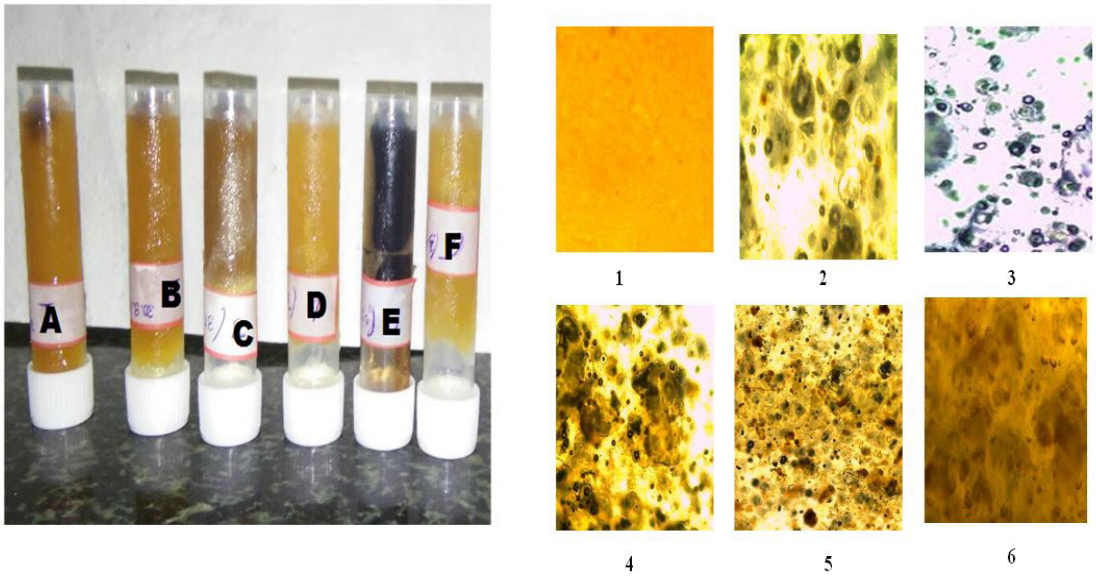
3.5. Changes in XRD

In order to determine the polymorphic nature and sub-cell type of crystals, the XRD pattern of the different oleogel samples was analyzed. The results are provided in Table 2. In order to present the patterns graphically, the oleogel curves are shown in Fig 2. The XRD data of sample 1 shows wide-diffraction peaks of 3.05Å. The XRD data of sample 2 shows wide-diffraction peaks of 4.52 Å and 3.75 Å. In the other four samples, there was a single diffraction peak between 4.36-4.56 Å observed. This observation suggested that samples 3-6 possessed finely developed crystals and caused clear diffraction intensity. On the other hand, no small peaks were observed. The short spacing spectra at around 4 Å observed in the case of the samples were characteristic of orthorhombic packing, which was similar to ß packing of triacylglycerols

The XRD data of oleogel samples 3-6 confirmed the presence of ß crystal form of the triacylglycerols in oleogel structures. This, in turn, facilitated the formation of a smooth and creamy texture of the prepared oleogel samples.

3.6. Effect of samples on the hardness

The hardness of oleogels, in general, is a contributory factor of oleogel characteristics such as appearance, workability, spreadability, and oil exudation. The hardness of oleogels usually increases due to the aggregation of the fat crystal network. The yield values of the oleogel samples are indicated in Table 2. The table indicates that product hardness increases with an increase in oleogelator concentration. The highest



1a 1b

Figure 1a. Pictographs of Oleogels formed by Olegelators (3-12%)
A: 3%; B:5%; C:6%; D:8%; E:10%; F: 12%

Figure 1b. Pictographs showing microstructure of oleogels
1: 3%; 2:5%; 3:6%; 4:8%; 5:10%; 6: 12% oleogelator concentration

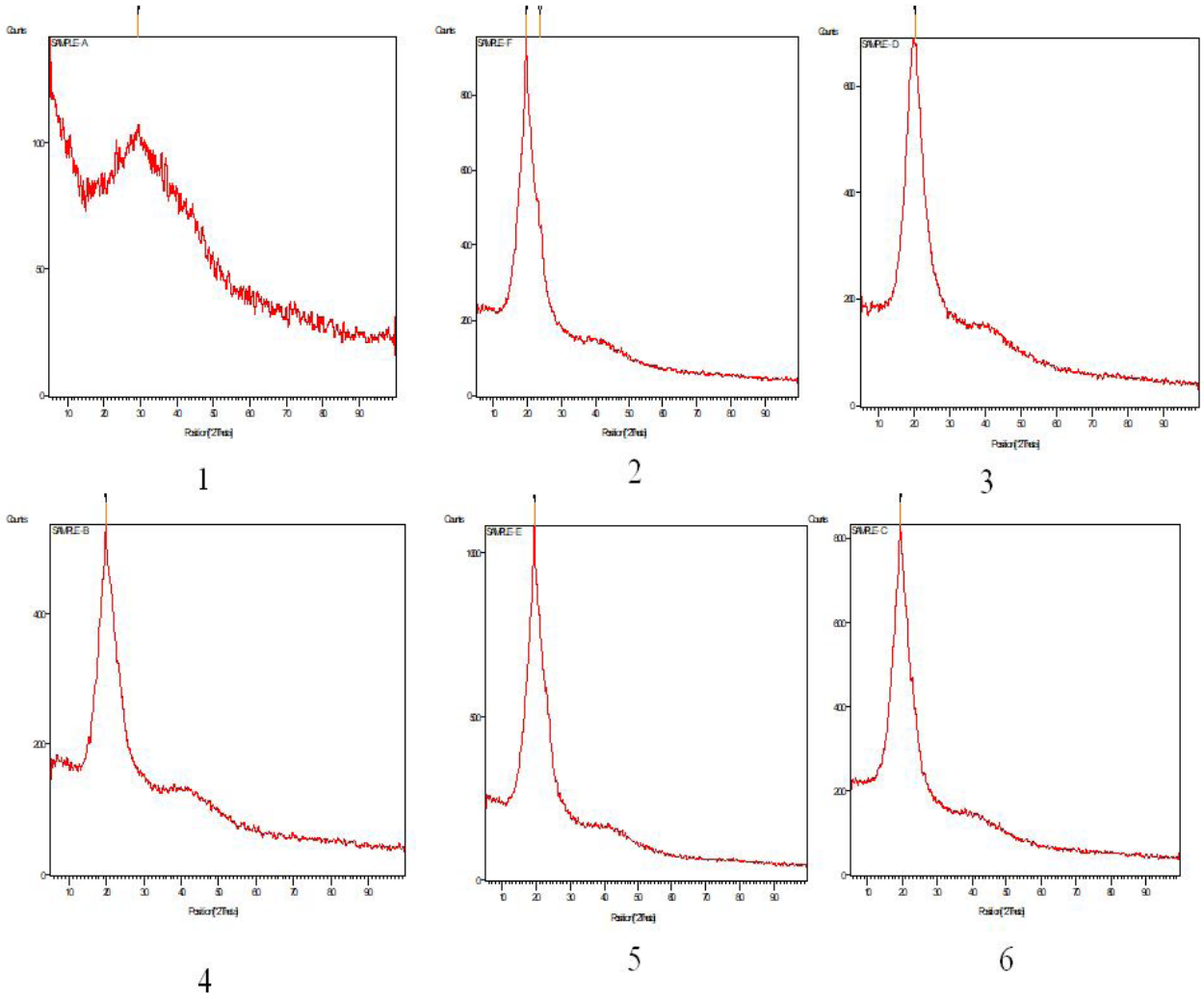


Figure 2. XRD pictographs of the oleogel samples. 1: 3%; 2:5%; 3:6%; 4:8%; 5:10%; 6: 12% oleogelator concentration

yield value belonged to sample 6, which contains the maximum amount of oleogelator compared to the other samples.

3.7. Changes in Physical Stability

Syneresis of the oleogel, observable by the separation of liquid from the gel, depicts an unstable formulation. Syneresis is also thought to be more likely in gels comprising larger aggregates due to the fibres or crystal structure in the gelation network sticking to the thick bundles. It is evident from the stability data that the physical stability of oleogels is highly dependent on the composition of oleogels. The physical stability data is depicted in Table 3. The table showed that samples 4, 5, and 6 were able to maintain their gal formation when kept in plastic containers.

3.8. Changes in Peroxide Value (PV)

The changes in the peroxide values of selected oleogels during storage are seen in Table 4. PV of oleogels (3, 4, 5, and 6) stored for 3 months registered a no significant increase with the storage period. The not much change in the PV indicates that there was not much oxidation caused by the formation of hydroperoxides during fat oxidation. It was observed that the change in PV of control was between 0.32 meq/kg and 2.09 meq/kg during the 3 months of storage. However, in oleogel 1 and 2, there was a slight change in the PV indicating oxidation caused by the formation of hydroperoxides during fat oxidation. The nutritional contribution of the tea polyphenols may have conferred this greater oxidative stability of the oleogel samples.

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Table 2. d spacing and the yield value (g/cm^2) of different oleogel samples

Sl. No.	Sample No.	Yield Value (g/cm^2)	d spacing (Å)
1	1	62.8±1.3	3.05
2	2	76.2±1.0	3.75 and 4.32
3	3	98.1±1.6	4.36
4	4	100.6±0.5	4.56
5	5	100.9±1.2	4.56
6	6	259.8±3.5	4.49

1: 3%; **2**:5%; **3**:6%; **4**:8%; **5**:10%; **6**: 12% oleogelator concentration

Table 3. Physical Stability of Oleogel Formulations : G=gel, L=liquid, GL=mixture of gel phase and liquid phase (partial syneresis)

Formulations	**Stability 0 months**	**Stability 1 month**	**Stability 2 months**	**Stability 3 months**
1	GL	L	L	L
2	GL	GL	L	L
3	G	G	GL	L
4	G	G	G	G
5	G	G	G	G
6	G	G	G	G

Table 4. Changes in Peroxide Value (PV) during storage

Sample No.	Initial (meq/kg)	1 month storage (meq/kg)	2 month storage (meq/kg)	3 month storage (meq/kg)
1	0.47	0.52	1.56	1.95
2	0.50	0.56	2.04	2.09
3	0.32	0.50	0.59	0.72
4	0.42	0.53	0.65	1.04
5	0.50	0.52	0.55	0.55
6	0.50	0.59	0.68	0.72

4. Conclusion

This study proved that successful oleogels could be produced with the help of Sal Starch as an oleogelator. The gels formed with 8 and 10% oleogelators showed the best properties in terms of spreadability, the test of gelation, microscopic structure, XRD studies, physical stability, and hardness. The storage stability data depicted that the samples with a low concentration of oleogelator showed high peroxide value after 3 months of storage, while with a high concentration of oleogelator, the storage stability of the oleogel was improved. Thus, a blend of rice bran oil and sesame oil can be successfully transformed into spreadable oleogels using Sal starch as oleogelator and can be used in the manufacture of bakery products.

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Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the manuscript's writing, or in the decision to publish the results.

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