# TRANS-FAT REPLACEMENT BY USING FOOD GRADE EDIBLE BIGELS

**SUMMARY OF**
**THESIS**
**SUBMITTED TO THE**
**BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY, LUCKNOW**
**FOR THE AWARD OF THE DEGREE OF**

## DOCTOR OF PHILOSOPHY

**in**

**HOME SCIENCE**

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

**Submitted By**


M. A. FIRDAUS
**RESEARCH SCHOLAR**
**ENROLLMENT No. 1051/19**


**Under the Supervision of**

PROF. SUNITA MISHRA

SUPERVISOR
पर्यवेक्षक
Supervisor 12/2/2024
DEPARTMENT OF HUMAN DEVELOPMENT & FAMILY S


**DEPARTMENT OF HUMAN DEVELOPMENT & FAMILY STUDIES**
**SCHOOL OF HOME SCIENCES**
**BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY**
**(A CENTRAL UNIVERSITY) (NAAC A++ ACCREDITED)**
**VIDYA VIHAR, RAEBARELI ROAD**
**LUCKNOW- 226025 (U.P.) INDIA**


**2024**

# ABSTRACT

**Trans-fatty acids (TFAs)** are the geometrical isomers of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids having at least one carbon-carbon double bond in the *trans* configuration rather than the more common *cis* configuration. TFAs are found naturally (*ruminants trans-fatty acid*) in meat and dairy products as a result of bacterial biohydrogenation, and artificially (*industrial trans-fatty acid*) in vegetable oils through partial hydrogenation of the oil. It is already proven that TFAs provide no nutritional benefits and are more harmful than any other fat, in terms of raising the risk of cardiovascular disease and many other health risks, and the consumption of partially hydrogenated vegetable oils (PHVOs) which are the primary source of industrially produced trans-fatty acids (iTFAs), is associated with a significantly increased risk of coronary heart disease, one of the leading causes of death worldwide. This is due to the effect on lipid levels, as trans-fat increases LDL cholesterol levels while lowering HDL cholesterol levels, promotes inflammation, and causes dysfunction in the lining of the heart and blood vessels. Trans isomers generally have more density, lower solubility and higher melting point. Often food manufacturers use artificial trans fat in food products because it is inexpensive and increases the food's shelf life, stability, and texture, which make them an important component of commercially produced processed food items such as margarine, vegetable shortenings, bakery products, and other snacks and fast food.

In recent years, consumer awareness of relation between diet and health has increased which caused worry about solid fat containing food products in terms of their high saturated fatty acid, and especially trans fatty acid contents. The growing trend towards healthier food products, e.g., products with less saturated fat and no trans fat, has prompted research into formulating alternative fats with high nutritional quality that also maintain the palatability, texture and functionality of conventional solid fats. Therefore, different attempts have been carried out, and one of the promising ways is using bigels or structuring oils with oleogelators.

The notion of **bigels or biphasic gel systems** is quite new as compared to the other gel formulations. These are uniform semisolid dispersion systems in which two gel phases (oleogel and hydrogel) are mixed together by applying a high shear rate and appear as a single gel when

seen visually. The two gels in each bigel are prepared individually using a specific gelator. The bigels possess advantages of both the phases and are very stable. This oleogel/hydrogel mixture has shown very interesting properties to reformulate food products containing almost zero amount of trans-fat without affecting the product's nutritional, textural, and organoleptic properties. Some of the advantages of bigels for food application are: it changes the physical state of the cooking oil from liquid to solid or semi-solid; it is a physical process (no trans fats or hydrogenated fats are added manually); it preserves the native nutritional profile of the oil (MUFAs and PUFAs) and textures the oil structure. The major areas for the food application of bigels include- baking industry, meat industry, confectionery industry, and dairy industry. Amongst others, the baking industry has benefited the most from the formulation of bigels since these can be used as replacers for shortenings and spreads which then contain no trans-fats.

In light of this, present research on the topic **"Trans-fat replacement by using Food Grade Edible Bigels"** was carried out in BBAU, Lucknow, to investigate the efficacy of synthesized bigels (B1, B2, B3) to use as substitution of margarine or shortening in food products mainly in bakery products, to replace trans-fatty acid without affecting product's nutritional and organoleptic properties. Characterization of synthesized bigels were done using various parameters and the overall acceptability of the formulated baked muffins was checked. In addition, estimation of trans-fatty acid was done thoroughly in oil samples heated at different intervals.

By observing the results, it was seen that, with the increase of oil phase, the system changed from oleogel/hydrogel to bicontinuous, and to hydrogel/oleogel state, which is a typical characteristic of a bigel system. Moreover, the findings provide an understanding that bigel properties depend on the formulation and preparation, which is valuable in the development of bigel fat replacers and other novel food applications. The overall result confirms that, by using food grade edible bigels we have successfully reformulated a food product (muffin) without affecting its nutritional, textural, and organoleptic properties, that also resulted in zero trans fat content. The shelf life study suggested that the bigel has a good storage life and can be used for more than two months (when stored at 4˚C to 0˚C). According to this study, synthesized bigels (B1, B2, B3) were more successful to be used as shortening in food product than control

(commercial margarine). All the data analyzed to check the hypotheses were statistically significant ($p<0.05$).

In conclusion, since FSSAI has implemented best-practice policy as specified by WHO to gradually reduce the trans-fat consumption in India by reducing the maximum limit of industrial trans-fatty acids to not more than 2% by weight in edible oils, fats and food products in which edible oils and fats are used as an ingredient, the use of bigels instead of margarine or shortenings present possible opportunities to formulate healthier baked goods by improving their fatty acid profiles (mainly trans fat elimination). In addition, bigels have been widely studied as drug delivery vehicles, particularly for transdermal applications, but in food products, until now, bigel applications have been very limited and largely restricted to solid fat replacers and delivery vehicles for functional compounds. Hence, based on the unique properties and potential usage of bigels, more research is needed to expand their food uses.

The research was conducted by using the following objectives:

1. **To study about the nutritional aspects of trans-fat and its consumption associated with various health risks.**
2. **To study about the estimation of trans-fat in popular fast food and bakery products of India.**
3. **To study about the physicochemical and antioxidant properties of cooking oils.**
4. **To study about synthesis of food grade edible bigels and their characterization using various technique.**
5. **To study the Shelf life of edible bigels during storage period.**
6. **To develop food item by using edible bigels.**
7. **To study the sensory evaluation, proximate analysis, fatty acid composition and other parameters of the developed food product.**

## Hypotheses:

1. **$H_0$1:** There is no significant effect of synthesized bigels (B1, B2, B3) on the physicochemical parameters during storage periods.

2. **$H_0$2:** There is no significant differences between the physicochemical parameters of commercial margarine (B0) and synthesized bigels (B1, B2, B3) during storage periods.

3. **$H_0$3:** There is no significant effect of synthesized bigels (B1, B2, B3) on the fatty acid composition of the developed muffins.

4. **$H_0$4:** There is no significant effect of synthesized bigels (B1, B2, B3) on the overall acceptability of the developed muffins.

## Methodology:

**Locale of the Study:**

The experiment was carried out in the research laboratory of the Department of Food and Nutrition, Department of Chemistry, and USIC Lab of Babasaheb Bhimrao Ambedkar University, Lucknow.

**Period of the study:**

The total period of the present research work was of three and half years. In the beginning, an extensive literature review of the study was done by going through various papers in journals and online publication, designing a protocol for research. The different materials used in the experiments and technique are outlined in this chapter. The whole research was divided into phases and each work was done in different phases one after the other in a planned way.

**Preliminary phase:**

The initial phase of the study was dedicated to in-depth understanding of the research topic. Subject experts were approached and literature review was done to prepare the protocol for experiment.

**Experimental phase:**

In this phase, all the experiments were carried out which include sample collection and analysis of different cooking oils, synthesis of edible bigels, characterization of bigels by using various technique, shelf life study of the synthesized bigels during storage period, bigels' application in food product and checking its efficacy by using various parameters.

**Research Design:**

Study was carried out by experimental research design. An experimental study design utilizing a qualitative and quantitative method for the analysis of the samples. This design was chosen as it will provide information on the role of edible bigels in reformulating food products and replacing trans-fat in the variety of food products. It made sure that it will be relatively inexpensive and can be used in a short space of time.

**Variables:**

The definition of a variable in the context of a research study is some feature with the potential to change, typically one that may influence or reflect a relationship or outcome. This study has two types of variable:

**Independent variables:**

An independent variable is one that the researcher controls or otherwise manipulates within a study. In order to determine the relationship between dependent and independent variables, a researcher will purposefully change an independent variable, watching to see if and how the dependent variable changes in response. Independent variables in this study were "heating temperature of cooking oils, days of storage period of edible bigels, etc."

**Dependent variables:**

A dependent variable is one being measured in an experiment, reflecting an outcome. Researchers do not directly control this variable. Instead, they hope to learn something about the relationship between different variables by observing how the dependent variable reacts under different circumstances. Dependent variables in this study were "Physicochemical parameters, Nutritional parameters, Sensory parameters, Antioxidant activity, Microbial activity, Trans-fat content, etc."

**Study Design:**

The study design of the present research is shown in the following Figure.

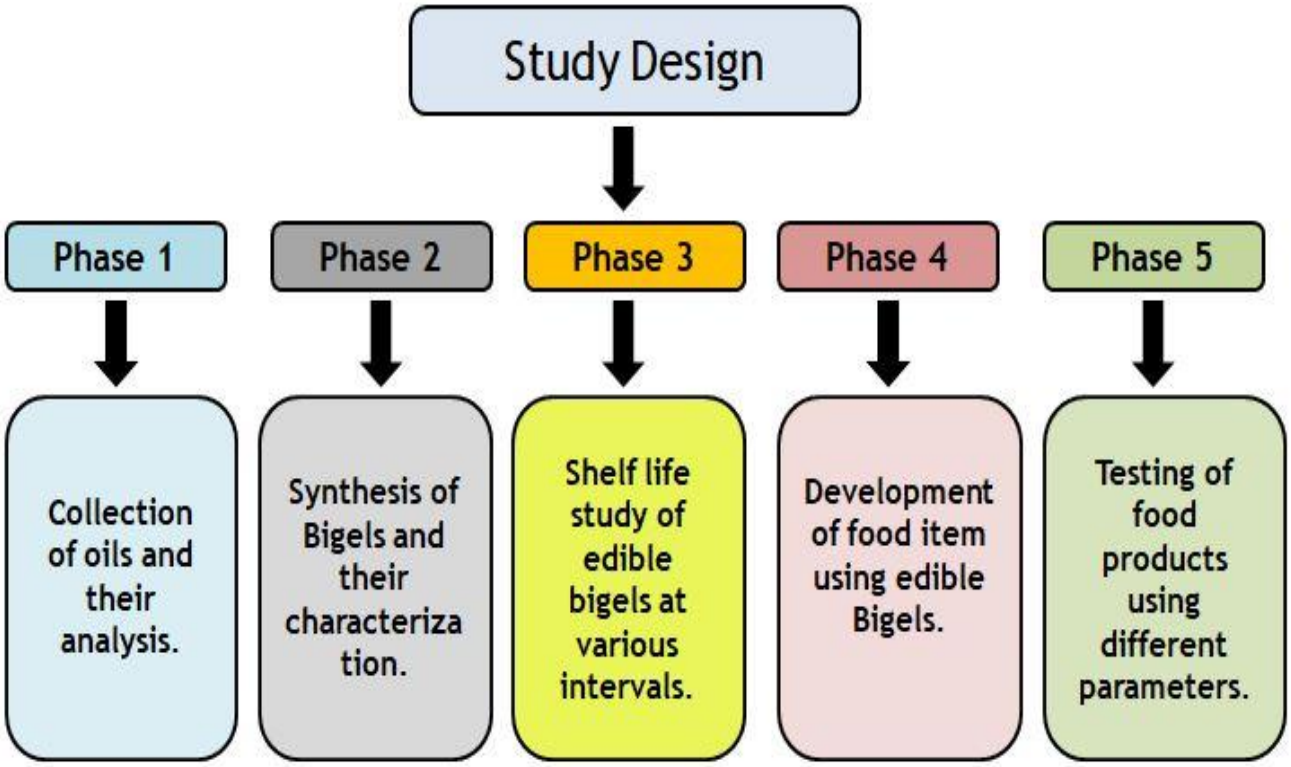


**Figure: Phase Wise Research Frame**

**Statistical Analysis:**

All values were shown as means and standard deviations (SD), for three replicates. The statistical analysis (where needed) was performed by using ANOVA function of IBM SPSS Statistical Analysis Software, version 24. The level of significance was defined as $p \leq 0.05$. All the graphs were plotted on Microsoft Excel 2007.

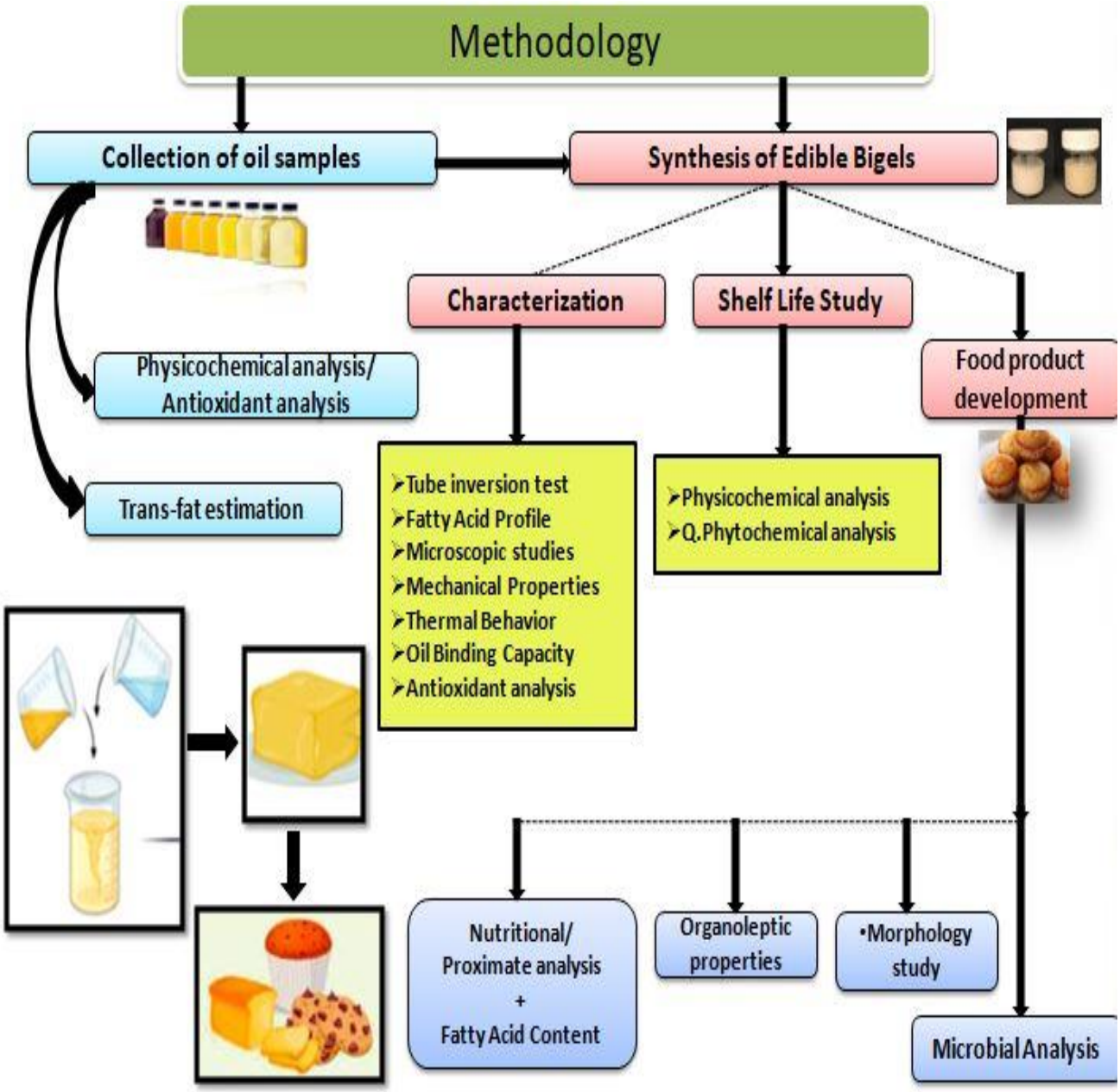


**Figure: Systematic diagram of Research**

There were four treatments that went through the analysis to check the hypotheses, and are mentioned below:

| Treatments | Sample |
|---|---|
| **B0** | **Control (Commercial Margarine)** |
| **B1** | **Coconut oil based Bigel** |
| **B2** | **Mustard oil based Bigel** |
| **B3** | **Rice bran oil based Bigel** |

The results obtained during the investigation have been summarized briefly under the following headings:

1. **Estimation of trans-fatty acid in popular fast food and bakery products of India.**
2. **Physicochemical and antioxidant analysis of cooking oils obtained from fried food vendors.**
3. **Physicochemical analysis of vegetable cooking oils after heat treatment at household level.**
4. **Synthesis and Characterization of Food-Grade Edible Bigels using various techniques.**
5. **Shelf Life Study of Edible Bigels during storage period (0, 15, 30 and 60 days).**
6. **Qualitative Phytochemical Analysis of all samples (0 and 60 days).**
7. **Nutritional Composition/ Proximate Analysis of all Muffin Samples.**
8. **Total fat composition/Trans-fat content of all Muffin Samples.**

9. **Morphology analysis of all Muffin samples using SEM (Scanning Electronic Microscope) with Energy Dispersive X-ray Spectroscopy (EDX).**
10. **Overall Acceptability of all Muffin samples through Sensory Evaluation.**
11. **Microbial analysis of all Muffin Samples on storage (0 and 15 days).**

## MAJOR FINDINGS OF THE RESEARCH

### 1. Estimation of trans-fatty acid in popular fast food and bakery products of India:

- In the present study, we evaluated the trans fatty acid (TFA) content in 15 food samples purchased from local markets of Lucknow, India. Total trans-fat content of baked foods (brownies, sweet bread, cookies, and pizza), confectionary (cream roll, and wafers), snacks (french fries, fried momos, noodles and patties), indigenous snacks (samosa, bread pakora, kachori, and veg kabab) were analysed.

- For determination, oils were first extracted from the food samples using Soxhlet apparatus and went through the trans-fat determination using ATR-FTIR spectroscopy. Some of the food products were labeled as containing zero trans fat, but found to have an appreciable amount of TFA after completion of analysis. The predicted percent TFA in food product was ranging from 1.32% to 3.95%.

- FTIR spectroscopy was successfully applied to trans-fat measurement using the absorbance bands at or near 966 $cm^{-1}$ in the formation of TFA. FTIR spectra of food samples of *Bread pakoda, Samosa, Kachori, Cream roll, Brownies, Sweet bread, Pizza, French fries, Manchurian balls, Patties, Fried momos, Wafers, and Cookies* clearly showed the intense peak at 966 $cm^{-1}$. These food samples contained trans fat more than 2% of the total fat/ 100 g of food; while *Noodles, and Veg kabab* didn't show any peak that can be calculated.

- From the results it was found that highest trans-fat content was present in indigenous snacks containing full of bad oil; like bread pakoda (3.95%), followed by samosa (3.59%) and kachori (3.52%) then cream roll (3.32%) per 100 gm of food sample. Trans-fat content in brownies, sweet bread, and pizza ranged from 3.13%, 3.12%, and 3.06% respectively. After that french fries (2.95%), manchurian balls (2.68%), patties (2.52%), fried momos (2.46%) and wafers (2.32%) and cookies (2.06%) found to have trans-fat content in the given range. Estimated trans-fat content found in the lowest range in noodles (1.46%) and veg kabab (1.32%) respectively.

- According to the result total trans-fat content in the samples analyzed was in the order: **Veg kabab > Noodles > Cookies > Wafers > Fried momos > Patties > Manchurian balls > French fries > Pizza > Sweet bread > Brownies > Cream roll > Kachori > Samosa >Bread pakoda.**

## 2. Physicochemical and antioxidant analysis of cooking oils obtained from fried food vendors:

- For this purpose, oil samples as fresh, after 5th batch and 10th batch of repeated frying (total 15 samples), were collected from five randomly selected street food vendors consumed regularly by the local people of Lucknow. Almost all shops were chosen as same in terms of oil type [mixture of ***refined soyabean oil (RSO)*** of Fortune brand and hydrogenated vegetable oil commonly known as ***dalda vanaspati ghee*** (10% of RSO)], food items being fried, the type of fryers, and frying conditions. All the samples were analyzed by evaluating physicochemical properties such as specific gravity (SG), viscosity, free fatty acid (FFA), iodine value (IV), peroxide value (PV) and saponification value (SV) using standard methods, and for antioxidant analysis oil's total phenolic content (TPC) and total flavonoid content (TFC) were assessed.

- The results revealed that almost all repeated frying oils collected were above the permissible limit for FFA, IV, and PV. It was noted that the highest values were obtained

in 10th batch of repeated frying oils. On the other hand, slight variation was noted for the SG and SV in all repeatedly heated oil samples. Increased usage of the repeated frying oils also resulted in a decrease in total phenolic content and total flavonoid content as compared to values obtained in fresh oil samples.

### 3. Physicochemical analysis of vegetable cooking oils after heat treatment at household level:

- Most of the research studies reported the characterization of vegetable oils after deep frying and found the deterioration in their physicochemical properties. In this context, ten different cooking oils - Coconut oil (virgin), Olive oil (extra virgin), Soybean oil, Palm oil, Mustard oil, Rice bran oil, Canola/ Rapeseed oil, Groundnut/ Peanut oil, Sunflower oil, and Safflower oil were assessed for their smoke point determination and physicochemical properties at various intervals (heating, frying and reheating after storage). The purpose of this analysis was to highlight the changes that appear in cooking oils profile when are exposed to high temperatures even for a short period of time, in order to take a picture of the massive use of vegetable oils at the household level as well as in food industry, especially in the case of deep and repeated frying.

- All fresh oil samples were first collected for the physicochemical analysis. All were heated above their smoke points. Once the oils reached their smoke points they were heated for continuously 30 min at 180˚C-200˚C. In the meantime we fried some potato chips in the oil. At this time oils were collected for their second trial of physicochemical testing. The remaining oils were transferred to glass bottles. The oil samples were filtered through a filter paper and stored for 2 days at room temperature. After that, the oils were re-heated again at the same temperature (180˚C-200˚C) for 30 min. And again we fried some mathri in the oil. These oils were then analyzed for physicochemical testing as third trial.

- The result showed increased values for the SG, FFA, PV and SV while decreased value for IV after the heat exposure for all investigated samples. We found that exposing edible oils

to high temperatures even for short period of time (30 min) impacted negatively on the physicochemical properties of vegetable cooking oils and resulted in an unpleasant odours and flavours in oils.

**4. Synthesis and Characterization of Food-Grade Edible Bigels using various techniques:**

- For the synthesis of bigels, oleogel and hydrogel were prepared separately. After that, the two gels were mixed together at room temperature to obtain a homogenized bigel. Three types of bigels were synthesized using Cold Pressed Coconut Oil, Cold Pressed Mustard Oil, and Cold Pressed Rice bran Oil. Beeswax was used as an oleogelator and veg gelatin (carrageenan K407) was used as hydrogelator. The synthesized bigels were stored in a glass container in refrigerator for 24 h before being analyzed. The ratio of OG and HG was prepared as 1:1 OG: HG.

- In the Tube Inversion Test, all the synthesized bigels (B1, B2, B3) were highly viscous and did not show any flow under gravity, thereby confirming the formation of a gelled structure.

- A careful observation of the images in confocal microscopy at different focal planes demonstrated the co-existent of two separate phases in bigels. Thus it was confirmed that the hydrogel and oleogel phases create a bi-continuous gel network within a bigel. The green droplets represent the hydrogel phase whereas the oleogel phase appearing as red in the images.

- GC analysis revealed that almost all fatty acids present in the coconut oil, mustard oil and rice bran oil were maintained in their bigel formulations. Moreover, trans fatty acids were not detected. The main fatty acids identified in coconut oil were lauric acid (C12:0), followed by Myristic acid (C14:0) and Palmitic acid (C16:0). It was observed that, in the bigel, palmitic acid and other fatty acids slightly increased in their amount as compared to

coconut oil. This could be due to the Beeswax being mainly a heterogeneous mixture of long-chain esters, n-alkanes, and fatty acids, where the amount of free fatty acids could be 9–11%, as pointed out in previous studies. Thus, Beeswax can contribute to the fatty acid content of oleogels. The main fatty acids identified in mustard oil were: Erucic acid (C22:1) followed by Linoleic acid (C18:2) and Cis-9 oleic acid (C18:1). In the bigel making process these fatty acids were maintained since bigel made with mustard oil oleogel (B2) are present in amounts very similar to mustard oil while very slightly increasing the amount existing in the beeswax. All fatty acids present in the rice bran oil were also maintained in their bigel formulation, along with no detection of any trans fatty acids. The main fatty acids identified in rice bran oil were: Cis-9 oleic acid (C18:1) followed by Linoleic acid (C18:2) and Palmitic acid (C16:0). In the bigel making process these fatty acids were maintained since bigel made with rice bran oil oleogel (B3) are present in amounts very similar to rice bran oil while slightly increasing the amount exist in beeswax.

- The XRD profile of formulations provided information about the modifications in the crystallinity. The diffraction pattern obtained from X-ray analysis confirmed that the resulting bigels were a semi-crystalline solid that presented a crystalline and an amorphous component. The crystalline component presenting a low degree of crystallinity, while the amorphous component contributing largely to the diffraction pattern. All the formulations showed a broad hump at ~20˚ 2θ. This kind of peaks is usually associated with the formulations with predominant amorphous nature. The strong diffraction peaks showed at a $2\theta$ about 19˚, 20˚, 22˚ in all bigel formulations with slight differences in each due to the presence of beeswax oleogel.

- FTIR analysis was performed mainly to obtain information on possible intermolecular interactions between components of both phases (oleogel and hydrogel) present in the bigel formulation. From B1 formulation (Coconut oil based Bigel), B2 formulation (Mustard oil based Bigel) and B3 formulation (Rice bran oil based Bigel) respectively, we can observe the intense peaks at 2916 and 2848 $cm^{-1}$. These two peaks correspond to C–H stretching vibrations of methylene ($-CH_2-$) and methyl ($-CH_3$) groups from cooking oil. The absorption peak in the region 3000–2800 $cm^{-1}$ was due to C−H stretching vibrations. All

of the peaks found in the gelatin hydrogel were conserved in the bigel. In the gelatin spectra, the broad peak between 3200–3400 $cm^{-1}$ range is related with O–H and N–H stretching and bending from water and gelatin, respectively. The peak observed at about 1640 $cm^{-1}$ was associated with the C=O stretching of the amide I bands, and the peak observed at about 1540 $cm^{-1}$ could be explained by the presence of amide II bands. Peak showing at 1636 $cm^{-1}$ corresponds to C=O stretching in gelatin, while Peaks at 1172 $cm^{-1}$ and 1463 $cm^{-1}$ correspond to C–H bending, and 1735 $cm^{-1}$ corresponds to C= O stretching in the bigels. In conclusion, the spectra did not show any major shifts or the occurrence of new peaks from interactions between the components of both phases (oleogel and hydrogel), suggesting that the system is a **"true" bigel**, as the gels are composed of two distinct phases that remain kinetically stable by structure due to the semi-solid structure present in both phases.

- The thermal properties of the bigel formulations (B1, B2 and B3) were studied using a differential scanning calorimeter. During heating, bigels (B1, B2 and B3) showed a prominent peak having a maximum intensity at around~ 56℃,~ 51℃ and~ 54℃ respectively. The water molecules associated with gelatin might have contributed to the formation of the broad endotherm in the bigels. During thermal analysis of the bigel formulation for B1, peak at ~34 ºC was shown which corresponds to the melting point of the bigel. Bigel with melting endotherm at ~32 ºC, shows for the formulation B2; suggesting that the thermal behavior of the bigels have not changed even after their incorporation into the gelatin network. This confirmed that the bigels have retained the thermal characteristics of Organogels (beeswax). In addition, peak showing at ~33ºC bigel formulation of B3 indicates an endothermic peak corresponding to the evaporation of free water present in the aqueous phase of the bigel.

- The antioxidant analysis of the synthesized bigels (B1, B2, B3) were done using DPPH Assay method. Since vegetable cooking oils pose good antioxidant activity**,** all three bigel formulations made from these oils, in their DPPH analysis, exhibited strong antioxidant activity too ($IC_{50}$ value of 10-50 µg/ml).

- Coconut oil based bigel (B1) indicate the best oil binding capacity i.e., 92.45% followed by Rice bran oil based bigel (B3) having 89.45% and the least oil binding capacity (85.24%) was observed in the mustard oil based bigel (B2). One thing that has to be noticed is, the homogenization process using high shear rates could destroy the crystal structure formed in the original oleogel, which can cause the resulting bigel matrix to have less oil-retention capacity.

## 5. Shelf life study of edible bigels during storage period (0, 15, 30 and 60 days):

- Study on the changes in physicochemical quality of all bigels throughout the storage period is crucial to ensure that the product do not lost its quality and safe to be consumed when store at a longer duration. For this purpose, one of the objectives of the present study was to conduct shelf life study of the bigels to determine their physicochemical properties at the different storage time (0, 15, 30 and 60 days). The changes in physicochemical parameters were studied by assessing the change in *Peroxide Value, Specific Gravity, Viscosity, pH value and Moisture content* of the Bigels (B1, B2, and B3).

- To check the efficacy of our synthesized bigels (B1, B2, B3) more accurately, we have taken margarine as control (B0) to be analyzed throughout the shelf life study of bigels. By observing the result it can be concluded that the formulated Bigels (B1, B2, B3) has the ability to be stored for >2 months like commercial margarine as the physicochemical parameters was not much affected by the storage period. The overall result indicates the significant effect ($p<0.05$) of synthesized bigels on peroxide value, specific gravity, viscosity, ph value and moisture content during storage.

- In conclusion, along this 60 days of shelf life study, it was found that there is an increment in the value of peroxide, specific gravity, viscosity and moisture content, while decreasing of pH value. However the values obtained were still in an acceptable range. This shows that the physicochemical parameters of all the synthesized bigels (B1, B2, and B3) product

changed but did not degrade along the storage time due to their unique properties, especially their large amount of phenolic compounds present in the oils. According to the analysis of all data, all treatments were statistically significant ($p<0.05$). However, differences in their significant value have been seen among all the treatments (B0, B1, B2, B3). Thus, we can say there were significant differences between the physicochemical parameters of commercial margarine (B0) and synthesized bigels (B1, B2, B3).

## 6. Qualitative Phytochemical Analysis of all samples (0 and 60 days):

- In the present study, qualitative analysis of phytochemicals was done at 0 day and 60 days. Phytochemical compounds such as Tannin, Saponin, Flavonoid, Alkaloid, Glycoside, Terpenoids, Phenols, and Sterols were screened in all control and bigel samples. Obtained result confirmed that the phytochemicals present in the Bigels (B1, B2 and B3) did not much affected even after 2 months of storage.

## 7. Nutritional Composition/ Proximate Analysis of all Muffin Samples:

- The parameters selected for nutritional analysis of treatments were Carbohydrate, Protein, Moisture, Ash, Water Activity and Total Fat. Result showed that the addition of bigels as a fat substitute modified the muffins' nutrient content, leading to changes in all nutritional parameters. However it is worth noting that the nutritional value of all muffins, including control, was not much differentiating from each other except the quantity of fat. Total fat content in muffin made from control was higher than muffin made from synthesized bigels.

- Thus it can be concluded that not only trans fat but bigel also helps to decrease the total fat content in a food product.

## 8. Total fat composition/Trans-fat content of all Muffin Samples:

- The fatty acid compositions of the muffins where commercial margarine (B0) was replaced with bigels (B1, B2 and B3) were investigated. By observing the result, it was seen that the muffins prepared with commercial margarine contained trans-fat (2.14±0.14). On the other hand, the proportions of trans-fat in muffins made by bigels (B1, B2, B3) had almost null.

- The major fatty acids of the muffins prepared with B0 were palmitic acid (60.7%) and oleic acid (20.1%), whereas lauric acid (41.7%) and myristic acid (25.2%) in B1, erucic acid (42.5%), oleic acid (22.6%) and linoleic acid (21.9%) in B2, and oleic acid (35.7%), and linolenic acid (29.5%) became more dominant in the bigel B3 muffin samples. Furthermore, significant differences in the value of PUFA, MUFA and SFA were observed between the B0 and bigels (B1, B2, B3) muffins. The level of trans fatty acids in the control (B0) prepared with commercial margarine were significantly higher than that of the bigel samples (B1, B2, B3).

- In conclusion, the synthesized edible bigel formulations (B1, B2, B3) were incorporated as a trans fat replacer in muffins for baking. The structuring of oils into solid bigels were effective in maintaining the quality attributes of muffins due to the presence of solid fat phases. Result suggests that margarine in the muffin formulation could be substituted with bigels without any sensory or nutritional quality loss, and the replacement of margarine with bigels could provide health benefits by replacing or decreasing the proportion of trans fatty acids. All the data tested were statistically significant ($p<0.05$).

## 9. Morphology analysis of all Muffin samples using SEM (Scanning Electronic Microscope) with Energy Dispersive X-ray Spectroscopy (EDX):

- In the current study, Scanning Electron Microscope (SEM) images were used to determine shape,surface morphology and surface features of all muffin treatments. The morphology

of muffin made from B0 (commercial margarine) is taken at X200 magnification, for B1 (coconut oil based bigel), B2 (Mustard oil based bigel), and B3 (rice bran oil based bigel) muffin samples, images were taken at X550 magnification.

- Hydrogel/ Oleogel concentration used during synthesis process plays an important role on morphology of bigels. Image showed that the air cell structure was more compact in case of control sample (B0) as compared to the bigel samples. Presence of fiber and protein in the muffin sample is more likely to be responsible for the compact structure of sample. However all muffin samples made from bigels (B1, B2, B3) were spherical in shape and having smooth surface as well.

- EDX analysis was done to know the elements present in developed muffins made by using Bigels (B1, B2, B3) along with the control (B0). Result reveals that the control muffin made from B0 sample had O (Oxygen) attached to K (potassium) with the highest content of 79.41%. The EDX spectra also showed the presence of other elements, such as Pt M, SK, KK and Cl K in the range of 9.88%, 7.36%, 1.95%, and 1.40% respectively. We observed that all the muffin made from B1, B2 and B3 formulation had the highest content of CK (carbon attached to potassium) in the quantity of 60.35%, 70.68% and 71.99% respectively. After that OK, Na K, and Cl K elements were present in the sample.

## 10. Overall Acceptability of all Muffin samples through Sensory Evaluation:

- Sensory evaluation of the muffin treatments made from Commercial margarine or B0 (control), B1 (Coconut oil based bigel), B2 (Mustard oil based bigel) and B3 (Rice bran oil based bigel) was done to check the overall acceptability of the treatments. Statistical analysis of all treatments were done and the pooled data showed that all the bigel treatments were significant to control (B0) in maintaining the overall acceptability of all samples.

- The combined result revealed that, all the analyzed data were statistically significant ($p<0.05$), but when it comes to preserving the overall acceptability of all samples, bigel treatments (B1, B2, B3) were significantly better than the control (B0). Thus, these results

suggest that bigels can substitute the margarine or shortening in the muffins without negatively affecting the main organoleptic attributes of marketed ones.

## 11. Microbial analysis of all Muffin Samples on storage (0 and 15 days):

- The microbiological change during the storage period was measured by total bacterial count, total yeast and mold count and total coliform count in the various muffin treatments (B0, B1, B2 and B3) stored at room temperature for 15 days.

- No microbial growth was found on the surface of muffin samples before storage (0 day), but the changes were seen after 15th days of storage. An increase in the total bacterial count, and total yeast and mold count with increase in the number of storage days (15) was observed. While for total coliform count no microbial growth was observed (not detected). However, in all the samples microbial growth observed for total bacterial count were not so high but should not be consumed due to unacceptable limit for food.

- In conclusion, edible oils being good source of antioxidant and antimicrobial activity do not have any microbial growth for long duration unless they are kept wrongly. Therefore, bigels formulated with these oils also possess good oxidative stability as we have seen in the previous results. However, muffin being a perishable food item generally have the shelf life of 3-5 days only when stored at room temperature in an airtight container. Thus, after storing them for 15 days, result revealed that muffin samples contained microbial growth.

# TESTING OF HYPOTHESIS

**$H_0$1: There is no significant effect of synthesized bigels (B1, B2, B3) on the physicochemical parameters during storage periods.**

From the table 4.14, 4.15, 4.16, 4.17, and 4.18, it was observed that synthesized bigels have significant effect ($p<0.05$) on the physicochemical parameters of all samples during storage period, which indicates null hypothesis was rejected and simultaneously proven.

**$H_0$2: There is no significant differences between the physicochemical parameters of commercial margarine (B0) and synthesized bigels (B1, B2, B3) during storage periods.**

From the table 4.14, 4.15, 4.16, 4.17, and 4.18, a significant difference was observed between the physicochemical parameters of commercial margarine (B0) and synthesized bigels (B1, B2, B3) during storage periods. However, all the data analyzed were statistically significant ($p<0.05$), which implies that null hypothesis was rejected and proven simultaneously.

**$H_0$3: There is no significant effect of synthesized bigels (B1, B2, B3) on the fatty acid composition of the developed muffins.**

From the table 4.21, it was observed that synthesized bigels have significant effect ($p<0.05$) on the fatty acid composition of the developed muffins, which proves that null hypothesis was rejected.

**$H_0$4: There is no significant effect of synthesized bigels (B1, B2, B3) on the overall acceptability of the developed muffins.**

It was observed from the table 4.22 that there is a significant effect ($p<0.05$) of synthesized bigels (B1, B2, B3) on the overall acceptability of the developed muffins. Therefore null hypothesis was rejected and simultaneously proven.

# CONCLUSION

- The current study explained an easy and economical method for the development of novel bigels using beeswax-edible oil based organogel and veg gelatin hydrogel, which resulted in a smooth, firm, fat-like texture. Different characterizations were done using various techniques. It was observed that, with the increase of oil phase, the system changed from oleogel/hydrogel to bicontinuous, and to hydrogel/oleogel state, which is a typical characteristic of a bigel system. Moreover, the findings provide an understanding that bigel properties depend on the formulation and preparation, which is valuable in the development of bigel fat replacers and other novel food applications.

- The overall result confirms that, by using food grade edible bigels we have successfully reformulated a food product (muffin) without affecting its nutritional, textural, and organoleptic properties, that also resulted in zero trans fat content.

- The shelf life study also suggested that the bigel has a good storage life and can be used for more than two months (when stored at 4˚C to 0˚C).

- According to this study, synthesized bigels (B1, B2, B3) were more successful to be used as shortening in food product than control (commercial margarine).