METHODS AND MATERIALS

CHAPTER 4

METHODS AND MATERIALS

This chapter deals with the materials and methods used to elicit necessary data on fried food intake by Gujarati housewives and the sensory quality of french fries and bhajias fried in Groundnut and Cottonseed oil intermittently, was studied with respect to various organoleptic attributes and thermal stability of these two commonly used edible oils used for frying french fries and bhajias was assessed with respect to different chemical and physical parameters. Finally the frying practices and other food safety practices of a railway food outlet were also determined. IEC material for safe frying practice was also developed. Various methods used during the course of the study are discussed under the following phases:

PHASE I: Fried food intake, knowledge on fats and oils and frying practices of the Gujarati housewives of urban Vadodara and its association with the prevalence of NCDs.

4.1.1 Experimental plan for phase I

4.1.2 Selection of the families

4.1.3 Tool used for collecting data

4.1.4 Statistical analysis

PHASE II: Sensory qualities of french fries and bhajias fried in cottonseed oil (CSO) and groundnut oil (GNO) during intermittent frying.

4.2.1 Experimental design of phase II

4.2.2 Screening of panelist for sensory evaluation

4.2.3 Threshold test

4.2.4 Development of score cards for sensory evaluation

4.2.5 Tool used for sensory evaluation

4.2.6 Procurement of oil, potatoes and raw material for french fries and bhajias

4.2.7 Standardization and preparation of french fries and bhajias

4.2.8 Oil uptake by french fries and bhajias during 25 h of intermittent frying

4.2.9 Statistical analysis

PHASE III: Chemical changes due to thermal degradation of intermittently deep fried cottonseed oil (CSO) and groundnut oil (GNO) as a result of french fries and bhajias frying.

4.3.1 Experimental design for phase II

4.3.2 Frying conditions used for french fries and bhajias

4.3.3 Collection of oil samples used for frying french fries and bhajias at intermittent durations

4.3.4 Analysis of CSO and GNO used for frying

4.3.4.1 Chemical methods used to check the quality of CSO and GNO used for frying

a. Fatty acid profile
b. Total polar components
c. Peroxide value
d. p-anisidine value
e. Totox value
f. Iodine value
g. Acid value

4.3.4.2 Physical parameters used to check the quality of CSO and GNO used for frying

a. Refractive Index
b. Color

4.3.5 Statistical Analysis

PHASE IV: Case study on prevailing food safety and frying practices in Jan aahar- a government run food outlet at Vadodara railway station.

4.4.1 To determine the current knowledge of kitchen staff on food safety practices in terms of personal hygiene, food hygiene, environmental hygiene, nutrition and health.

4.4.2 To determine the oil procurement and storage practices and frying practices of kitchen staff working at Jan aahar -a Government run food outlet at Vadodara railway station.

PHASE V: Development of Nutrition Health Education (NHE) material in two languages on intake of edible oil, types, and on choices of oils for healthy living and problems during frying of edible oil and its storage.

4.5.1 To develop IEC material on intake of edible oil, types, and on choices of oils for healthy living.

4.5.2 To develop IEC material on frying and problems during frying.

PHASE I

4.1 Fried food intake, knowledge on fats and oils and frying practices of the Gujarati housewives of urban Vadodara and its association with the prevalence of non-communicable diseases (NCDs).

Frequent/high oil or fried food intake are implicated as one of the reasons for increased prevalence of obesity, diabetes and CHDs across the world. In present phase of the study, Gujarati housewives were surveyed for their fried food intake, knowledge on fats and oils and frying practices and to assess its association with prevalence of non-communicable diseases. Under the survey methods-selection of families, tools used for collecting information are discussed and the use of statistical analysis applied on the obtained data. The study was approved by Medical Ethical Committee under the reference no. F.C.Sc./FND/HE/88.

4.1.1 Experimental plan for phase I

- 120 Gujarati housewives- 30-65 years of age were randomly selected from five different zones of Baroda (Central, North, East, West and South). Baseline information will be collected on family monthly income, education level and exercise pattern as well as their knowledge on fats and its use.
- Morbidity profile of housewives was collected to determine the presence of common NCDs and gastrointestinal problem.
- Association between fried food intake and morbidity profile was determined using chi-square and odds ratio.
- Knowledge on amount of oil intake, refrying, changes during storage of fresh, *trans* fats and oil blends was determined using semi-structured questionnaire.

4.1.2 Selection of the families

Using purposive sampling method, one hundred and twenty Guajarati house wives in the age group of 30-65 years belonging to middle income group (Kuppuswamy, 2007) were surveyed from five different zones (Central, North, East, West and South) of urban Vadodara.

4.1.3 Tool used for collecting data

In total 54 questions were asked relating to the general information on various aspects such as education level, exercise, morbidity profile, and specific information on type of oil used for cooking was collected using a pre-tested structured questionnaire (Appendix 11.1). The questionnaire was specifically aimed at collecting information on the consumption pattern of popularly consumed deep fried, shallow fried and sweets prepared at home and purchased from the markets of Vadodara. Frequency of consumption of these foods was determined using a food frequency questionnaire (Appendix 11.1).

Anthropometric measurements were determined by measuring the body weight using calibrated bathroom weighing scale and height was measured using a flexi tape. BMI (Body Mass Index) was calculated using the standard formula (WHO, 2004; Sheth M and Shah N, 2008):

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (mt}^2\text{)}}$$

4.1.4 Statistical analysis

Statistical analysis was performed using Epi info-2006 and Microsoft Excel-2007. Chi square test and Odds ratio used to determine the association between frequency of deep and shallow fried food and deep fried sweets intake and prevalence of NCDs and GI problems. Association between the knowledge level on *trans* fats and education level of the subjects was also determined using chi square test.

PHASE II

4.2 Sensory qualities of french fries and bhajias fried in Cottonseed oil (CSO) and Groundnut oil (GNO) during intermittent frying.

Sensory evaluation can be defined as scientific method used to analyze and interpret the quality of a food product perceived through the senses of sight, smell, touch taste and hearing (Srilakshmi B, 2004). Frying of foods has unique sensory properties of color, flavor, texture, and palatability.

This phase of the study was conducted to assess the changes occur in sensory quality of fried foods during intermittent frying in CSO and GNO. Intermittent frying can be defined as frying of a food product at predicted time interval. The experimental design of phase 2 is depicted in Figure 4.2.1.

4.2.1 Experimental design for phase II

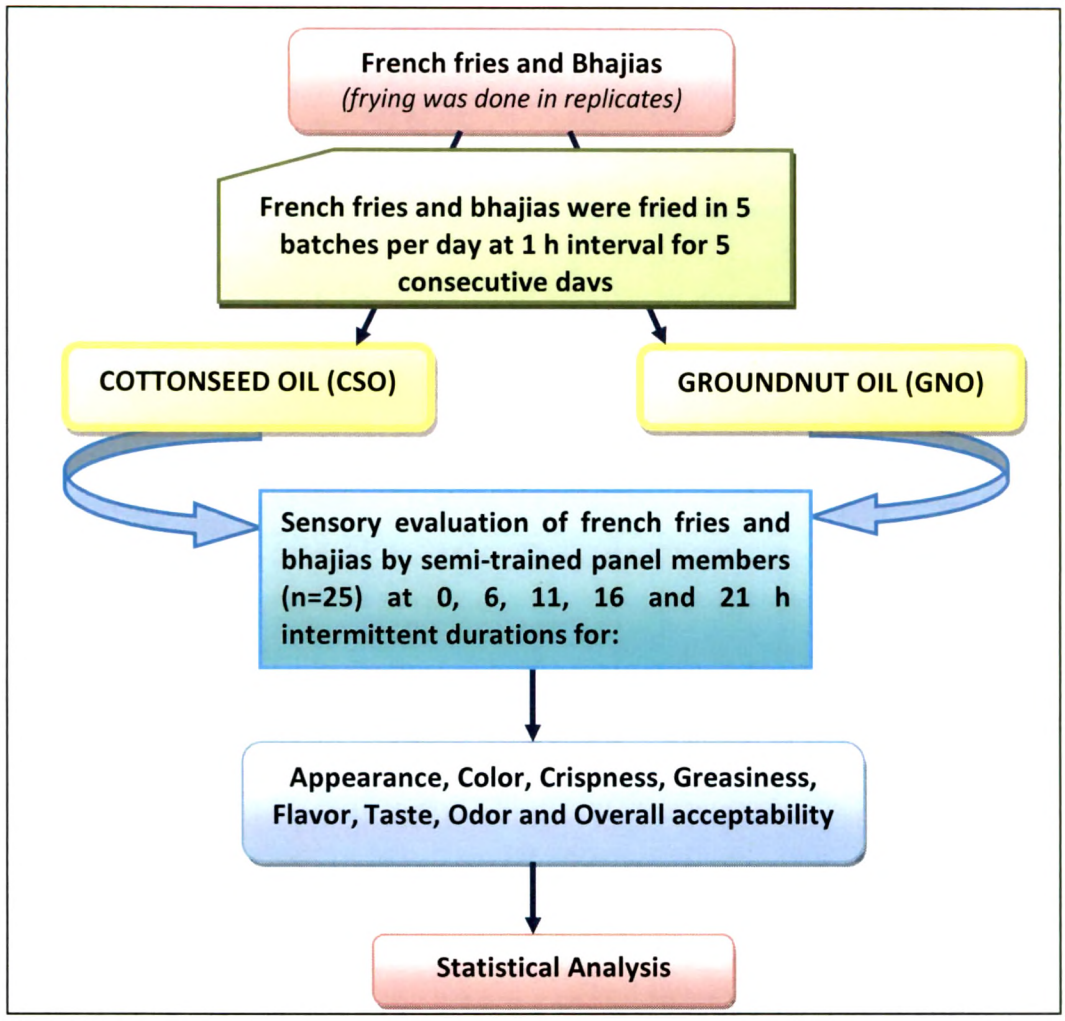


Figure 4.2.1: Experimental design for phase II

4.2.2 Screening of panelists

In this section, selection of panel members was carried out. Students and staff of Department of Foods and Nutrition were subjected to threshold testing.

4.2.3 Threshold test

Threshold is defined as a stimulus scale at which a transition in a series or judgment occurs. For conducting this test, score card for the same was formulated and pre tested (Appendix 11.2). Each perspective panel member was given two sets of the solution i.e. Set 1 and Set 2 having six solutions of

different concentrations of salt and sugar respectively and was arranged in random order (Joshi VK, 2006). The participants were asked to identify and rank the samples in increasing order of concentration of taste from the test solutions offered. Three successive trails were conducted for screening of the panelists.

4.2.4 Development of score cards for sensory evaluation of french fries and bhajias

Score cards were developed for sensory evaluation (Appendix 11.3) where in tools for evaluation is listed.

4.2.5 Tool used for sensory evaluation

Hedonic rating scale was used to determine the acceptability of fried foods. It is ascertained for 'likes' and 'dislikes' of foods., Scales with different range of scores or with suitable terms expressing the interest of pleasure can be used (Joshi VK, 2006).

Sensory evaluation of french fries and bhajias were carried out at 0, 6, 11, 16 and 21 h of intermittent frying, without replenishing the oil. Twenty five semi-trained sensory panelists who were familiar with the quality of french fries and bhajias were selected. Freshly fried (hot) french fries and bhajias were judged for appearance, color, crispness, greasiness, taste, flavor, odor, and overall acceptability. French fries and bhajias were evaluated on 9-point hedonic scale where, 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4- dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely (Joshi VK, 2006).

4.2.6 Procurement of oil, potatoes and raw material for french fries and bhajias

One month old double filtered groundnut oil (GNO) and refined cottonseed oil (CSO) procured from Ankur Oil Industries (Ahmedabad).

For the preparation of **French fries,** *Kufri surya* variety of potatoes were selected and procured from Potato Research Station, Deesa (Gujarat). This variety is widely used by food chains for the preparation of French fries as it is known to have good frying quality.

For the preparation of **Bhajias** bengal gram flour (*Gaaya brand*) and other ingredients like sodium bicarbonate (Tata), salt (Tata), turmeric powder, red chilli powder and bishop seeds were purchased from local market. Potatoes were procured from potato research station located at, Deesa, Gujarat.

4.2.7 Standardization and preparation of french fries and bhajias

French fries were prepared using similar size of potatoes selected and washed under running water, peeled and sliced in 7×1×1 cm dimension using a stainless steel knife and the excess moisture was removed by spreading them on paper napkins before frying. Raw potato slices were fried in cottonseed oil (CSO) and groundnut oil (GNO) for 5 minutes.

Bhajias were prepared by deep frying thinly sliced circular potatoes that were dipped in a batter prepared out of 75 g Bengal gram flour with 70 ml water, a pinch of sodium bicarbonate, salt, turmeric powder, red chilli powder and bishop seeds were added to the batter (Pasricha S, 2004). Bhajias were fried in cottonseed oil (CSO) and groundnut oil (GNO) for 5 minutes.

4.2.8 Oil uptake by french fries and bhajias during 25 h of intermittent frying

Oil uptake was measured by taking difference between the initial amount of oil used for frying french fries and bhajias in CSO and GNO and total amount of oil left after 25 h of intermittent frying.

Oil uptake= Initial amount of oil-Total oil left in fryer after 25 h of intermittent frying.

4.2.9 Statistical analysis

Mean and standard deviation of values obtained by conducting the experiments in replicate was calculated using Microsoft Excel 2007. Analysis of variance (ANOVA) was used to determine significant difference amongst the french fries and bhajias sensory attributes at intermittent intervals of frying. Student't' test was used to find the significant difference between the two means. Pearson's coefficient was used to calculate correlation between flavor, taste, odor and overall acceptability of french fries and bhajias.

PHASE III:

4.3 Chemical changes due to thermal degradation of intermittently deep fried Cottonseed oil (CSO) and Groundnut oil (GNO) as a result of french fries and bhajias frying.

During deep-frying, thermal, oxidative, and hydrolytic reactions take place and, thus results in physical and chemical changes in the oil or fat consequence of the formation of new compounds. Repeated frying in same oil brings some undesirable modifications in the frying medium. Foods fried in these oils absorb this fat or oil of variable degradation, which contributes considerably to the quality of the dietary fat. Consumption of foods prepared in such oils is responsible for various health ailments.

This phase of the study was designed to study the changes in chemical and physical properties of two dominate oils of Gujarat. The experimental design of this phase is presented in Figure 4.3.1.

4.3.1: Experimental design for phase III

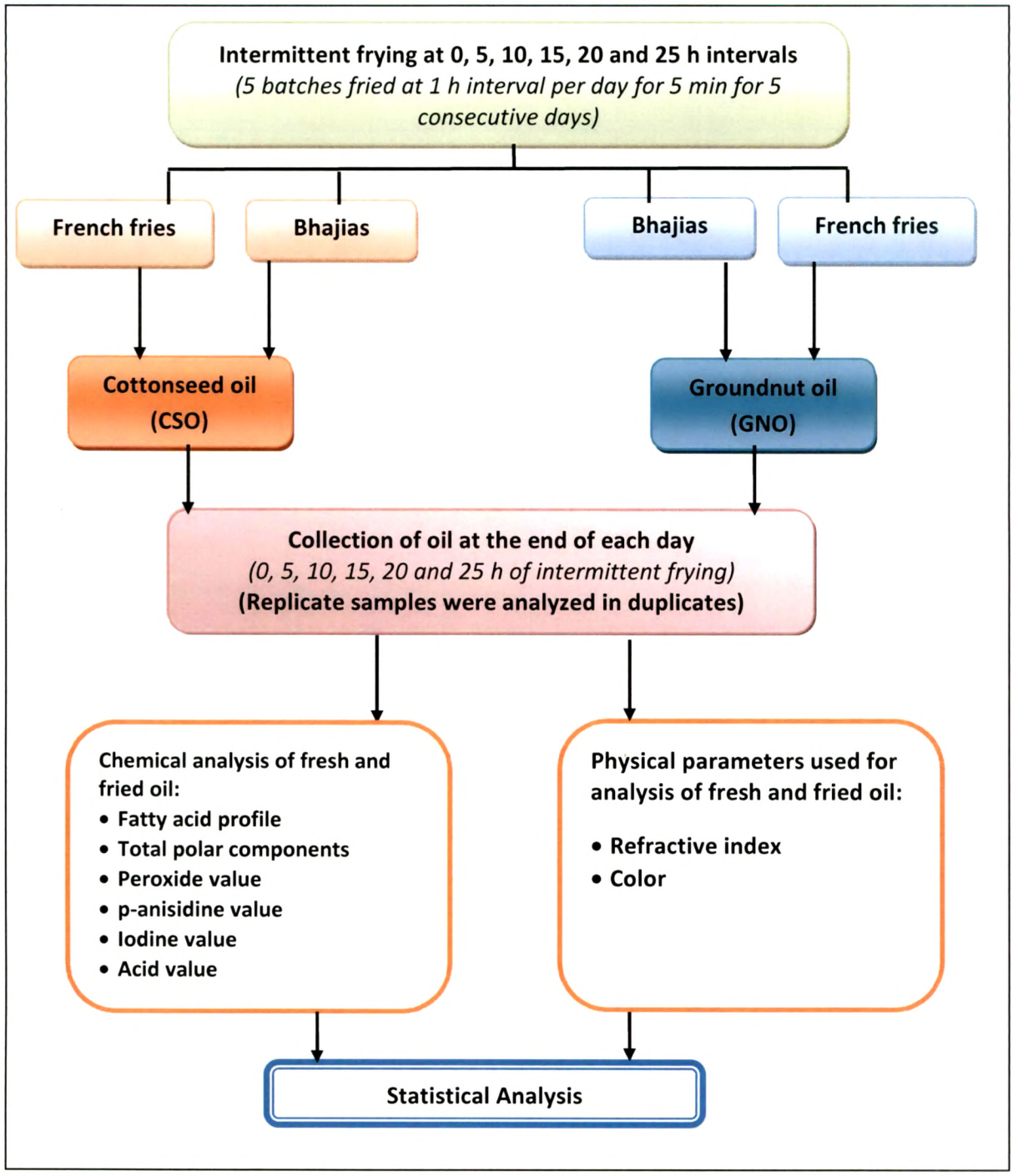


Figure 4.3.1: Experimental design for phase III

4.3.2 Frying conditions used for frying french fries and bhajias

Frying experiment was performed in replicates. Frying was conducted in 2L capacity hindalium domestic frying pan (diameter-12", depth- 7.2"). Mitsubishi (ISI) mark auto ignition stainless steel LPG stove was used during entire frying experiments. 1.5L GNO was placed in a frying pan and heated between 160^0-180^{0}C for frying. French fries were dipped in oil at 160^{0}C and then the temperature was raised to 180^{0}C. This helped in avoiding french fries from excess browning and aided in restricting cooking time within 5 min. A batch of 170g sliced raw potatoes for french fries and 200g of batter coated potatoes for bhajias were fried in separate oil for 5 min at an interval of 1 h; five times a day for 5 consecutive days. The frying temperature was monitored every minute with the help of mercury thermometer. Frying pan was not covered during the frying operation. The fried oil was not replenished during or post frying. French fries fried oils were not filtered at any interval of frying. Oils used for frying bhajias were filtered at the end of each day to remove the fried debris.

Table 4.3.2.1 and 4.3.2.2 shows the summary of frying operation used for frying french fries and bhajias in CSO and GNO.

Table 4.3.2.1: Frying operation variables for french fries

Frying variables	Oils	
	Cottonseed oil (CSO)	Groundnut oil (GNO)
Food type	Potatoes	Potatoes
Frying vessel	Domestic fryer	Domestic fryer
Total oil quantity (L)	1.5	1.5
Proportion of food to frying oil (g/batch)	170±5	170±5
Temperature (^{0}C)	160-180	160-180
Frying time/batch (min).	5	5
Total frying time (h)	2.5	2.5
Number of frying times	25 intermittent batches of five frying days	25 intermittent batches of five frying days
Total amount of food fried (kg)	4.250	4.250

Table 4.3.2.2: Frying operation variables for bhajias

Frying variables	Oils	
	Cottonseed oil (CSO)	**Groundnut oil (GNO)**
Food type	Bengal gram coated potatoes	Bengal gram coated potatoes
Frying vessel	Domestic fryer	Domestic fryer
Total oil quantity (L)	1.5	1.5
Proportion of food to frying oil (g/batch)	200±5	200±5
Temperature (^{0}C)	160-180	160-180
Frying time/batch (min).	5	5
Total frying time (h)	2.5	2.5
Number of frying times	25 intermittent batches of five frying days	25 intermittent batches of five frying days
Total amount of food fried (kg)	5.0	5.0

4.3.3 Collection of oil samples used for frying french fries and bhajias at intermittent durations

At the end of each day an aliquot of cooled 75ml oil was pipetted out from the pan and stored in amber colored air tight glass bottles (Plate 4.3.3). The fried oil samples collected at the end of each day for 5 consecutive days were kept in a deep freezer for further chemical analysis for peroxide, p-anisidine, Iodine, and Acid value as well as color and refractive index determination.

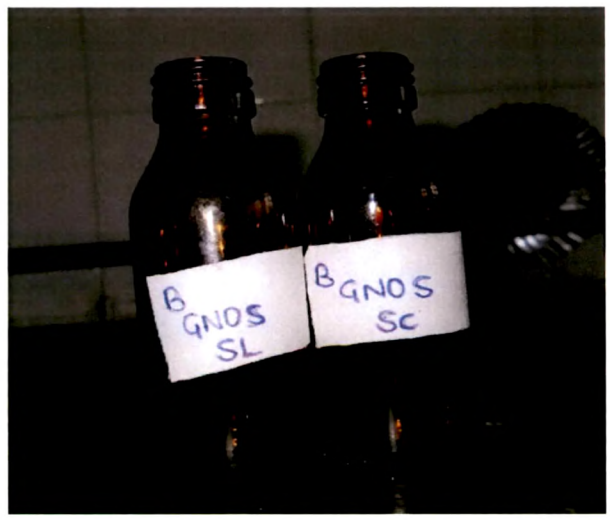


Plate 4.3.3: Amber color glass bottles used for collection of fried oil samples

4.3.4 Analysis of CSO and GNO used for frying

4.3.4.1 Chemical methods used to check the quality of CSO and GNO used for frying

4.3.4.1.1: Fatty acid profile

Methyl esters of fatty acid (MEFA) were prepared according to AOCS Ce 2-66 method, 1974.

Fatty acid composition was determined using Gas Chromatography (GC) (Perkin Elmer, US) with auto system column PE-FF-AP (30m×0.25μm×0.25mm), a flame ionization detector (FID), and nitrogen as the carrier gas (10psi). GC split ratio was 1:70. The oven temperature was adjusted to 80°C and held for 2 min. Thereafter, the temperature was raised to 220°C at the rate of 10°C per min and held for 10 min. Injector and FID temperature was kept at 250°C. Standard MEFA were used as authentic samples and peak identification was done by comparing the relative retention times.

4.3.4.1.2: Total polar components

Total polar components were determined by AOAC method 982.27, 1995.

4.3.4.1.2.1 Principle

Total polar component method assesses deterioration of used frying fats, and it's applicable to all fats and oils. Polar components are those components of fats determined by column chromatography under specified conditions and include polar substances such as monoglycerides, diglycerides, free fatty acids that occur in unused fats, as well as polar transformation products formed during frying of food stuffs and/or during heating. Non-polar components are mostly altered triglycerides. Frying fats are separated by column chromatography on silica gel into non polar components. Polar components are determined indirectly by subtracting concentration of non

polar components. Quality of separation can be checked by thin layer chromatography.

4.3.4.1.2.2 Preparation of Reagents

1. *Adsorbent*: Silica gel 60, particle size 0.063-0.200mm (70-230 mesh ASTM). Silica gel was dried ≥ 4 h in porcelain dish at 160^0 C in an oven and cool in desiccator to room temperature.
2. *Eluting solvent mixture*: 100ml petroleum ether (bp 40-60^0) and ether mixture was prepared by adding 87ml petroleum ether and 13ml ether.
3. Sea sand (analytical reagent grade) was purified by acid and calcined
4. *Spray reagent*: 10% molybdophosphoric acid was prepared in alcohol.

4.3.4.1.2.3 Preparation of sample

Samples were heated to temperature slightly above melting point and mix thoroughly. Visible impurities were removed by filtration.

4.3.4.1.2.4 Preparation of column

1. Column was filled with 30 ml petroleum ether-ether (87+13). Cotton wool wad was placed in the bottom of column and air was removed by pressing with glass rod.
2. 25g silica gel slurry was prepared in 100ml glass beaker by adding approximately 80 ml petroleum ether-ether (87+13). This slurry was poured into column through 8cm glass funnel (Plate 4.3.4.1.2.4).
3. Beaker, funnel, and sides of column were rinsed with same solvent.
4. Solvent was drained to 10cm above silica gel by opening the stopcock and silica gel was leveled by tapping the column.
5. 4g sea sand was added into column and solvent was drained to sand layer.

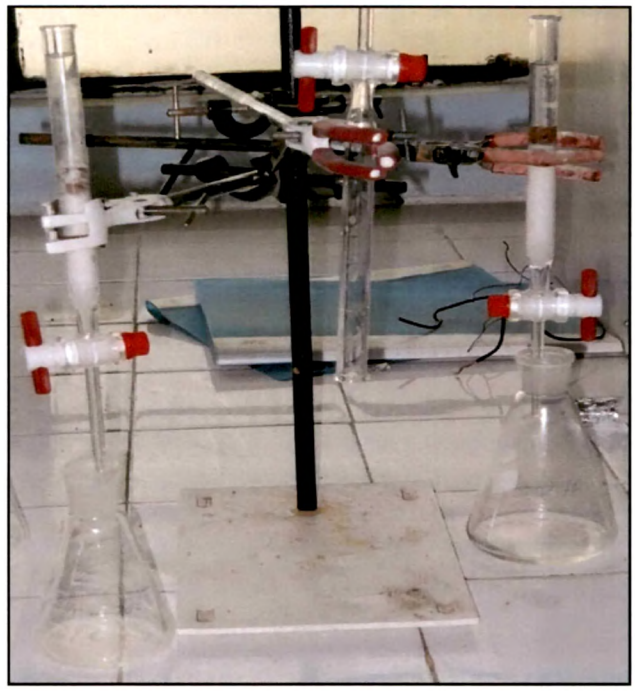


Plate 4.3.4.1.2.4: Preparation of column chromatograph for determination of total polar components in fried oils.

4.3.4.1.2.5 Procedure

1. Polar components were determined by difference, only non polar fraction was used. Both polar and non-polar fractions were required for calculations.

2. Samples were accurately weighed 2.5±0.1g into 50ml volumetric flask, and dissolved in approximately 20ml petroleum ether-ether while warming slightly. After that samples were allowed to cool at room temperature and dilute to volume with same solvent.

3. 20ml sample aliquot to was pipette and transferred using volumetric column, without disturbing surface.

4. Two 250ml round-bottom flask were dried in 103±2^{0}C oven, cool to room temperature, and accurately weigh to 0.001g. One flask was placed under column and allows sample solution to drain to level of sand layer by opening stopcock.

5. Non polar components were eluted with 150ml petroleum ether-ether contained in 250ml dropping funnel. Flow rate was adjusted so that 150ml passes through column within 60-70 minute. After elution, any substance adhering to outlet of column into round-bottom flask was washed with petroleum ether-ether (87+13).

6. Polar components were eluted into second 250ml round-bottom flask with 150ml ether. Silica gel was discarded after elution of one sample.

7. Solvent from each fraction were removed with an evaporator and $\leq 60^0$ water bath.

8. Residues were allowed to cool at ambient temperature and then flasks were weighed.

4.3.4.1.2.6 *Thin layer chromatography to check efficiency of column chromatography*

1. Polar and non-polar fraction (1+9) diluted in $CHCl_3$.

2. 2µl spots were applied using capillary dispensing pipette. Thin layer chromatograph was developed with petroleum ether-ether-CH_3COOH (70+30+20) in tank lined with filter paper for approximately 35 min (17 cm).

3. Plate were removed from thank and allowed solvent to evaporate. Plate was kept in iodine chamber. After evaporation of alcohol, heat plate at 120-130^{0}C in drying oven. Fraction 1(non polar) should be free of polar substances (Plate 4.3.4.1.2.6).

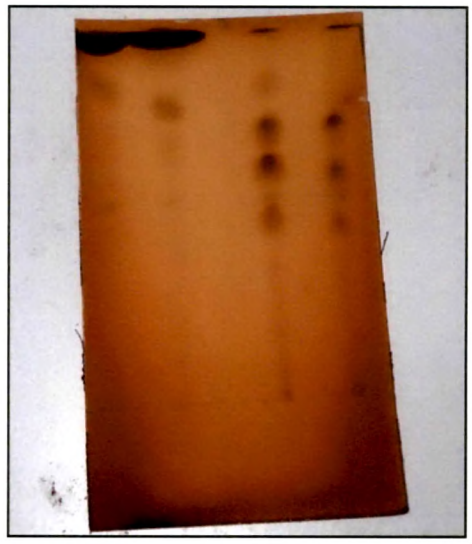


Plate 4.3.4.1.2.6: Thin layer chromatograph sheet for separation of polar and non-polar components in fried oils

4.3.4.1.2.7 Calculation

Polar components were calculated as % (wt/volume) by formula-

Polar components; %= [(E-A)/E] X 100

Where, A= g non polar fraction

E= g sample in 20ml aliquot (approximately 1g)

(Results were reported to 1 decimal place)

4.3.4.1.3: Peroxide value

Peroxide value of oils used for frying was assessed by AOAC method 965.33, 1995.

4.3.4.1.3.1 Principle

Peroxide value is commonly used as an indicator to measure peroxides of the early stages of oxidation in fats and oils. The peroxides present are determined by titration against sodium thiosulphate in the presence of potassium iodide. Starch is used as indicator.

In oxidative rancidity oxygen is taken up by the fat with the formation of peroxides. The degree of peroxide formation and the time taken for the development of rancidity differ among oils.

4.3.4.1.3.2 Preparation of reagents

1. *Acetic acid-chloroform solvent mixture (3:2):* 3 volumes of glacial acetic acid were mixed with 2 volumes of chloroform.
2. *Saturated potassium iodide (KI) solution*: Saturated potassium iodide solution was prepared in water. Potassium iodide was added to water till it stops dissolving in water.
3. *0.1 N Sodium thiosulphate* ($Na_2S_2O_3$) *solution:* 25g of sodium thiosulphate was weighed and dissolved in 1L of distilled water. Solution was boiled, cooled and filtered in 1000ml volumetric flask. Sodium thiosulphate solution was standardized against standard potassium dichromate solution.
4. *1% Starch solution*: 1g starch was weighed and mixed in 100ml lukewarm distilled water with continuous stirring.

4.3.4.1.3.3 Procedure

1. 5 ± 0.5g sample was weighed into a 250ml stopper conical flask.
2. 30ml acetic acid-chloroform solvent mixture was added to weighed sample and swirl to dissolve.
3. 0.5ml saturated KI solution was added add with a pipette. KI added sample was allowed to stand for 1 min in dark with occasional shaking, and then 30ml of water was added.
4. 0.1 N sodium thiosulphate was filled in 25ml burette. Sodium thiosulphate solution was slowly titrated to the liberated iodine solution, with vigorous shaking until yellow color was almost gone (Plate 4.3.4.1.3.3).

5. 0.5ml starch solution was added as indicator and titration was continued by shaking vigorously to release all iodine from $CHCl_3$ layer until blue color disappears.

6. One blank was conducted with each sample determination.

(When less than 0.5ml of 0.1 N $Na_2S_2O_3$ was used during titration, sample was repeated using 0.01 N $Na_2S_2O_3$).

4.3.4.1.3.4 Calculation

Peroxide value expressed as mili equivalent of peroxide oxygen per kg sample (meq/kg)

$$\textbf{Peroxide value} = \frac{\textbf{Titre X N X 100}}{\textbf{Weight of the sample}}$$

Where, Titre = ml of Sodium thiosulphate used (blank corrected)

N = Normality of sodium thiosulphate solution

Plate 4.3.4.1.3.3: Determination of peroxide value by titration method

4.3.4.1.4: p-anisidine value

AOCS Cd 18-90 method (1998) was used to determine the p-anisidine value of oils. This method defined as 100 times the optical density measured at 350 nm in a 1cm cell containing 1g of oil in 100ml of mixture of solvent and reagent is used to determine the secondary changes in oils.

4.3.4.1.4.1 Principle

Secondary oxidation products were measured by determining the p-anisidine value. Aldehydic compounds in fats and oils react with p-anisidine, in the presence of acetic acid, to form yellowish reaction products. According to the method the intensity of the yellowish compounds is not related only to the amount of aldehydic compounds present, but also to their structure. A double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four to five times. p-anisidine determines the quantity of aldehydes (principally 2-alkenals and 2,4-dienals) present in oils and fats.

4.3.4.1.4.2 Preparation of reagents

1. *Isooctane (2,2,4-trimethylpentane)* or *n-hexane*: Analytical grade n-hexane reagent was purchased from authenticated chemical dealer.

2. *p-anisidine (analytical reagent quality):* 0.25g p-anisidine was mixed in 100ml glacial acetic acid.

4.3.4.1.4.3 Procedure

1. 0.5–4.0 ± 0.001g of sample was weighed into a 25ml volumetric flask. Dissolved and diluted to volume with isooctane (Plate 4.3.4.1.4.3.1).

2. Absorbance (Ab) of the solution was measured at 350nm in a cuvette with the spectrophotometer (Shimadzu UV-VIS spectrophotometer 1201) shown in Plate 4.3.4.1.4.3.2, using the reference cuvette filled with solvent as a blank.

3. Exactly 5ml of the fat solution was pipette into one test tube and exactly 5 ml of the solvent into a second test tube. By means of a pipette, exactly 1ml of the solvent was added into a second test tube (Plate 4.3.4.1.4.3.3). By means of a pipette, exactly ml of the p-anisidine reagent was added to each tube, and shake.

Plate 4.3.4.1.4.3.1: Dilution of oil sample with isooctane in 25 ml volumetric flask

4. After exactly 10 min, solvent in the first test tube was filled in the cuvette and absorbance (As) was measured at 350nm, using the p-anisidine solution from the second test tube as a blank in the reference cuvette.

Plate 4.3.4.1.4.3.2: Shimadzu UV-1201 used for determination of p-anisidine value

Plate 4.3.4.1.4.3.3: Addition of p-anisidine reagent in diluted samples of oils for second absorbance (As)

4.3.4.1.4.4 Calculation

The p-anisidine value (p-AnV) is given by the formula

$$\text{p-AnV} = \frac{25 \times (1.2\ As - Ab)}{m}$$

Where,

As = absorbance of the fat solution after reaction with the p-anisidine reagent

Ab = absorbance of the fat solution

m = mass of the test portion in grams

4.3.4.1.5: Totox value

The TOTOX (i.e. total oxidation products) value was calculated by AOCS Cc13e-92 method. The TOTOX value was calculated as:

TV=2 peroxide value + p-anisidine value

4.3.4.1.6: Iodine value

Iodine value was determined by AOAC 993.20 Wij's method, 1995.

4.3.4.1.6.1 Principle

Fat or oil sample is mixed with iodine monochloride solution to halogenate double bonds in fat or oil. Excess iodine monochloride is reduced to free iodine in presence of potassium iodide, and free iodine is measured by titration with sodium thiosulphate ($Na_2S_2O_3$) using starch as indicator. Iodine value, calculated as approximate iodine absorbed per g of sample (% iodine absorbed), is a measure of unsaturation of fats and oils.

4.3.4.1.6.2 Preparation of reagents

1. *15% potassium iodide (KI) solution*: 15g KI dissolved in 100ml distill water.
2. *Wij's iodine solution*: 13g iodine was dissolved in 1L acetic acid, and passed in dried (through H_2SO_4) Cl until original $Na_2S_2O_3$ titration of solution was not quite doubled. (Characteristic color change at end point indicated

proper amount of Cl. Convenient method is to reserve some of original iodine solution, add slight excess of Cl to bulk of solution, and bring to desired titer by re-additions of reserved portion). Wij's solution is sensitive to temperature, moisture and light. Solution was stored in amber color bottle in dark at <30^{0}C.

3. *Soluble starch solution:* 1g starch was mixed with small amount of cold water. While stirring 200ml boiling water was added.

   Test for sensitivity of starch solution: 5ml starch solution was mixed in 100 ml water and 0.05ml 0.1N iodine solution was added in to it; deep color was produced and discharged by 0.05ml 0.1N $Na_2S_2O_3$.

4. *Sodium thiosulphate* ($Na_2S_2O_3$) *solution*: 25g of sodium thiosulphate was weighed and dissolved in 1L of distilled water. Solution was boiled, cooled and filtered. Standardized against standard potassium dichromate solution.

5. *Cyclohexane-acetic acid solvent:* cyclohexane and acetic acid were mixed in equal parts (1+1) (volume/volume).

   Absence of oxidizable matter in cyclohexane-acetic acid solvent: Determined by shaking 10ml solvent with 1ml saturated aqueous potassium dichromate solution and 1ml H_2SO_4. No green color indicates absence of oxidizable matter.

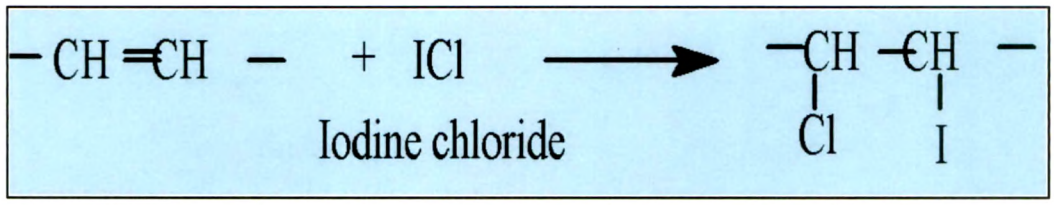
4.3.4.1.6.3 Procedure

1. Sample was filtered to remove any solid impurities and traces of water.

2. Sample was weighed in a conical flask and 15ml cyclohexane-acetic acid solvent added to test sample and swirled to ensure that sample was completely dissolved.

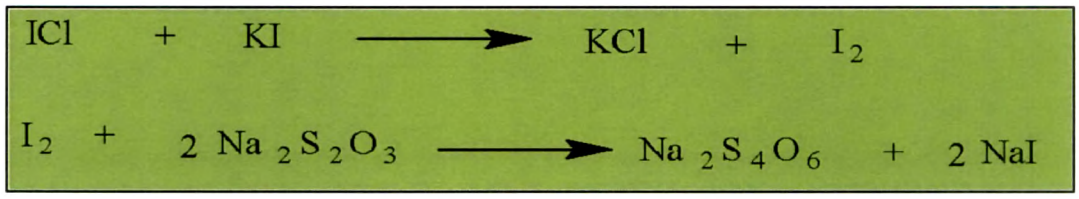
3. 25ml Wij's solution was added into flask containing test sample, stopper flask, and swirl to mix. Immediately timer was set for 1 or 2 h, depending

on IV of sample (IV<150, 1 h; IV≥150, 2 h) and flasks were store in dark at 25±5^0 for reaction.



4. Flasks were removed from dark; 20ml KI solution was added. 150ml water was added and gradually titrate with 0.1N standard $Na_2S_2O_3$ solution with constant and vigorous shaking.

Excess unreacted ICl



5. Titration was continued until yellow color almost disappeared; 1-2ml starch indicator solution was added to flasks and continued titrating until blue color disappeared.

4.3.4.1.6.4 Calculation

$$\underline{\text{IV= [(B-S) XNX12.69]}}$$
$$\text{weight of sample}$$

Where,

B= titration of blank (ml); S=titration of sample (ml); N= Normality of $Na_2S_2O_3$ solution

4.3.4.1.7: Acid value

AOCS Cd 3a-63 method (1974) was used for determination of acid value.

4.3.4.1.7.1 Principle

The acid value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids in 1 gram of sample.

4.3.4.1.7.2 Preparation of reagents

1. *Standard potassium hydroxide (KOH) solution:* 0.1N. 6g of KOH was added to 1 litre of water in a 2 liter flask, and then boiled for 10 minutes with stirring. 2g barium hydroxide ($Ba(OH)_2$) added to boiled solution and again boil for 5-10 minutes. Boiled solution was cooled and allowed to stand for overnight, filtered through funnel. KOH solution was standardized by titration with pure potassium acid phthalate using phenolphthalein indicator.

2. *Isopropyl alcohol-toluene solution:* Equal parts by volume of isopropyl alcohol and toluene were mixed and filled in a volumetric flask. Isopropyl alcohol-toluene solution gives a distinct and sharp end point with phenolphthalein in the titration.

3. *1% Phenolphthalein indicator solution:* 1g of phenolphthalein was mixed in 100ml isopropyl alcohol.

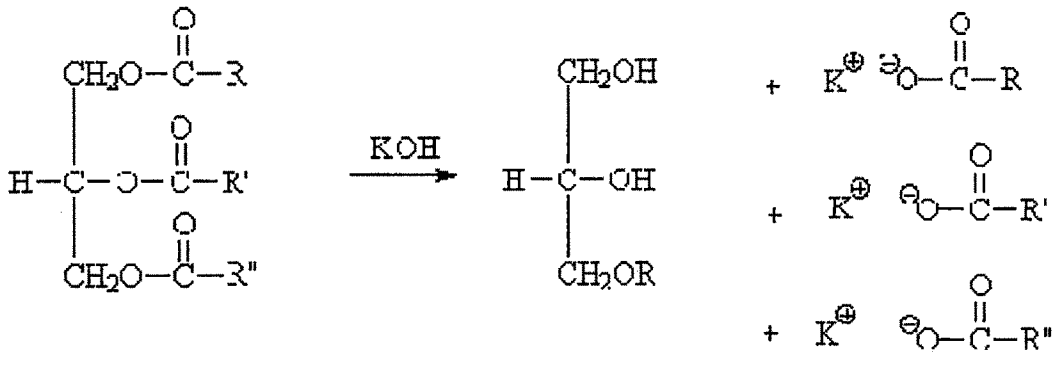
4.3.4.1.7.3 Procedure

1. 10±0.02g of sample was weighed into 250ml conical flask.

2. 2ml phenolphthalein indicator solution was added to 125ml isopropyl alcohol-toluene solution and neutralize with alkali to a faint but permanent pink color.

3. 125ml neutralized solvent mixture was added to sample and dissolved completely before titrating.

4. Sample was whirled while titrating with standard alkali to the first permanent pink color of the same intensity persist for 30 seconds as that of neutralized solvent before the latter, was added to the sample.



TAG + 3 KOH ⟶ glycerol + 3 alkali salt

4.3.4.1.7.4 Calculation

$$\text{Acid value, mg KOH per g of sample} = \frac{\text{ml alkali X N X 56.1}}{\text{Weight of sample}}$$

Where, ml alkali= amount of KOH solution (ml) used

N=normality of KOH solution

4.3.4.2 Physical parameters used to check the quality of CSO and GNO used for frying.

4.3.4.2.1: Refractive Index

AOAC 921.08 method (1995) was used to estimate the refractive index of oils with the help of Abbe refractometer shown in Plate 4.3.4.2.1.

4.3.4.2.1.1 Principle

The refractive index of a medium is the ratio of the speed of light at a definite wavelength in vacuo to its speed in the medium. When light passes from air to fat its direction changes at the interface of two media. The actual change depends on the angle at which the light strikes the fat and order to get a significant value. The refractive index of a given substance varies with the

wavelength of the light and with the temperature. The refractive index of fat is related to molecular structure and unsaturation.

4.3.4.2.1.2 Procedure

1. Instrument is based upon observation of position of border line of total reflection in relation to faces of flint glass prism.
   Bring the border line into field of vision of telescope by rotating double prism by means of alidade in following manner: hold sector firmly and move alidade backward or forward until field of vision is divided into light and dark portion. Line dividing these portions is "border line", and as a rule, will not be the sharp line but band of color. Colors are eliminated by rotating screw head of compensator until sharp, colorless line is obtained. Adjust border line so that it falls on point of intersection of cross hairs. Read n substance directly on scale of sector, estimating 4^{th} decimal place.

2. Double prism opened by means of screw head and few drops sample were placed into funnel-shape aperture between prisms. Prisms closed firmly by tightening screw head. And allowed the instrument stand few min before reading, so the temperature of sample and instrument is same. Clean prisms between readings by wiping off oil with cotton pod moistened with solvent (e.g. toluene or petroleum ether), and allowed to dry. Refractive index was noticed from the scale sector of Abbe refractometer (Plate 4.3.4.2.1).

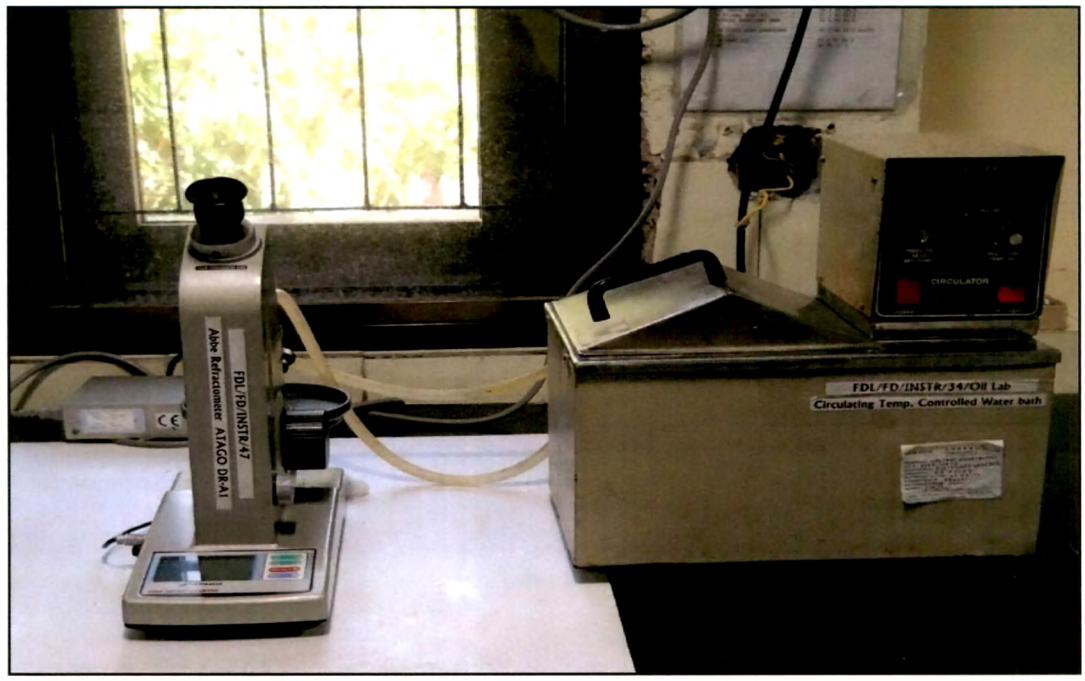


Plate 4.3.4.2.1: Abbe refractometer used for determination of refractive index of oil

4.3.4.2.2: Color

Lovibond Tintometer (Plate 4.3.4.2.2) was used to determine the color of oils by using oils and fats, Manual of methods of analysis of foods, Lab manual 2 (7.0), 2005.

4.3.4.2.2.1 Principle

Determines the color of oils by comparing with Lovibond color racks of known color characteristics. The color is expressed as the sum total of the yellow and red slides used to match the color of the oil in a cell of the specified size in the Lovibond Tintometer .

4.3.4.2.2.2 Procedure

1. Oil sample was filtered through a filter paper to remove any impurities, traces of moisture, clear and free from turbidity.

2. Oil sample color was matched by adjusting yellow (Y) and red (R) racks of the instrument. A glass cell was filled with the sample and placed inside the lightening cabinet. Match the color with sliding red and yellow color racks.

4.3.4.2.2.3 Calculation

Color was reported in terms of Lovibond units as:-

Color reading = (a Y + 5 b R)

Where,

a = sum total of the various yellow slides (Y) used

b = sum total of the various red (R) slides used

Y + 5R is the mode of expressing the color of oils

Plate 4.3.4.2.2: Lovibond tintometer for estimation of color of oil used for frying french fries and bhajias

4.3.5 Statistical analysis

Mean and standard deviation of duplicate values obtained by conducting the experiments in replicate were calculated using Microsoft Excel 2007. Analysis of variance (ANOVA) was used to determine significant difference amongst

the fried samples at different intervals of frying for their chemical analysis. Student't' test was used to find the significant difference between the two means. Pearson's coefficient was used to calculate correlation between dependent and independent variables. Regression equations in the regression analysis were used to determine if the independent variable (time) had any effect on the chemical indicators.

PHASE IV:

4.4 Case study on prevailing food safety and frying practices in Jan aahar- A government run food outlet at Vadodara railway station.

To study the food safety practices regarding personal hygiene, food hygiene, environmental hygiene, oil storage and frying practices of government run food outlet was selected.

4.4.1 To determine the current knowledge of kitchen staff on food safety practices in terms of personal hygiene, food hygiene, environmental hygiene, nutrition and health.

A pre-tested structured questionnaire was used to assess the knowledge of cooks, waiters, cleaners and supervisors on food hygiene, nutrition and health, and personal hygiene. An observation table was used to assess the prevailing practices of cooking staff on personal, unit and environment hygiene (Appendix 11.5).

4.4.2 To determine the oil procurement and storage practices and frying practices of kitchen staff working at Jan aahar -a Government run food outlet at Vadodara railway station.

Current practices used by cooks for procurement and storage of cooking oil, and frying practices were also reviewed (Appendix 11.5).

4.4.3 Statistical analysis

Per cent mean scores were calculated for knowledge scores of food safety. ANOVA (F-test) was determined to obtain difference amongst the knowledge scores of kitchen staff.

PHASE V:

4.5 Development of Nutrition Health Education (NHE) material in two languages on intake of edible oil, types, and on choices of oils for healthy living and problems during frying of edible oil and its storage.

Nutrition Health Education material was developed with the objective to educate people about recommended intake of edible oils, common types of oils, and to choose correct oil for healthy living. An attempt was made to develop an education material on safe frying practices in two languages (Hindi and English).

4.5.1 To develop IEC material on edible oil, types of oil, and its composition.

"KNOW YOUR FATS AND OILS" education material was developed in two languages with following points:

- Oils and fats
- Types of oils and their sources
- Recommended allowances of fats and oils
- What is the difference between oils and fats
- Do vegetable oils contain cholesterol?
- How to store oil?
- Which fat is heart friendly?
- Recommended combination of oils for optimal health benefits in Indians consuming cereal based diets

- Why fried foods are bad for health?
- What are *trans* fats?

4.5.2 To develop IEC material on frying and problems during frying.

Frying, a cooking method used in various food outlets for preparing variety of foods. Selection of oils and frying practices should be such that they are safe for human consumption.

Hence the Information Education Communication (IEC) material was developed on safety of fried foods, common problems envisaged during frying and calorie content of common fried foods that comprised of following points:

- What is frying?
- What are good frying practices?
- Problems envisaged during frying and their solutions:
  a. Causes of foaming and its solutions
  b. Causes of greasiness and its solutions
  c. Causes of rapid oil breakdown and its solutions
  d. Causes of darkening of oil and its solutions
  e. Causes of smoking and its solutions
- Calories and fat content of some common non-vegetarian fried foods
- Calories and fat content of some common vegetarian fried foods