CHAPTER 3
DESIGN OF RESEARCH

3.1 Area of Selection

The experiment was carried out at Department of Food Technology, Sri Padmavathi Mahila Viswa Vidyalayam, Tirupathi, A.P., INDIA.

3.2 Procurement of Oils

Test eatable oils accessible locally were obtained in mass from the Oil Millers Association of Tirupathi, Andhra Pradesh, India. Sunflower oil, Soybean oil, Rice bran oil, Groundnut oil, Olive oil and Palm oil have been utilized as control and furthermore were utilized as test oils.

3.3 Oil Blending

Mixed oils were set up by blending in the proportions of 50:50 in the research facility utilizing a blender cum blender and 15 mixes were arranged and put away in PET jugs. Every one of the specimens were gathered for the local oil and after each arrangement of bunch browning. The oil tests were kept at -18°C in nitrogen climate, in impenetrable, golden shaded glass bottles. The mixing was done by blending foreordained measures of oils with mixing for 1h at 400°C on an attractive stirrer subsequent to flushing with nitrogen. The blending proficiency was checked by assessing unsaturated fat synthesis of the mixed oils occasionally and contrasting it and hypothetical esteem [133].

3.4 Physico-compound Parameters

The examples were put away for a time of eleven months at a temperature of 25±5°C and relative dampness of 60-70% individually. The examples were opened after like clockwork for the assessment of different physico-substance parameters. Distinctive physic-synthetic and healthful parameters were considered for the individual, mixed oils and also items created utilizing mixed oils.

3.4.1 Physical parameters

3.4.1.1 pH

The pH of the items was dictated by utilizing a pH meter.
3.4.1.2 Color

Shade of the oil was measured by utilizing Lovibond tintometer (Model F, Effem Technologies Pvt. Ltd., New Delhi, India)

3.4.1.3 Spreadability

Spreadability of the oils were contemplated by utilizing a Brookfield synchro-electric Rheometer (M/s Brookfield Engineering Company, Middleboro, MA, USA) with LVT show shaft (axle No.2) at 25±1°C.

3.4.2 Chemical parameters

Free unsaturated fats (FFA), Peroxide esteem (PV), Iodine esteem (IV), Saponification esteem (SV) were dictated by utilizing standard strategies (AOCS, 2004).

3.4.2.1 Determination of free unsaturated fats

The free unsaturated fat (FFA) substance of the oil was dissected by AOCS Official Method (AOCS, 2002). It delineates level of hydrolysis by the measure of acidity show in the oil which impacts the flavor and is measured by the volume of potassium hydroxide required to kill it. The killed liquor was added to the oil test and permitted to bubble, and afterward titrated against sodium hydroxide utilizing phenolphthalein pointer. The FFA esteems were communicated as % oleic acid premise.

%FFA as oleic acid = salt volume (mL)X soluble base normalityX 28.2/specimen weight (g)

3.4.2.2 Peroxide esteem (PV)

3.4.2.2.1 Principle

Peroxide esteem is a measure of the peroxides contained in the oil. The peroxides exhibit are dictated by titration against thiosulphate within the sight of KI. Starch is utilized as pointer

3.4.2.2.2 Methodology

The peroxide esteem (PV) of the two oils prior and then afterward progressive browning was controlled by utilizing standard techniques (AOCS, 2002). The oil test was broken down in acidic acid/chloroform blend (3:2), trailed by the expansion of
immersed KI and held aside under the shut condition for a time of one min. The response was ended with the expansion of refined water and was titrated against standard sodium thiosulfate (0.1 N) utilizing newly arranged starch marker until the point when the blue shading vanished. The PV esteems are communicated as milli equivalents of oxygen/kg of oil.

3.4.2.3 Determination of Saponification Value (Number) (AOCS Method album 3-25, 1993)

3.4.2.3.1 Principle

Fats and oils are the rule put away types of vitality in numerous living beings. They are exceedingly diminished mixes and are subordinates of unsaturated fats. Unsaturated fats are carboxylic acids with hydrocarbon chains of 4 to 36 carbons, they can be soaked or unsaturated. The most straightforward lipids built from unsaturated fats are triacylglycerols or triglycerides. Triacylglycerols are made out of three unsaturated fats each in ester linkage with a solitary glycerol. Since the polar hydroxyls of glycerol and the polar carboxylates of the unsaturated fats are bound in ester linkages, triacyl glycerols are non polar, hydrophobic particles, which are insoluble in water.

Saponification is the hydrolysis of fats or oils under essential conditions to bear the cost of glycerol and the salt of the relating unsaturated fat. Saponification truly signifies "cleanser making". It is essential to the modern client to know the measure of free unsaturated fat present, since this decides in expansive measure the refining misfortune. The measure of free unsaturated fat is assessed by deciding the amount of salt that must be added to the fat to render it unbiased. This is finished by warming a known measure of the fat with solid watery scathing pop arrangement, which changes over the free unsaturated fat into cleanser. This cleanser is then expelled and the measure of fat remaining is then decided. The misfortune is assessed by subtracting this sum from the measure of fat initially taken for the test.

The saponification number is the quantity of milligrams of potassium hydroxide required to kill the unsaturated fats coming about because of the total hydrolysis of 1g of fat. It gives data concerning the character of the unsaturated fats of the fat-the more drawn out the carbon chain, the less acid is freed per gram of fat
hydrolysed. It is likewise considered as a measure of the normal atomic weight (or chain length) of all the unsaturated fats show. The long chain unsaturated fats found in fats have low saponification esteem since they have a generally less number of carboxylic useful gatherings per unit mass of the fat and in this way high sub-atomic weight.

### 3.4.2.3.2 Methodology

A 0.002kg of the oil test was weighed into a volumetric cup. At that point 25mL of 1.0N alcoholic KOH was pipetted and permitted to deplete for around 1 minute into the blend. A condenser was associated with the jar and the blend test permitted to bubble delicately however consistently for 45 minutes for finish saponification. The jar and the condenser were then cooled yet not adequately to shape a gel, within the condenser was washed down with around 1ml of refined water. The condenser was detached and 1ml of phenolphthalein marker included. The arrangement was titrated with 0.5N hydrochloric acid (HCl) until the pink shading just vanished.

**Computation:**

\[
\text{Saponification Value} = 56.1 \times \frac{(B-S)N}{W}
\]

Where, 
- \(B\) = Volume in ml of standard hydrochloric acid required for the clear.
- \(S\) = Volume in ml of standard hydrochloric acid required for the specimen
- \(N\) = Normality of the standard hydrochloric acid and
- \(W\) = Weight in gm of the oil/fat taken for the test.

### 3.4.2.4 Determination of Iodine Value

#### 3.4.2.4.1 Principle

Unsaturated fats respond with a halogen [iodine] bringing about the expansion of the halogen at the C=C twofold security site. In this response, iodine monochloride responds with the unsaturated securities to create a di-halogenated single security, of which one carbon has bound an iota of iodine.

After the response is finished, the measure of iodine that has responded is controlled by including an answer of potassium iodide to the response item.
ICl + KI -> KCl + I₂

This causes the rest of the unreacted ICl to shape atomic iodine. The freed I₂ is then titrated with a standard arrangement of 0.1N sodium thiosulfate.

I₂ + 2 Na₂S₂O₃ -> 2 NaI + Na₂S₂O₄

Soaked unsaturated fats won't give the halogenation response. On the off chance that the iodine number is between 0-70, it will be a fat and if the esteem surpasses 70 it is an oil. Starch is utilized as the marker for this response so the freed iodine will respond with starch to give purple hued item and in this way the endpoint can be watched.

3.4.2.4.2 Methodology

A 0.001kg of oil test was weighed into a 500mL volumetric flagon. 15mL of carbon tetrachloride was added to the specimen and twirled to guarantee that the example is totally broken up. 25mL of Wijs arrangement was then apportioned into the flagon containing the example utilizing a pipette. The flagon was stoppered and twirled to guarantee finish blending. The specimen was then set oblivious for 30 minutes at room temperature. The carafe was expelled from capacity and 20mL of 10% potassium iodide (KI) arrangement included, trailed by 150mL of refined water. The blend was titrated with 0.1N thiosulphate (Na₂S₂O₃) arrangement, including slowly and with steady and fiery shaking until the point when the yellow shading had practically vanished. 1.5mL of starch pointer arrangement was included and the titration was proceeded until the point that the blue shading vanished.

3.5 SELECTION OF THE PRODUCTS

For the present examination, the items chose are Potato Chips, Bhoondi and Chekodi and for the most part on the grounds that these are the basic nibble things which are all around acknowledged by the general population of all wage bunches in India.
3.5.1 Preparation of Potato Chips

3.5.1.1 Ingredients

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Potatoes’</td>
<td>100grams</td>
</tr>
<tr>
<td>Salt</td>
<td>to taste</td>
</tr>
<tr>
<td>Stew powder</td>
<td>2 grams</td>
</tr>
<tr>
<td>Oil</td>
<td>0.5 litre</td>
</tr>
</tbody>
</table>

3.5.1.2 Method of Preparation

100grams of Potatoes were taken and washed well. At that point cleaned with a fabric and kept for 5min. Utilizing a sharp blade the potatoes were cut into thin layers and again washed with water and air dried. At last these were seared in the chosen oils and mixed oils.

3.5.2 Preparation of Bhoondi

3.5.2.1 Ingredients

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal flour</td>
<td>100grams</td>
</tr>
<tr>
<td>Red Chili Powder</td>
<td>1 table spoon</td>
</tr>
<tr>
<td>Salt</td>
<td>to taste</td>
</tr>
<tr>
<td>Oil</td>
<td>0.5 litre</td>
</tr>
</tbody>
</table>

3.5.2.2 Method of Preparation

In a blending dish included besan flour, rice flour, hing, one tsp red bean stew powder and required salt. To start with add little water to make a thick glue. At that point included more water little by little to shape a streaming player, at that point include the two tsp oil (this is guarantee pouring the hitter easily). Here is the place we should be extremely watchful. In the event that the hitter consistency is not flawless the balls won't be round and it will have tails. To check if the hitter is in remedy consistency: First take a spoon and plunge the posterior of the spoon into player and hold it over the preheated oil. On the off chance that it drops as immaculate adjusts then the player is perfect. Turn over to cook on the two sides
evenly. Once it turns brilliant darker and take it out from oil and deplete in tissue. Do this for the rest of the hitter also.

3.5.3 Preparation of Chekodi

3.5.3.1 Ingredients

- Wheat flour: 100 grams
- Ghee: 500 grams
- Salt: to taste
- Oil: 0.5 litre

3.5.3.2 Method of Preparation

Wheat flour bought from the nearby market, Tirupathi was altogether blended with adequate measure of water to make the mixture, made into little rings to achieve same size and width through the Chekodi. The measurement of the Chekodi is observed to be 2 cm. The arranged Chekodi are subjected to profound fat broiled in the chose oils and mixed.

Profound fat fricasseeing of the snacks expelled snacks was led in each oil mix while keeping up singing temperatures at 180°C±5°C. Indistinguishable broiling tests were additionally directed with control oils. The snacks expelled nibble was put away at room temperature in polythene packs similarly as they are put away under typical showcasing conditions. These were put away for 60 days and were opened occasionally (0, 30, and 60 days) for tangible assessment took after by synthetic investigation to direct a correlation of oil quality, and taste of the item arranged in various mixes of oil.

3.6 Product Analysis

3.6.1 Oil content investigation

The aggregate oil content in the snacks was controlled by soxhlet extraction (AOAC technique, 1984) utilizing oil ether (b.p. 40-60°C) as dissolvable, for 6 hours. The oil ether was vanished under vacuum at 60°C by a rotational evaporator (M/s Eyela Corp., Tokyo, Japan). The remaining oil division weight was evaluated by an
electronic adjust (M/s Sartorious Inc., Bangalore, India). The heaviness of the portion of extricated oil was utilized to evaluate the % of aggregate oil volume of the Snacks and by subtracting it with the crude potato oil content, % oil take-up amid searing was ascertained.

3.6.2 Storage

The singed snacks were wrapped in aluminum thwart and bundled in LDPE sacks. Nitrogen was flushed into them before fixing. From there on, the parcels were put away at -18ºC for 10, 20, 30 and 40 days. The parcels were marked for the length of capacity and were pulled back as needs be for organoleptic and surface investigations.

3.6.3 Sensory assessment:

The examples put away in polythene packs were assessed by board individuals for their discernible tangible properties, similar to shading, season, surface, taste, lastly the general worthiness at first at 0 day, following 30 days, and following 60 days utilizing five point hedonic scale (Ranganna, 1992). A calendar was created for tangible assessment i.e. for appraisal of shading, enhance, surface, taste and general worthiness of the item. Keeping in mind the end goal to take out predisposition, the items were undisclosed with regards to the reality to the kind of oil that was utilized, and were allocated codes as mix 1, mix 2 et cetera till mix 12. The expectation of the examination was to decide how well the oil mixes performed healthfully, in tactile assessment and oil solidness and use however a controlled 60 day stockpiling.

3.6.4 Chemical examination

The snacks were put away for a time of 60 days and the oil was removed from the item utilizing soxhlet device at 0, 30, 60 days. The separated oil tests were examined for different rancidity parameters, for example, acid esteem, peroxide esteem, free unsaturated fats (% oleic acid), para anisidine esteem, totox esteem, thiobarbituric acid esteem and kreis test by institutionalized techniques. The information was organized and subjected to investigation of difference, trial of criticalness, means
and standard deviation. The bundle utilized for the examination was SPSS 15.0, Windows form.

### 3.6.5 Texture examination

The surface of the broiled snackswas dictated by Instron Texture Analyzer (M/s Instron Inc., Buckinghamshire, UK) display number 4301. The browned wedges were cracked longitudinally with a wedge molded test (60° cutting point, 7 cm width) at a crosshead speed of 20 mm/min. A compel remove outline was built from the resistance that the test experienced amid the break. The pinnacle acquired for each situation spoke to the hardness of the singed wedge. The lower shear esteems delineated a gentler item and higher, a harder item. Surface investigation parameters of the seared snackswere communicated as hardness (N). The trials were directed in triplicates and qualities were accounted for as mean ± SD.

### 3.6.6 Proximate investigation

The dampness content, rough fiber content, protein content, fat substance and fiery remains content were resolved (AOAC technique, 1984). The aggregate starches were ascertained by distinction (West et al., 1988). For Reducing and non-lessening sugar estimation, dried and defatted potato test was grounded to powder by mortar and pestle. The sugar was removed from powder with water for 10 min at 60oC and arrangement was utilized to dissect sugar content by Lane and Eynon technique (Lane and Eynon, 1923).

### 3.7 SHELF LIFE STUDY OF THE PRODUCTS

The readied items were put away both at room temperature and frosty stockpiling (5-10°C) to consider the time span of usability of the items. The items are at first dissected synthetically, microbiologically and organoleptically before capacity.

### 3.7.1 Observations recorded

The items were broke down at an interim of at regular intervals in a day for the accompanying parameters and recorded.
3.7.2 Organoleptic assessment of the items

The items created were surveyed each month by a board of 10 judges. The qualities considered amid the investigation were appearance, shading, flavor, taste and general adequacy.

3.7.3 Development of score card:

With a specific end goal to assess the tactile characteristics of created items, elucidating test, which diagnostically depicts the tangible characteristics of an item, was utilized. So as to rank the tactile qualities, ordinal scoring technique (positioning) was utilized. Five – point scale was utilized for positioning i.e., from 1 to 5 and subtle elements of positions/scores are as per the following.

5-Excellent
4-Good
3-Fair
2-Poor
1-Very poor

A score card was created to assess the adequacy of the items. The investigation was completed in a room, which was free from all aggravations in mid-evening.

3.8 Microbiological assessment of the items

Microbiological contemplates were directed at first and third month of capacity. Add up to plate check (TPC), yeast and form number, coliform and E.Coli were embraced.

The strategy of Cruick Shank et.al, (1975) was utilized for add up to plate number and yeast and shape tally. The microbiological consider was done at Department of Food innovation, Sri Padmavathi Mahila Viswa Vidyalayam, Tirupathi.

3.8.1 Total plate number and yeast and form tally by pour-plate technique

Ten – overlay serial weakening of the bacterial suspension was readied. Typical saline was utilized as a diluents for the living being. 9 ml of the diluents was pipette into a few sterile test tubes. The bacterial suspensions were consistently blended. Utilizing a clean one ml pipette, one ml of the suspension was moved into
the main container of diluents and blended completely. From this blended weakening, 1 ml was exchanged to the following diluents. Comparable weakenings were made similarly utilizing new pipettes. One ml of every weakening (from the best weakening) was pipetted into sterile petri plates and 15 ml of liquid agar medium which was cooled around 450°C was filled plates containing weakened examples. The agar medium was promptly disseminated by delicately blending the petridish in round developments both clock insightful and hostile to clock savvy on a level seat and afterward permitted to set equitably and the modified plates were hatched for 1-2 days and 3-5 days at 370°C for bacterial and yeast and shape separately. For add up to plate check, add up to plate number agar and potato dextrose agar for yeast and form tally was utilized.

3.8.2 Coliform and E.coli tally:

10 ml of the example was immunized in twofold quality lactose juices in 5 test tubes, one ml in 5 test tubes single quality lactose soup and 0.1 ml in other arrangement of lactose juices test tubes and hatched at 350°C for 24+/ - 2hours. After brooding they were watched for gas generation. Gas creation in Durham's tubes shows positive test. Positive test tubes were isolated and vaccinated in Brilliant green lactose bile soup and brooded for 24-48 hours. Nearness of gas creation demonstrates positive test. From the positive tubes, vaccinated in EMB agar and streaking was finished. The plates were brooded for 24 – 48 hours. After the advancement of states, they were separated by watching province morphology and Gram's recoloring.

3.9 GC-MS technique

Unsaturated fats of triglycerides were broke down by get ready methyl esters as per a regular technique comprising of saponification took after by acidification lastly methylation utilizing diazomethane according to the revealed strategy. Gas chromatographic (GC) examination of unsaturated fat methyl esters was completed utilizing a NUCON SERIES 5700 of information station 0-2.5 mV run and < 1.5s reaction rate. A 2m x 2 mm stainless steel 10% Silar 7C section pressed with 60-120 work Gas Chrom Q was utilized. The injector and indicator temperatures were kept up at 240°C. The section temperature was set at 160°C for 5 min and afterward inclined at a rate of 5°C for every min to a last temperature of 220°C and kept there for 20
min. The aggregate time for examination was 37 min. Unsaturated fats were likely distinguished by correlation with maintenance times of credible reference tests.

3.10 FT-IR technique

Biodiesel tests were described by FT IR, utilizing a Bio-Rad Excalibur Model FTS3000MX in the range 4000 - 400 cm\(^{-1}\). The determination was 1cm\(^{-1}\) and 15 filters.

3.11 NMR strategy

NMR examinations were performed at 7.05 T utilizing Avan CE 300 MHz spectrometer outfitted with 5mm BBO tests. Deuterated chloroform and tetramethylsilane were utilized as dissolvable and inward standard separately. \(^1\)H (300 MHz) spectra were recorded with heartbeat span of 30o, a reuse postponement of 1.0 s and 8 filters. The \(^{13}\)C (75 MHz) spectra were recorded with a heartbeat span of 30o, a reuse postponement of 1.89 s and 160 outputs.

3.12 STATISTICAL ANALYSIS

The information were investigated utilizing univariate and multivariate measurable examination. Investigation of Variance (ANOVA, proc blended, SAS form 8.2, 2001) was performed to decide huge impacts of the characteristic forces in each of the items. A critical F-proportion (\(\alpha < 0.05\)) from the ANOVA showed that an ascribe was utilized to discover contrasts among the items. Multivariate Analysis of Variance (MANOVA) was utilized to decide contrasts among the items, communicated as far as mean vectors of the tangible characteristics. Unmistakable Discriminant Analysis (DDA, proc candisc SAS variant 8.2, 2001) was connected to recognize tactile traits that basically accentuated contrasts among the items. While applying this procedure, authoritative coefficients are computed. The most noteworthy incentive for standard coefficient is 1.0, which demonstrates consummate segregation. At the point when the sanctioned coefficient of a variable gets more like one, such factor is a segregating variable.