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Preparation of oleogel from black sesame seeds

Swathi Shree Sangam, S Shruthi, Samreen and R Swamy

Abstract
Preparation of Oleogels utilizing food- approved polymers is an arising procedure which is an alternative method for making solid fats with low saturated fatty acids and limiting trans fatty acids contents. In this article, oleogels of various treatments (containing black sesame oil) were made utilizing the emulsion-template approach, and subsequent drying of the emulsions made utilizing a blend of water-soluble food polymers (Hydroxypropyl methylcellulose (Hpmc) and xanthan gum (XG)) and further assessed for physico-chemical and microbial analysis. The peroxide value of sample BS12(containing HPMC 3 g, XG-3 g) was 21.8 meq/kg and microbial count was 16 log-109 cfu\ml after 21 days of storage while the oil loss was observed as 15.55 g and a pH value of 7.68 for the same sample. Preparation of cookies with the incorporation of the significant oleogel i, e., sample BS12 and sensory evaluation was carried out.

Keywords: Hydroxypropyl methylcellulose, oxidation, xanthan gum, peroxide values

Introduction

Food items are regularly formulated with significant amounts of solid fats that have a high amount of saturated and trans fatty acids. Solid fats contribute to numerous purposes in food such as texture, flavour, firmness and functionality which are desirable both for the consumer and the food industry. However, there is a relationship between the consumption of solid fats and increased risk of cardiovascular disease, diabetes and ischemic incidents (Estadella *et al*., 2013) [15]. From past 10 years, the adverse consequences of saturated and trans-fat utilization on human wellbeing have been conspicuously featured in several scientific publications. Besides, the consumer's interest for better eating regimens without compromising quality have put forth the food business focuses their attempts on lessening the degrees of saturated and trans fats. Thus, research strategies to replace saturated and trans fats have been conducted in which solid fats used in the industry are replaced with a variety of lipid sources, carbohydrates and proteins (Paglarini *et al*., 2019) [25}. In any case, the aggregate or fractional substitution of the solid fat adversely influences the sensory and mechanical properties of the food. Accordingly, the advancement of new solid fat substitutes with low saturated and high unsaturated fatty acids and trans-fat-free is the prime concern of many investigations.

Oleogelation is one such exciting procedure which has been effectively explored by food researchers in the beyond couple of years. Oleogelation, implies change of a fluid oil into a 'gel- like' structure with viscoelastic properties. Making structured gels that contains a maximum of eatable fluid oil (over 90 wt%) opens new possibilities of replacing harmful saturated fats with unsaturated fats, resulting in food products that have a relatively better nutritional profile. However, though very promising, oleogelation has a major limitation in terms of the available food approved structures that can be used for gelling liquid oils. Polymers appear to be the most promising, but most food approved polymers are inherently hydrophilic in nature and cannot be dispersed easily in oil to achieve the necessary structure for gelation. Therefore, a way should be introduced for these water-soluble polymers into oil and drive the subsequent network formation, the polymer oleogelation could be used to revolutionize the way lipid-based food products are formulated.

Consumable oleogels are gelled frameworks where an oil constant stage is immobilized in a three-dimentional network, with the help of an oleogelator or a blend of gelators. The process of formation for oleogels requires the use of gelling agents at low concentration, which have the ability to give structure to oils (>90%) (Abdolmaleki *et al*., 2019) [1] and impart a solid-like material with the functionality (rheological, texturing, oil binding and stabilizing properties, etc.) of solid fats but an improved nutritional profile (Stortz *et al*., 2012) [32].

In this study, a novel way of utilizing water soluble food polymers to create oleogels containing a high concentration of edible liquid oil (>97 wt.%) (Patel *et al*., 2014) [27].

is introduced. Some of the food polymers for oil gelation include hydroxypropyl methylcellulose (HPMC) based oleogels, oil gels and pickering emulsion-based oil gels. Hydroxypropyl methyl cellulose is an odorless and tasteless, white to slightly off-white, excellent biocompatibility, low toxicity, free- flowing powder. Involving HPMC as an oleogelator is favorable since it is reasonable compared to more studied low molecular weight organogelators and is generally recognized as safe (GRAS) and has beneficial effects on health. However, there are limited studies considering the production of oleogels using HPMC, because of their hydrophilic nature. Hydrocolloids are unable to structure liquid oil because of their limited dispersibility in oil. Therefore, to disperse HPMC in oil, to achieve the required network formation for gelation, an indirect method is necessary, like the emulsion template approach, which was first reported by Romoscanu & Mezzenga (2006) [34].

Xanthan gum is a polysaccharide used as an effective thickening agent and stabilizer to prevent ingredients from separation. Xanthan gum is used to prepare water gels. It is also used in oil-in-water emulsions to enhance droplet coalescence; it also possesses shear thinning or pseudoplasticity

Black Sesame seeds (*S. radiatum*) are widely consumed oil seed. It is an antibiotic and known to treat or prevent hepatic insufficiencies. The leaves are also used for treating various sickness including stomach ailments, cataract, eye pains, bruises and erupted skins. Vitamin E is highest in black sesame seed which also had the highest lipid content indicating highest oil content. These seeds contain highest calcium, magnesium, zinc copper and iron (Okoronkwo *et al.*, 2020) [22]. It also has good medicinal values and is cost effective, therefore used in the preparation of oleogels.

The process of formation for oleogels requires the use of indirect physical gelation, using the emulsion-template method, which is a multistep process; for which first hydration of the hydrocolloid, then preparation of an oil-in-water emulsion followed by removal of the water, to obtain dried products driving the network formation which results in the physical trapping of oil droplets in the polysaccharide matrix network (Patel *et al.*, 2014) [27]. The drying of emulsions in the current work was carried out in a tray drier which can be considered as the main energy intensive step. The addition of other hydrocolloids, like thickening agents, such as xanthan gum (XG), can increase the stability of HPMC oleogels by enhancing the bulk phase viscosity of the aqueous continuous phase and prevent oil droplet coalescence (Mingming *et al*., 2021) [21].

The aim of this study is to analyze the impact of black sesame oil is used in oleogel formulation, regarding the physico-chemical and microbial properties, when HPMC and XG are used as gelling agents.

Material and Methods

Raw materials

Black sesame, hydroxypropyl methyl cellulose (HPMC), Xanthan gum (XG) etc., were used as raw materials for the conduct of research work. The required chemicals were procured from Bangalore Fine Chem Company, Bangalore and Telangana scientific Pvt Ltd, Hyderabad, Telangana State. Black Sesame seeds of Parchi variety were purchased from local market, Bhodan, Nizamabad.

Experimental procedure

- Preparation of oleogels using HPMC solutions
- Physico- chemical analysis of prepared oleogels
- Preparation of cookies using oleogels

Preparation of oleogels by using HPMC solutions

The preparation of oleogels was carried out based on the procedures described by Bascuas *et al.* (2020) [8] (Fig 1). HPMC solutions were prepared by dispersing it in cold water and mixed thoroughly using a stirrer for 30 min. The aqueous solution obtained after stirring was stored at 8°C for a period of overnight. Subsequently XG was added to the HPMC solution and stirred for 5 min. 60 g of oil was added to aqueous solution and homogenized for 6 min. The solutions were dried in tray dryer at 80 °C for 6 h to form oleogels. The time- temperature combination was decided based on preliminary trails. The dried oleogels were further used by incorporating during preparation of products.

Disperse HPMC in cold water and stir for 30 min

↓

Prepared aqueous solution is stored at 8 overnight

↓

Add xanthan gum to the HPMC aqueous solution

↓

Stir for 5 minutes

↓

60 g of black sesame seed oil is added and homogenized for 6 min

↓

Pour the solution in the moulds

↓

Put the moulds in tray drier at 80 for 6 h to form oleogels

Fig 1: Flow chart for preparation of oleogels

The different compositions for the preparation of black sesame oleogels were shown in Table 1

Table 1: Different compositions for preparation of black sesame oil oleogels.

	Composition	
Sample Name	**HPMC (g)**	**Xanthan gum (g)**
BS1	1	1.2
BS2	1	1.8
BS3	1	2.4
BS4	1	3.0
BS5	2	1.2
BS6	2	1.8
BS7	2	2.4
BS8	2	3.0
BS9	3	1.2
BS10	3	1.8
BS11	3	2.4
BS12	3	3.0

For the preparation of HPMC aqueous solution 39g of water was used. In addition to HPMC and XG, 60 g of black sesame oil was added to all the samples to make the solutions.

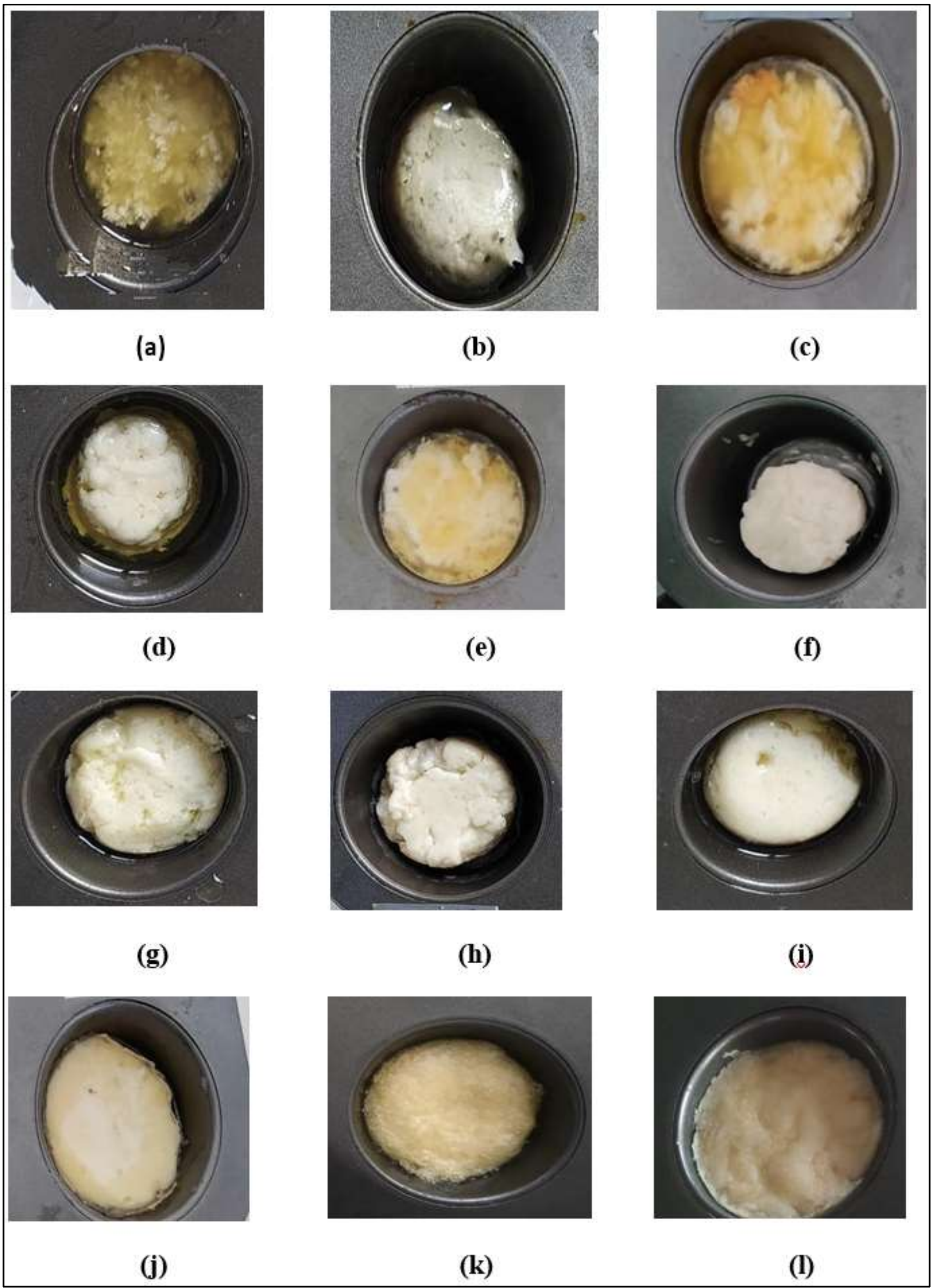

Plate 1: Different oleogels obtained as prescribed compositions (a) HPMC 1 g and XG 1.2 g HPMC 1 g and XG 1.8g (c) HPMC 1 g and XG 2.4 g (d) HPMC 1 g and XG 3.0 g (e) HPMC 2 g and XG 1.2 g(f) HPMC 2 g and XG 1.8g (g)HPMC 2 g and XG 2.4 g (h) HPMC 2 g and XG 3.0 g (i) HPMC 3 g and XG 1.2 g(j) HPMC 3 g and XG 1.8g (k) HPMC 3 g and XG 2.4 g (l) HPMC 3 g and XG 3 g.

The oleogel samples obtained were subjected to physic-chemical analysis explained in section 2.3

Physico-chemical Analysis

The physico-chemical analysis was conducted to the prepared oleogels when subjected to storage for 21 days. The analysis was conducted at intervals of 7 days to assess physico-chemical properties of prepared oleogels.

Peroxide value

The peroxide value was determination according to procedure given by Cho and Lee (2015) [9]. 1 g of sample was taken and mixed thoroughly with a mixture of acetic acid and chloroform solution (3:2, v/v). The solution obtained is added with 1 ml of potassium iodide and kept in dark for 10 min. About 30 ml of distilled water was added to solution. Titration was performed with 0.01 N sodium thiosulfate standard solutions and 1% starch solution (1 ml) was used as an indicator. The peroxide value was expressed as milli equivalents of active oxygen per kilogram of oil (meqO2/kg).

Oxidative Stability (OS)
The oxidative stability was determined according to the procedure of ISO 3656: 2011. The OS was analyzed at k270 in spectrophotometer. 1 gram of sample was taken and dissolved in 100 ml of cyclohexane and measured at k270.

Oil loss of oleogels
The percentage of oil loss was determined by Bascuas *et al*. (2020) [8]. The weight of oil released was measured at 0, 7, 14, 21, and 28 days of storage. A funnel with a filter paper was arranged above an Erlenmeyer flask where the liquid oil from the oleogels dropped into it. The weight of the funnel, the filter paper and the Erlenmeyer flask were measured as (Ml), 10 g of oleogel was weighed as (M3) and set into the funnel. The weight of the funnel, the filter paper and the flask with the liquid oil released was measured again as (M2). The results were expressed as g oil loss per 100 g oleogel. The measurement was recorded in triplicate for each sample.

$$Oil\ loss\ (\%) = \frac{M_2 - M_1}{M_3} \times 100$$

pH
The pH was determined according to the procedure given by Huang *et al*. (2018) [19]. Using a digital pH meter (Labline Digital pH meter). all the samples pH values were recorded immediately after the samples were prepared. All values were recorded in triplicates.

The oleogel samples obtained were subjected to microbial analysis as explained in section 3.4

Microbial Analysis
The bacterial count was determined according to the procedure given by Niyoyitungiye *et al* (2020) [24]. The dilutions were made by taking 1 ml of sample into a tube containing 9 ml of buffer resulting in 10-1 dilution. Similarly, the decimal dilutions were prepared by transferring 1ml of previous dilution. These dilutions were inoculated onto the solidified nutrient agar (NA) media of petri plates weighing 15-18 ml and incubated at ambient temperature for 21± 3h. The number of microorganisms per milliliter in sample was calculated from the number of colonies obtained on NA plate from selected dilutions.

$$CFU/ml = \frac{(Number\ of\ colonies \times dilution\ factor)}{(volume\ of\ culture\ plated\ in\ ml)}$$

The oleogel samples obtained were incorporated in the preparation of cookies as explained in section 2.5

Preparation of cookies using black sesame oleogel
Cookie making was carried out according to the AACC method (AACC 10- 50.05, 2000). The ingredients used to prepare cookies include flour 225 g, sugar 163 g, salt 2 g, distilled water 25 ml, and shortening/oleogel 64 g. The dough was divided into few portions and lightly flattened using a dough rolling pin. The dough was then cut into desired shapes using a knife and placed onto a greased baking sheet. Cookies were baked in an oven at 120°C for 28 min. After baking, the cookies were removed from baking sheets and served after cooling.

Plate 2: Preparation of cookies using black sesame oleogel

The sensory evaluation of cookies prepared with oleogel was carried out by few semi-trained panel members who had some previous experience in sensory evaluation. Judgment was made through rating products on 9-point hedonic scale with corresponding descriptive terms ranging from "9- like extremely to 1- dislike extremely" with respect to the different quality attributes such as flavor, appearance, texture, taste and overall acceptability. The overall rating was calculated by doing average of the scored obtained. The most significant sample BS12 was taken for the preparation of cookies.

Results and Discussion
In this chapter, the details of the results obtained for the oleogel prepared using black sesame oil were presented. Physico-chemical and microbial properties of oleogel prepared have been evaluated and discussed.

Peroxide Value
The peroxide value (PV) measurements shown in (Fig 2) indicates the stability of black sesame oleogel. The oleogels prepared show a significant rise in the PV values from 0th day and gradually increased throughout the storage period. The highest PV was observed on 21st day for sample BS1 (HPMC 1 g, XG-1.2 g) with 42.2 meq/kg, while the least PV was observed in BS12 (HPMC 3 g, XG-3 g) with 21.8 meq/kg. The freshly made black sesame oleogels have shown least range of PV.

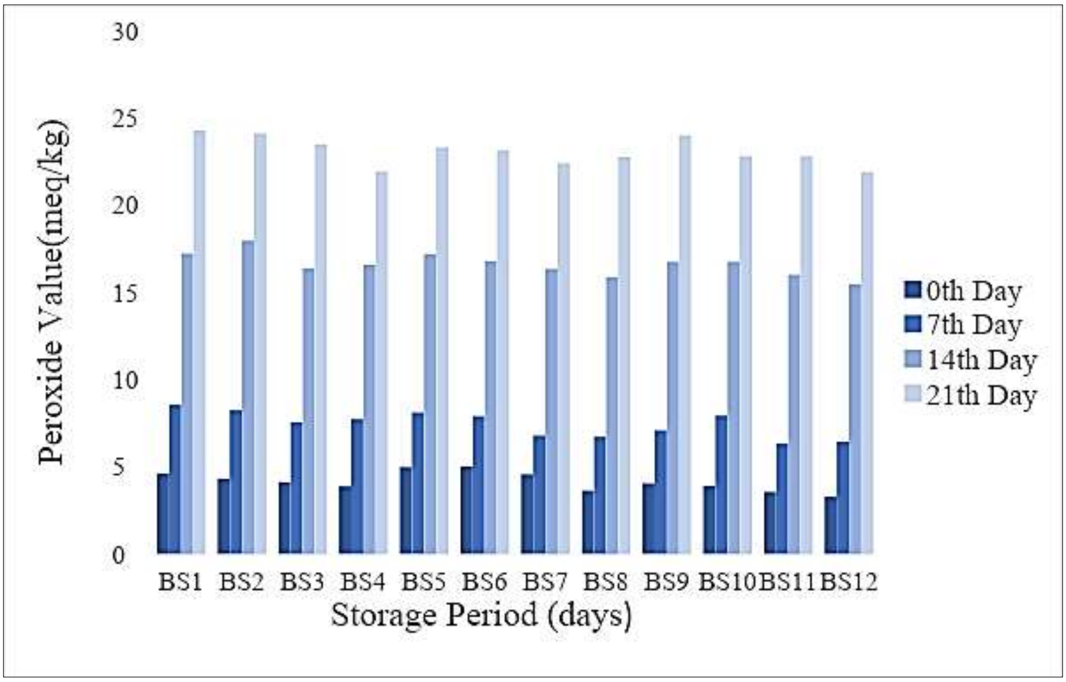

Fig 1: Changes of peroxide value of oleogels upon storage (meq/kg)

Results show that all the oleogels had PV values below the limit of acceptance (<25 meq/kg) after 21 days of storage. Freshly made oleogels show least PV while the significant increase in PV was observed on storage of samples due to the composition of fatty acids, since the high content of polyunsaturated fatty acids present in oil are more susceptible to autoxidation (Santiago *et al*., 2020). The PV of fresh oils is <10 meq /kg (Codex Alimentarius, 2005). The acceptance limit in vegetable oils ranges between 30 and 40 meq /kg, a rancid taste is noticeable beyond this range of PV. least PV while the significant increase in PV was observed on storage of samples due to the composition of fatty acids, since the high content of polyunsaturated fatty acids present in oil are more susceptible to autoxidation (Santiago *et al*., 2020). The PV of fresh oils is <10 meq /kg (Codex Alimentarius, 2005). The acceptance limit in vegetable oils ranges between 30 and 40 meq /kg, a rancid taste is noticeable beyond this range of PV.

Oxidative stability of oleogels

The Oxidative stability (OS) of black sesame oleogels shown in Fig 2 was evident to say that OS increased upon storage. A gradual increase in k270 values throughout storage was detected for all the samples. The lowest OS was observed in sample BS12 (HPMC-3 g, XG-3 g) with the oxidative value of 6.38 and highest value was observed in sample BS6 (HPMC- 2 g, XG-1.8) on 21st day. The least OS values was observed on the 0th day for the freshly prepared samples.

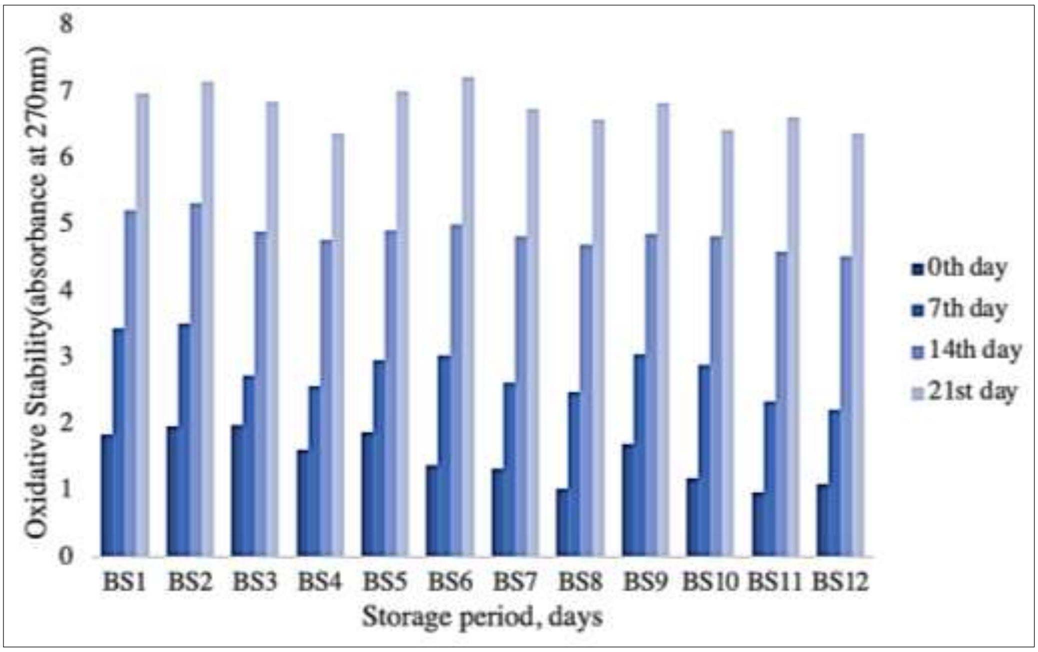

Fig 2: Changes of oxidative stability of oleogels upon storage

The measurement of absorbance in the ultraviolet range (k270) provides indications about the quality of oil and the state of oil preservation. The degree of oxidation (regarding PV values) was significantly high because of air bubbles formed inside the gel. The k270 are indicative, of conjugated trienes and the presence of carbonyl compounds (Santiago *et al*., 2020). The water removing process adopted during drying could decrease the oxidative values of oleogels as reported by Meng *et al.* (2018) [35].

Oil loss in oleogels

The values shown in the fig 3. indicate the oil loss factor for the prepared black sesame oleogel. The oil loss analysis was observed maximum on 28th day as compared to the 0th day. The highest oil loss was seen in sample BS1 (HPMC-1 g, XG-1.2 g) measuring 21.89g of oil loss and the lowest oil loss was observed in BS12 (HPMC- 3 g, XG- 3 g) measuring 15.55 g. Samples BS1 (HPMC-1 g, XG-1.2 g) and BS2 (HPMC-1 g, XG-1.8g) show 0.5 g of oil loss on the 0th day.

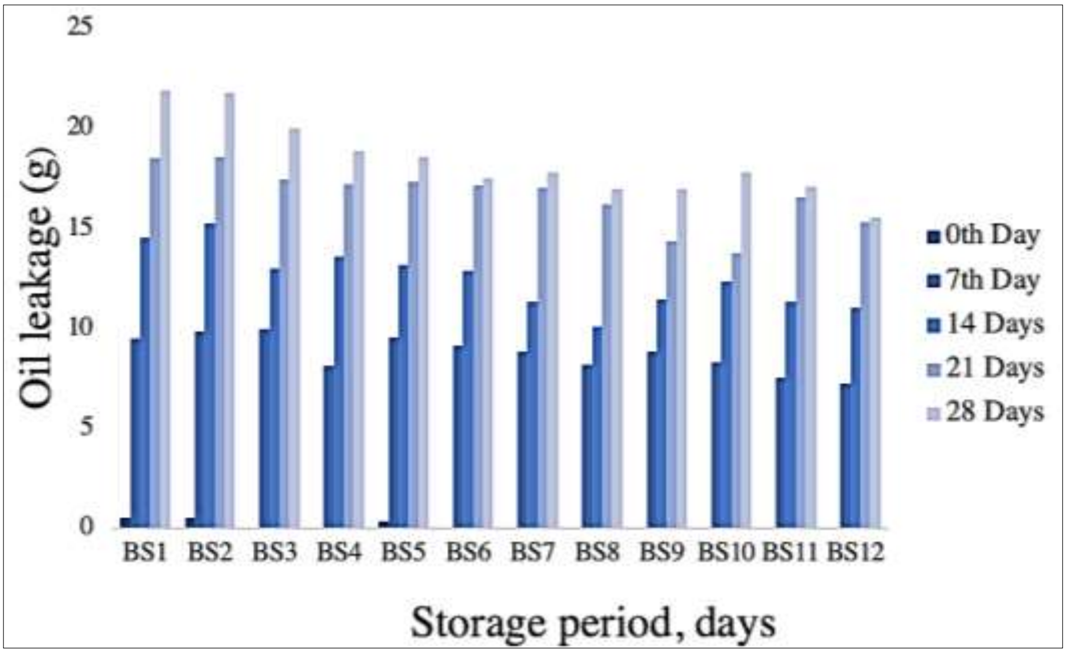

Fig 3: Changes in oil loss (g of oil per 100 g of oleogel) upon storage

The results show that the freshly made oleogels had no oil loss and other samples had very little loss of oil; the greatest loss occurred during the first week of storage indicating the increasing tendency of oil loss.

This could be because of semi- crystallization of the polymer network and this would be related to the structure of the oleogels due to the composition of the sample (Stortz *et al.*, 2012) [32].

pH

The pH of freshly made black sesame oleogels is recorded in fig 4. The maximum pH was observed in sample BS12 (HPMC- 3 g, XG- 3 g) with a value of 7.68 and BS11 (HPMC- 3 g, XG- 2.4 g) with a value of 7.45, the least values were depicted as 5.51 of sample BS1 (HPMC- 1 g, XG- 1.2 g) and 5.55 of sample BS2 (HPMC- 1 g, XG- 1.8g). The graph shows an increasing tendency of pH with the increase in composition of the sample.

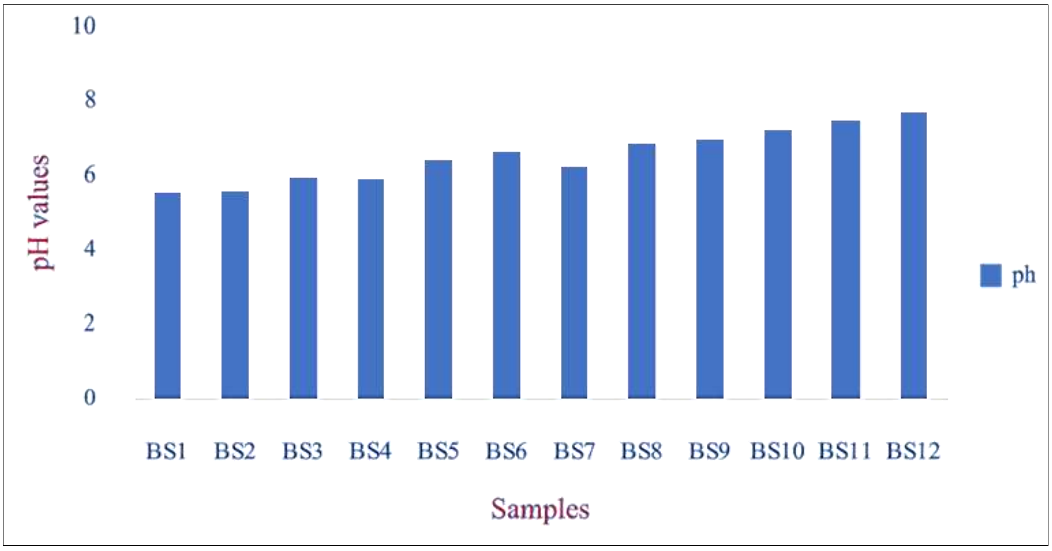

Fig 4: Changes in pH values of the freshly prepared oleogels

From the results, it is evident that oleogel after the analysis can be said that the pH mostly ranged from 5-8. The significant oleogel can be said as the BS11, BS12 as the visual structure seems smoother. This behavior is related to the lower mass loss (water) during the drying process. In this case, the electrostatic repulsion between biopolymers results in stable systems because XG keeps dispersed in the continuous phase and the intermolecular bonding of the HPMC increases with increase in concentration (Punitha *el al*., 2020) [28].

Microbial analysis

The values shown in the fig 5 indicate the bacterial count on storage of the black sesame oleogel samples. The highest values were observed in BS1 (HPMC- 1 g, XG- 1.2 g) with the value 18.5lo g-109 cfu\ml and the lowest values was observed in BS11 (HPMC- 3 g, XG- 2.4 g) with the value 15.9 log-109 cfu\ml and BS12 (HPMC- 3 g, XG- 3 g) with the value 16log-109 cfu\ml. The results show that the bacterial group as an indicator of the possible presence of pathogens in the systems. There was an increase in total bacterial count at each interval of analysis from 0 to 20 days, however the increase was not significant (p<0.05). Samples which signify lesser water activity and hence more oil binding capacity which can be related to the oil loss of the same samples. (Qureshi *et al.*, 2020) [12].

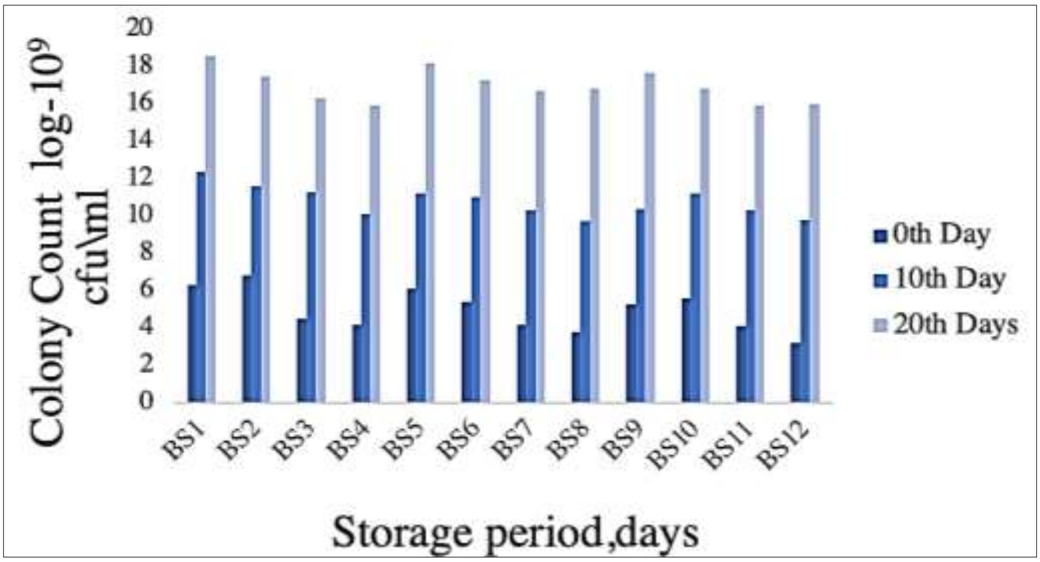

Fig 5: Preparation of cookies using prepared oleogels

Sensory evaluation conducted for the oleogel incorporated cookies revealed that there was no significant difference between the cookies made with the rest of the shortenings. Cookies were slightly hard in texture but there was no taste difference observed. There was no bitter taste as black sesame oil is rich in flavonoids, the smell was sweet rather than oily. Sensory analysis (or sensory evaluation) is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch and hearing) for the purpose of evaluating consumer products. The discipline requires panels of human assessors, on whom the products are tested, and recordings the responses made by them. By applying statistical techniques to the results, it is possible to make inferences and insights about the products under test most large consumer goods companies have departments dedicated to sensory analysis.

Summary and Conclusions

Study on production of oleogel was undertaken with an aim to replace saturated and trans fats with the use of oleogels by structuring vegetable oil (black sesame seeds). In this study, preparation of an emulsion from HPMC, xanthan gum and oil-based templates were made to prepare oleogels, analysis of physico-chemical and microbiological properties of the oleogels are carried out and their summary and conclusions from the study are presented here:

1. The freshly made oleogels show less peroxide values as the oil does but as the storage time increased, the PV also showed significant increase due to the auto-oxidation. The samples BS12 showed lowest range of PV indicating the least peroxide content after 21 days of storage. While sample BS1, BS2, BS9 recorded highest values indicating least preservation status of the samples.
2. Oxidative stability is the chemical reaction that occurs between the oil and oxygen. This oxidative value increases with time, the samples BS4, BS12, recorded least value indicating lesser affinity towards rancidity of the oleogel while the samples BS1, BS2 recorded greater affinity.
3. Oil loss is the factor which represented the fact that loss of oil is more from samples (BS1, BS2) with less concentration of the additives (gelators) while least in samples (BS11, BS12) with high percent concentration due to the semi- crystallization of the polymer network. It was also observed that the samples with high concentrated gelators presented stability of oil loss after 21 days of storage.
4. pH of oleogel is an indicative factor which affect the micro-structure of the oleogel formed and hence has an influence on the over-all effect of the sample and also the oil loss. pH was increased as the concentration of HPMC was increased.
5. The bacterial count of the oleogels indicated the storage life of the oleogel without degradation. It can be concluded that the prepared samples showed stability with gradual and very slow increase in bacterial count even after 20th day of analysis.

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