Development of Rice Bran Oil and Flaxseed oil based Oleogels using Beeswax for Food Application

Shreya Singh, Vivek Kumar*Ashwani Kumar* and Ajit K. Singh

Department of Food Technology, Harcourt Butler Technical University, Nawabganj, Kanpur, Uttar Pradesh-208002, India
E-mail: viveksachan99@rediffmail.com, ashwanikumar.tu@gmail.com

Abstract—*Transand saturated fat reduction is one of the main challenges faced by food industry which is why it has been a tremendously active area of research for the food industry. Oleogelation is a concept of structuring liquid oil into a three-dimensional network by using a small amount of one or more gelator molecules. Unlike polymers used for hydrogels, oleogels require small, amphiphilic molecules that self-assemble via highly specific, non-covalent interactions entrapping liquid oil via capillary forces. Edible applications of oleogels have replaced the need for transunsaturated and saturated fatty acids to structure processed foods. The aim of this research was to convert vegetable oil in to oleogel without altering their unsaturated fatty acid profile. Rice bran oil and flaxseed oil have been gelled with food grade wax i.e. beeswax at a very low concentration (2 to 5%). Oleogel prepared using rice bran oil showed higher oil binding capacity i.e. 37.59±8.22 and higher firmness i.e. 22.91 g than the other oleogels. Crystal formation time was also lower for rice bran oleogel at a concentration of 5% beeswax.The resultant oleogel showed solid like properties that can be used to replace saturated fat.*

Keywords: *Oleogel, Rice bran oil, Bees wax and Crystal formation.*

Highlights

- *Minimum 2% of bees wax concentration was required to form oleogel at room temperature (28 ± 2ºC).*
- *Crystal formation time was decreasing with increasing concentration of beeswax.*
- *Gel strength was found highest at 5% bees wax concentration*
- *Oil stability was increased after gel formation.*

1. Introduction

Regarding the consumption of dietary fats many revisions have seen in nutritional recommendation. However, dietary guidelines consistently emphasized the need to lowerthe consumption levels of *transand* saturated fats. *Transand* saturated fats have negative effects on cardiovascular health and they increase the levels of LDL (low density lipoprotein) whereas mono and polyunsaturated fats tend to lower LDL levels, that is why it is recommended by nutritional guidelines to replac e*transand* saturated fats with unsaturated fats. Among different approaches that can be explored for *transand* saturated fat replacement, oleogelation (converting liquid oilin to gel-like material without modifying the chemical characteristics of oil) has been hailed as a feasible approach. At a relatively lower mass fraction gelator molecules are capable of gelling liquid oil approximately 90% wt or more that is making oleogelation a very efficient means toimprove the nutritional profile of food products (*trans*-fat free,low in saturated fats and high in unsaturated fats) (Patel et al. 2016).

For replacement of *trans*and saturated fat from unsaturated sources, researchers have tried to use vegetable oil instead of saturated fat but the use of vegetable oil produces some quality problems in bakery products. The low viscosity of the oil creates handling and shaping problems. Vegetable oil also provides more greasy and less crispy texture to the bakery products. It also decreases flavor stability and shelf life of the products because of oil oxidation. Therefore, direct replacement of shortening with vegetable sources in bakery products has been presented a challenge. Consequently, rather than the replacement of solid fats with liquid oils, it has generally been accepted by the food industry that modification or structuring of the oils is essential to create a plastic fat having solid-like properties but also possesses a healthier fatty acid profile (Mert et al. 2016).

Due to this reason, oil is modified using oleogelation technique. Oleogelation is a novel technique, in which the entrapment of an organic liquid within a thermo-reversible and threedimensional gel networks is created. Recently, the use of organogel, which is also called as oleogel when the organic phase is edible oil, has gained use in cosmetic and pharmaceutical industries due to its specific consistency and firmness without chemical modifications (Mert et al. 2016).Rice feeds half the world's population and to produce white rice, the branlayer is removed. This bran layer makes up 8%-10% of the rice grain so it is a sizable portion of the total crop harvest. Rice bran contains 18%-24% oil, consisting of palmitate (12%-28%), oleate (35%-50%), and linoleate (29%-45%), with a significant quantity of unesterified fatty acids, especially when the bran is stored. India (59%), China (14%),and Japan (10%) are the main countries producing rice bran oil. AlthoughTAG is the main class, there are glycerolipids, phospholipids, waxes, and theaforementioned non-esterified fatty acids. Notable amounts of tocols and oryzanolsgive rice bran oil high oxidative stability so it is good to use as asalad or frying oil, or as coating oil for biscuits and nuts (Harwood et al., 2017).Rice bran oil has low content of saturated fatty acids (≈20 % w/w) and higher amounts of nutritional substances like oryzanol and antioxidants (Doan et al. 2015).Flax (or linseed) is a traditional source of α-linolenic acid (35%-60%)(Harwood et al. 2017).

In oleogelation technique, there is no change in fatty acid composition of stock oil. This technique is safer as compared to hydrogenation and itgive solid-fat like properties to vegetable oil. Oleogels made up of rice bran oil and flaxseed oil has a good nutritional profile because rice bran oil contain oryzanol and antioxidants and low amount of saturated fatty acids and flaxseed is a good source of omega-3 fatty acid. So the objective of this study was to develop oleogel using rice bran oil and flaxseed oil as a base material and beeswax as a gelator molecule.

2. Materials and methods

2.1 Materials

Rice bran oil was purchased from market and flaxseed oil was extracted from flaxseed in university laboratory. Beeswax was purchased from chemical supplier of the university. Refined wheat flour, sugar, butter, egg, milk, baking powder was purchased from local market.

2.2 Oleogel preparation

To form oleogels, beeswax was dispersed in rice bran oil (RBO) at the concentration of 1,2,3,4, and 5 % w/w named as RBO1, RBO2, RBO3, RBO4 and RBO5 respectively. The samples were heated at 70 °C under mild agitation (200 rpm) using a magnetic stirrer for 10–30 min until clear dispersions was obtained. The clear oily dispersions was subsequently cool down to 5 °C at a cooling rate of approximately 2°C/min and was stored at 5 °C overnight in a thermal cabinet for further experiments (Doan et al. 2015). Similarly flaxseed oil (FSO) FSO1, FSO2, FSO3, FSO4 and FSO5 was prepared using flaxseed oil as base material.

2.3 Gel strength of oleogel

A texture analyzer (TA.XT Plus Stable Micro System) equipped with a 50 kg load cell and cylindrical probe (13 cm in diameter) was used to perform texture profile analysis (TPA). Samples were compressed to 70% of their original height, at room temperature, for gel strength at a testing velocity of 1.5 mm/s. The probe was a kept at a distance of 30 mm from the experimental samples. The resultant force-deformation curves were used to derive gel strength of oleogel samples (Tanti et al. 2016).

2.4 Crystal Formation Time (CFT)

The previously formed oleogel samples were first completely melted in water bath (90^{o}C) and kept for 2h for isothermal setting. Then they were taken out from the water bath to room temperature, and meantime chronometer was started. The CFT was recorded when the tubes turned 90^{o}and no flow observed (Öğütcü et al. 2015).

2.5 Oil Binding Capacity (OBC)

First, 1 mL of the melted oleogel sample was put into previously weighed eppendorf tube (weight a) and conditioned in refrigerator for 1 h. Then the tube was weighed again (weight b). The tubes were centrifuged at 9167 g for 15 min at roomtemperature, and turned over onto a filter paper for drainage of released oil. Finally, the tubes were weighed (weight c) again and OBC was calculated by Eq.

OBC (%) = 100 − Released Oil (%) and Released Oil (%) = [(b − a) − (c − a)]/ (b − a) × 100(Öğütcü et al. 2015).

2.6 Peroxide value (PV) measurement

Peroxide value was determined according to AOCS Cd 8-53 method (AOCS 1996) (Naeli et al.2016). Same procedure was followed by Park et al. 2018. In this research, same procedure was followed with slight modification. To measure PV, about 5.00 g of the samples was placed in a flask. Then, 30 mL of the acetic acid/chloroform solution (3:2, w/w) was added, followed by the additional agitation for 20 s. Once the chemicals dissolved, 0.5 mL of saturated KI was added to the flask, and swirled for 1 min. immediately after; 30 mL of deionized (DI) water was added and shaken to liberate the iodine from the chloroform layer. Then, the flask was placed back on a hot plate and swirled for 20 s, followed by an addition of 3 mL of 1% starch indicator and titration with 0.01 N $Na_2S_2O_3$. Volumes of 0.01 N$Na_2S_2O_3$ required for the titration were recorded. Samples' PV was calculated using the following equation:

$$PV(meq\ peroxide\ per\ 1000g\ sample) = \frac{(\mathrm{S}-\mathrm{B}) * \mathrm{N} * 1000}{\mathrm{Wt\ of\ Sample\ (g)}}$$

Where B and S are the volume of titration for blank and test portion, respectively, and N is the normality of the sodium thiosulfate solution.

2.7 Statistical Analysis

All experiments were performed in duplicate. Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure in SPSS16 software, and mean comparisons were carried out by Duncan's multiple range test ($p < 0.05$)(Park et al. 2018).

3. Results and Discussion

3.1 Preparation of oleogels

Oleogels were prepared by following aforementioned method (material and methods section). Addition level of beeswax was in the range of 1-5%. Figure 3.1 shows the visual appearance of oleogel. Oleogel of flaxseed oil had a darker color than that of oleogel of rice bran oil. This may be due to the color of oil.Oleogel was not formed at 1 % beeswax addition in both oils.Oleogels prepared from rice bran oil at an addition level of 5% showed most stable gel at room temperature followed by rice bran oleogel of 4% & 3% beeswax. Oleogels of 2% addition level for both oils showed a weaker gel formation at room temperature. But all samples showed a stable gel at refrigerated temperature.

Figure 3.1: Oleogels of Rice bran oil (RBO) and Flaxseed oil (FSO)

3.2 Oil binding capacity

It is an important physicochemical parameter. It allows relating the strength of oleogel with the ability to retain oil phase within oleogel network. The oil binding capacity is one of the key factors in determining whether a new fat formulation will be successful in novel food applications. In food manufacturing, it is desirable to have oil exude as little and as slowly as possible. The oil binding capacity (OBC) of the oleogel samples is shown in Table 3.1. Clearly, the oil binding capacities (OBC) of the beeswax and rice bran oleogel at 2% addition level was the lowest value obtained followed by that of beeswax and flaxseed oleogel at 3%, 4% and 5% addition level. Oil binding capacity was found increasing with increasing beeswax concentration. Same trend was also found in flaxseed based oleogel. However ricebran oil based oleogel was found higher than the flaxseed based oleogel at 5% beeswax concentration. A similar finding was reported by Pandolsook & Kupongsak 2017. They found increased OBC with increasing concentration of wax. Yang et al. 2018 reported the increase in oil binding capacity as the concentration of gelator mixture increased from 15-20 g/100 g.

Table 3.1: Physicochemical properties of oleogels

Sample	CFT	OBC	PV
RBO1	NA	NA	NA
RBO2	77.21 ± 2.58^{g}	14.97 ± 1.89^{a}	9.16 ± 0.34^{d}
RBO3	52.54 ± 1.15^{e}	17.29 ± 1.92^{ab}	8.01 ± 0.16^{c}
RBO4	19.56 ± 0.02^{d}	23.01 ± 3.69^{bc}	6.05 ± 0.35^{b}
RBO5	5.26 ± 0.02^{a}	37.59 ± 8.22^{d}	5.37 ± 0.13^{a}
FSO2	90.97 ± 0.66^{h}	17.24 ± 0.68^{ab}	10.18 ± 0.49^{e}
FSO3	64.53 ± 2.11^{f}	18.88 ± 2.68^{a}	7.60 ± 0.48^{c}
FSO4	16.92 ± 0.55^{c}	26.46 ± 1.18^{c}	7.56 ± 0.30^{c}
FSO5	9.20 ± 0.06^{b}	27.59 ± 1.37^{c}	6.54 ± 0.53^{b}

3.3Crystal formation time

The crystal formation time was lowest for rice bran oleogel at addition level of 5% beeswax followed by flaxseed oleogel at 5% and flaxseed oleogel at 4%. RBO2 and FSO2 took longer time for crystal formation i.e. 77.2033 ± 2.5816 and 90.9733 ± 0.6645 minutes respectively. There was a significant difference in the CFT value of all samples. A similar result can be seen in study done by Yılmaz&Öğütcü2014, where they have reported that crystal formation time is decreasing as increasing beeswax concentration. The decrease in time shows faster crystallization.

3.4 Peroxide value

Oxidation of lipids is a major problem in the food industry because in addition to changing the quality of the product, it also affects the mechanical properties, color, and nutritional values, which reduces the shelf life of the product and consequently generates economic losses. Peroxide value is one of the best parameter to evaluate oxidation. The peroxide value of RBO5 was the lowest value obtained indicating low level of oxidation. Highest oxidation level was found in FSO2. This may be due to the weaker network of gel formed. There was significant difference in the oxidation of samples. With increasing concentration of beeswax, oxidation is decreased. A similar finding is reported by Martins et al. 2017, where they found lower oxidation with addition of higher concentration of gelator. They also found a trend of increasing oxidation with storage time.

3.5 Gel strength of oleogels

Textural properties of the plastic fats can be as important as the thermal properties, if they are to be used as margarines, spreads or other similar products. In some food products (ice cream, chocolate etc.), the textural properties of the solid fat are the main quality determining factor. Shortenings, as baking ingredients, are plastic range products made by blending. In this study wecompared measured gel strength in terms of firmness of the RBO and FSO oleogels. Figure 3.2 and figure 3.3 shows the texture curve. The obtained values of firmness are shown in table 3.2.As the force applied, when it reaches its maximum, the point is measured as the firmness value, and under that curve, the area is calculated as the work of shear value.It can be seen from table 3.2, that firmness isincreasing with increasing concentration of beeswax in both oils i.e. rice bran oil and flaxseed oil. Rice bran oleogel with 2% beeswax had lowest firmness value (6.76 g) and highest value for rice bran oleogel with 5% beeswax (22.91 g). A similar result can be seen in study done by Öğütcü et al. 2015 and Yılmaz&Öğütcü2014. They also found increasing firmness with increasing concentration.

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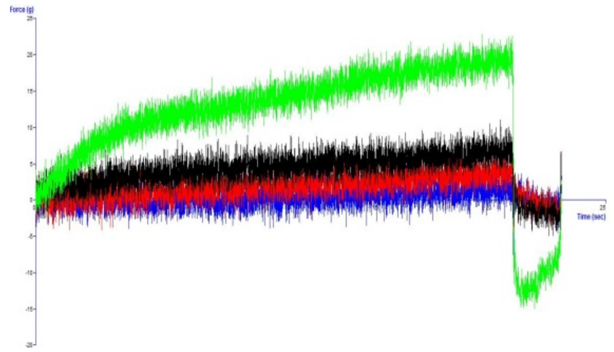

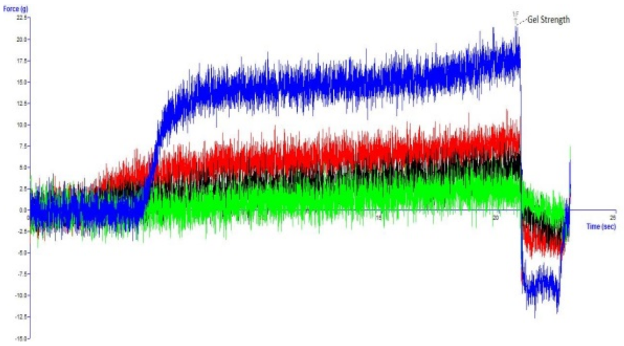

Figure 3.2: Texture curve of RBOFigure 3.3: Texture curve of FSO

Table 3.2: Firmness of oleogels

S No.	Sample	Firmness (g)
1.	RBO2	6.76
2.	RBO3	8.07
3.	RBO4	11.02
4.	RBO5	22.91
5.	FSO2	7.42
6.	FSO3	8.51
7.	FSO4	11.89
8.	FSO5	21.60

Conclusions

In this study some important properties of beeswax containing oleogel of rice bran oil and flaxseed oil were determined. The aim of this research was to convert vegetable oil in to oleogel without altering their unsaturated fatty acid profile and to investigate the usage of vegetable oil based oleogel to replace shortening in muffins. For this purpose, oleogels were prepared with two oils i.e. rice bran oil and flaxseed oil using beeswax as a gelator at a concentration of 2-5%. Oleogels of rice bran oil with 5% beeswax showed the most stable gel and crystal formation time was also less. Oil binding capacity of oleogel of rice bran oil was highest. In terms of firmness, oleogels of rice bran oil with 5% beeswax had a maximum firmness value of 22.91 g.Overall from the determined properties of rice bran oleogel, it can be concluded that this study has proved a good suitability of rice bran oil oleogel prepared with beeswax as plastic fat product.

Declaration of conflict of interest

Authors declare no conflict of interests.

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