EFFECT OF DIFFERENT SYNTHETIC ANTIOXIDANTS IN THE STABILITY OF PALM OIL ON DEEP FAT FRYING OF INSTANT NOODLES

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Approval Letter

This dissertation entitled *Effect of different synthetic antioxidants in the stability of palm oil on deep fat frying of Instant noodle* presented by *Basanta Bhattarai* has been accepted as the partial fulfillment of the requirement for the B. Tech. in Food Technology.

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_____

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Abstract

The effect of three different synthetic antioxidants viz.-Tertiary Butylated Hydroxyquinone (TBHQ), Butylated Hydroxyaisole (BHA), Butylated hydroxyquinone (BHT) in the stability of palm oil on deep fat frying of instant noodles was well studied at ambient condition taking peroxide value and acid value as the objective parameters to measure the oxidation of frying oil. Souped cake(noodles) as frying samples were taken from Yaoxian Machinery (YM) plant of Chaudhary Group (CG) Foods (Nepal Pvt. Ltd.) then fried in Refined Bleached Deodorized (RBD) palm oil of standard grade at 170-180°C . The RBD palm oil was analyzed for its physiochemical parameters. The fried noodles were also analyzed for moisture content , acid value(AV), peroxide value (PV), sapnification value ,unsaponifiable matter , density (gm/cc), iodine value (IV) .

The three synthetic antioxidants in their proportion 100 ppm, 150 ppm, 200 ppm mixed in RBD palm oil in three batches. Oil sample collected in each hour of frying, it was found that higher the proportion of antioxidants lesser will be the oxidation of frying oil which increased the stability. For three antioxidants the frying quality were analyzed such as AV, PV, specific gravity and saponification value. Control was taken as without using antioxidant.

The results showed that TBHQ of 150 ppm was statistically positive significance over two antioxidants (BHA and BHT) on the basis of AV and specific gravity where correlation coefficient(r), r >6 PE whereas 100ppm of TBHQ was found to be positive sstatistically significance(r > 6PE)on the basis of PV over BHA and BHT . It extended the stability of frying oil upto 10 hr whereas control (without using TBHQ) exceed the quality of frying oil within 6 hrs as Nepal standard. In case of BHA and BHT while using 200 ppm it extend the stability of frying oil upto 14 and 16 hrs respectively on the basis of AV. It was statically posotive significant (r > 6PE) but in case of PV it extend upto 12 hr and 14 hr which was not statistically positive significance where r< 6PE. In conclusion 150ppm of TBHQ was more significance over BHA and BHT to control the oxidation of oil on the basis of PV, AV and specific gravity.

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Part I

Introduction

1.1 Background and Justification

Fats and oils, important and the most concentrated source of energy furnishing about 9 kilo calories/ gram of oils and fats as compared with 4k.calories per gram each furnished by protein and carbohydrate (Arwater-Bryant, 1974). Since Fats and oils have higher energy value, foods containing a high proportion of fat form a compact energy source and thus give variety of our preparations of foods (Formo,1979). From a nutritional point of view a dietary intake of at least 30% of energy as Fats and oils has been recommended (MooLayil, 1982)

Fats and oils are oxidative in nature even at room temperature (Potter, 1979). When they are stored for a long period, they undergo oxidation and become rancid. Autoxidation is the major cause of rancidity of fats and oils (Allen and Hamilton, 1983)

Sanders (1983), States that oxidative rancidity leads to the formation of both unpalatable and toxic compounds, and is thus nutritionally undesirable. Different classes of substances occurring in oxidized fat have been shown to have toxic effects and physiological disorders (Artman, 1969; Wilson, 1976). Additional data on human subjects supports the concept that lipid peroxides may inhibit other metabolic systems, particularly glycolysis accounting oxygen toxicity (Mengel et al., 1964). Therefore it is necessary to control oxidation of oils and fats.

Deep fat frying is a process in which the food is cooked by immersion in hot oil. Despite the fact that the deep fat frying industry is well-established and highly automated, the deep fat frying process is considered to be an art rather than a science (Blumenthal, 1991). It is a complex process. During deep fat frying, thermal, oxidative, and hydrolytic reactions take place resulting in physical and chemical changes in the oil and the formation of new compounds (Li, 2005).

Regarding the control of oxidative deterioration of fats and oils, a number of methods have been developed. Many problem of oxidation can be solved or at least kept under control, by adequate technological interventions by food manufacture. Choice of the appropriate operational condition is very often crucial to the keeping quality of foods otherwise easily oxidized. The correct packing material, use of inert atmosphere and suitable storage condition are ways to retard the oxidative deterioration. There are however, many situations where

reasons do not allow one to utilize any of these ways of protecting food against oxidation. In many of these desperate situations, well known antioxidants are very useful. Antioxidants are a good all around remedy against the lipids oxidation in foods, or oils and fats (Hamilton & Allen, 1983).

Synthetic antioxidants are used. In recent years, the possible toxicity of synthetic antioxidants has been investigated by several workers (Imaida et al, 1983, Djuthuus et al,1982). Therefore, emphasis has been given for using natural antioxidants rather than synthetic antioxidants.

1.2 Statement of problem

In the developing and least developed country fat and oils used for frying in commercial purpose until its colour changes. Small and medium processor is unaware of impact of such oil in public health. Even organized and large industries use burnt oil (fried oil) with raw oil in low amount which is harmful for public health causes different cardiovascular diseases. Sharp rise in peroxide value (PV) and acid value (AV) is another problem for industry of instant noodles. It is necessary to control the sharp fluctuation in AV and PV within limit. There are so many findings in past decades about deep fat frying, all of them recommended for natural antioxidants but in least developed country like Nepal there are not sufficient sources for natural antioxidants. So it is compelled to use synthetic antioxidants but in low amount, codex recommends the food grade synthetic antioxidants upto 200ppm. AV is food safety indicator and PV is public health indicator, it is necessary to control within limit by using synthetic antioxidants. So in industrial point of view different synthetic antioxidants are used in frying oil to prevent it from being rancid but banned in some countries due to long term toxic effect. Hence it is necessary to optimize the concentration of synthetic antioxidants lying within the permitted standard without sparing the economic benefit of the industry and human health. Synthetic antioxidants in low amount (100 to 200 ppm) or synergistic antioxidants individually or combination with other antioxidants or chelating for its more stability should be used for health concern as well as economy.

1.3 Objective

1.3.1. General objective

To study the stability of palm oil using different synthetic antioxidants on deep fat frying of Instant noodles.

1.3.2. Specific Objective

*To analyze the change in physiochemical parameter (AV, PV, Specific gravity, Saponification value) of palm oil in each hour of frying using different concentration of antioxidants on deep fat frying of instant noodles.

* To evaluate the relationship of these physiochemical parameters of frying oil using different concentration of antioxidants against frying time (hrs)

*To compare the stability of oil with the effect of synthetic antioxidants e.g.: tertiary butylated hydroxyquinone (TBHQ), butylated hydroxytoluene (BHT) and butylated hydroxyl anisole (BHA) on the basis of AV, PV, Specific gravity and Saponification value.

* To recommend these for industrial purposes according to the result obtained.

1.4 Significance of the study

The finding applies on oil industry and frying industry to save large amount of oil by increasing its stability towards oxidation and also increase the sensory quality of fried product. The changes in palm oil composition during deep fat frying of instant noodles using synthetic antioxidants may serve as better antioxidants options for frying food industries The finding of this work minimizes the volume of burnt oil (rancid oil) while using different synthetic antioxidants to save more or less economy as well as health of the consumer.

Thus the present study may serve as the general guidelines by the effect of different synthetic antioxidants to increase stability of frying oil.

1.5 Limitation

Due to the limited laboratory facility the secondary oxidation couldn't be analyzed.

Part II

Literature Review

2.1. Fats and Oils

Oils and fats belong to a larger group of naturally occurring called lipids that are fatty acid derivations (Fox and Cameron, 1982).Oils and fats are of great importance in the food we eat because they are readily digested, broken and utilized in body to serve as energy source for living organism (Lake et al, 1980).They are widely distributed and almost every natural food has considerable quantities of them. Natural fats and oils can be classified according to their origin as animal, marine or vegetable.

On the other hand, oils and fats are prone to spoilage which result in the production of unpleasant odours and flavours; such spoilage is usually described by the general term rancidity (Lee; 1975).Different types of oils and fats show its varying degree of resistance to spoilage; thus vegetable oils vegetable oils deteriorate only slowly where as animal fats deteriorate rapidly and marine oils, which contain relatively high proportion of highly unsaturated free acid radicals deteriorate more rapidly (Bailey, 1982).Therefore; control of rancidity seems to be a must.

a. Edible fats and oils are foodstuffs which are composed of glycerides of fatty acids. They are of vegetable, animal or marine origin. They may contain small amounts of other lipids such as phosphatides, of unsaponifiable constituents and of free fatty acids naturally present in the fat or oil. Fats of animal origin must be produced from animals in good health at the time of slaughter and be fit for human consumption (CODEX, 1999).

b. Virgin fats and oils are edible vegetable fats and oils obtained, without altering the nature of the oil, by mechanical procedures, e.g. expelling or pressing, and the application of heat only. They may be purified by washing with water, settling, filtering and centrifuging only CODEX, 1999).

c. Cold pressed fats and oils are edible vegetable fats and oils obtained, without altering the oil, by mechanical procedures, e.g. expelling or pressing, without the application of heat. They may have been purified by washing with water, settling, filtering and centrifuging only.
(CODEX, 1999)

2.1.1 Crude oils and fats

Oils as obtained by expression or extraction from oil seed etc, and those from animals and marine animals, in this natural state are referred to as "crude oils" (Williams, 1966). The fat content reported in foods is often this crude fat and represent total lipids content rather than true fat. Actually the lipid fraction contain not only true fat but also waxes, complex lipid (such as the sterols) and many pigments, hydrocarbons, and volatile oils (Dollear, 1958).In this present study the different one variety of crude oils namely palm oil has been taken into consideration.

2.1.2 Chemistry of Fats and Oils

Both fats and oils have similar chemical composition in that they consists mostly of triglycerides, with lesser amounts of mono and diglycerides, unsaponifiable compounds such as sterols, and pigments, smaller amounts of phospholipids glycolipids and lipoprotein. If the triglycerides are solid at room temperature they are designated as fats, and if liquid, as oils. (Dugan, 1986)

Natural fats and oils can be defined as mixture of mixed triglycerides or triacylglycerols (Each molecule containing more than one type of fatty acids), are the major cause of rancidity in lipids.

Chemically, fats and oils belong to class of substance known as esters, which result from the reaction of acid with alcohols. The three hydroxyl groups of the glycerol molecule can each combine with a fatty acid molecule and the resulting esters is called a triglyceride. (Jain, 1979).

The mechanism of fats (Oil) molecule formation

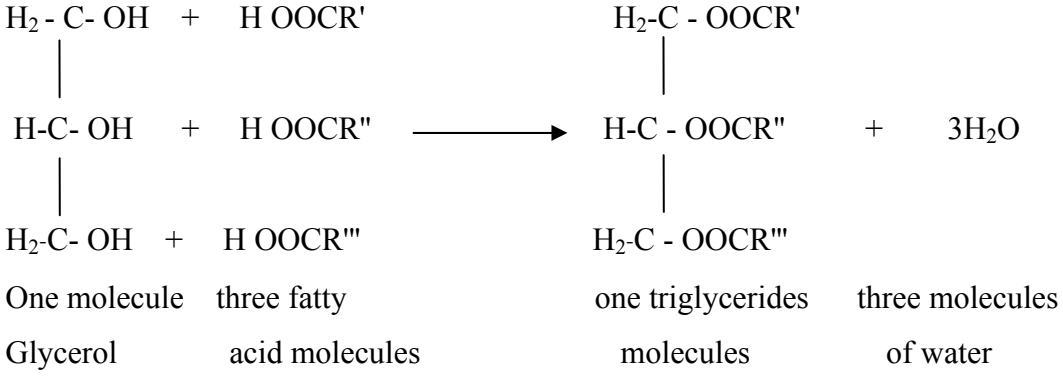

Fig 2.1 Mechanism of formation of triglycerides

This fatty acid may have different chain lengths, different degree of saturation, different amounts of geometric isomers, different amounts of positional isomers and in some cases considerable amounts of hydroxy acid. (Berk, 1967).Most natural oils contain small quantities of a variety of combined fatty acids, although usually two or three predominate. (Fox et al, 1982)

The chain of fatty acid may be saturated (Containing single bond) or unsaturated (Containing one or more double bond). Triglycerids containing unsaturated fatty acids have lower melting points. The oils are higher in unsaturated fatty acids than fats. Edible oils contain more unsaturated than saturated fatty acids. (De Man, 1976).

Saturated fatty acids consists palmitic (Most widely distributed), stearic acid, myristic acids and lauric acids. The unsaturated acids are palmitoleic acids, olic acids, linolenic acids are widely distributed in animal and plant fats and oils however olieic acid probably the most generally found of all fatty acids, linolenic acids is widely distributed and linolenic acid with its three double bond is found in a number of drying oils. Arachidenic acid occurs in animal fat. (Bailey, 1982). The unsaturated acids have the possibility of either cis or trans isomerism, in nature only cis-isomer occurs. (Meyer, 1987).

The rate of autoxidation reaction of fats and oils is connected mainly with unsaturated

acyl groups (the acyl radicals is R- C= O) with the hydroperoxides group appearing in the alpha position relative to the double bond (Gaddis, 1961). Saturated fatty acids derivatives autoxidize very slowly. Cis-isomers of the unsaturated fatty acids are more susceptible to autoxidation than the trans (Knight et al,1951).Saturated hydrocarbon chain are relatively inert, but the unsaturated chain are affected by both oxidizing and reducing agents (Tooley, 1971).The commonly found saturated and unsaturated fatty acids structure or given in table-1 of appendix-1.

2.2 Deep fat frying

Fried foods have continued to be popular in spite of the current guidelines which recommend a decrease in the content of fat in our diet (Li, 2005). Frying is a fast and convenient technique for production of foods with unique sensory properties including color, flavor, texture, and

palatability that are highly appreciated by consumers. Therefore, it is important to understand the frying mechanism in order to manufacture, preserve, and market fried foods optimally (Moreira et al., 1999).

Deep fat frying is a process in which the food is cooked by immersion in hot oil. Despite the fact that the deep fat frying industry is well-established and highly automated, the deep fat frying process is considered to be an art rather than a science (Blumenthal, 1991). It is a complex process. During deep fat frying, thermal, oxidative, and hydrolytic reactions take place resulting in physical and chemical changes in the oil and the formation of new compounds (Li, 2005). Cooking oils are generally treated to separate insoluble material in order to prolong the useful life of the cooking oil. Without any treatment, the cooking oil is generally used for only about 1 to 3 days and must be discarded after such time. According, there is a need to increase the useful life of such cooking oils (Clewell and Friedman, 1976).

The useful life of cooking oil can be able to increase by the addition of various adsorbents such as natural adsorbents and synthetic adsorbents. Natural adsorbent include such materials as bentonite, zeolite, activated carbon, diatomaceous earth, active alumina and active magnesia. Synthetic adsorbents have included blends of silicates with magnesium and aluminum oxides, and aluminum oxides with diatomaceous earth (Akoh and Reynolds, 2001).

Adsorbents materials or their combinations were found to effectively useful for the control of free fatty acids, polar compounds, dielectric constant and color of used frying oil (Mancini-Filho et al., 1986; Yates and Caldwell, 1992, 1993). In addition, the oil replenishment techniques have to be reported to reduce the rate of oil deterioration. It was found that fresh oil replenishment at 15% every 4 h significantly delayed an increase in polymer contents and a decrease in the induction period of frying oil (Chuand Lin, 1996)

2.2.1 Changes during frying

During deep-frying of food at temperatures in the region of 170° – 200°C, the oil used come under a heavy region of 170° – 200°C, the oil used come under a heavy three-prong attack, namely:

a. Hydrolysis*:* Moisture from the food being fried vaporises and hydrolyses triglycerides (TGs) in the frying oil to glycerol, free fatty acids (FFAs) monoglycerides (MGs) and diglycerides (DGs)(Dana, 2001)

b. Oxidation: Triglyceride molecules in the frying oil undergo primary oxidation to unstable lipid species called "hydroperoxides" which cleave to form secondary oxidation products which comprise non-volatile and volatile compounds. Some of these secondary products polymerize (tertiary oxidation), increasing the oil viscosity, cause browning on the surface, and darken the oil (Dana, 2001); and

c. Thermal Polymerization: High temperatures of the frying operation produce high molecular cyclic fatty acid (FA) monomers, and triglycerides dimers and oligomers (Billek, 1983).

The volatile secondary oxidation products (aldehydes breakdown products, alcohol and hydrocarbons such as pentane), acrolein formed from glycerol, and short-chain fatty acids move to the surface, aided by steam formed from moisture in the food fried. Both pleasant fried flavours (contributed mainly by 2, 4 decedienal from linoleic acid) and obnoxious odours (e.g. from acrolein) are formed. Several chemical and physical processes follow, namely: i) the food being fried absorbs oil as well as releases some of its own lipid content (sometimes coloured) into the frying medium, ii) charring of food particles and lipid browning darkens the oil. The immediate environment of the kitchen area gets unpleasant, especially when the smoke point of the frying medium is exceeded, as often happens when animal fat such as lard is used as the frying medium (Henry and Chapman, 2002)

Meanwhile, the potentially hazardous non-volatile compounds gradually build-up in the fried oil. The majority of these products are called "polar compounds" (PC) formed as secondary oxidation products- e.g. epoxides, polar dimmers, oxidized polymers, ketones and aldehydes (carbonyls), as well as hydrolysis products of triglycerides such as free fatty acids, monoglycerides and diglycerides. There is much uncertainty whether these products are actually harmful to humans at habitual intakes (Tony Ng Kock Wai, 2007).

2.2.2 Principles of Deep-Fat Frying

Frying technology is important to many sectors of the food industry: supplier of oils and ingredients, fat-food shop and restaurant operators, industrial producers of fully fried, par-fried and snack food, and manufacturers of frying equipment. The amount of food fried and oils used at both the industrial and commercial levels are huge. A deep-fat fryer consists of a chamber where heated oil and a food product are placed. The speed and efficiency of the frying process depend on the temperature and the quality of the oil. The frying temperature is usually between 150 and 190°C. Oil turnover time (mass of used oil/oil usage rate) is around 10 hours. Frying is defined as the process of cooking and drying through contact with hot oil and it involved simultaneous heat and mass transfer. The oil not only acts as the heat transfer medium, but also enters into the product, providing flavor.
(Source: http://phoenix.eng.psu.ac.th/chem/ram/8Note.pdf)

The following factors can affect the frying process of foods:

A. Depending on the process
   a. Temperature
   b. Frying time
   c. Fryer type – batch vs. continuous

B. Depending on the frying oil
   a. Properties of the oil – chemical and physical
   b. Additives and contaminants

C. Depending on the food

   a. Properties of the food – chemical and physical

   b. Preparation
   c. Ingredients interchange with oil

2.2.3 The Chemistry of Frying

The common element of all fats is a substance called glycerol. Also occurring in nature are compounds called fatty acids. Glycerol can combine with these fatty acids through esterification to form mono-, di-, and tryglycerides. All fats and oils are made up of a mixture

of triglycerides. Some fatty acids possess double bonds and their presence tends to make these particular fatty acids more sensitive and more unstable. Many chemical reactions take place during frying and affect the quality and storage time of the oil. Several of these factors lead to the spoilage of the oil (Warner, 1985).

a. Isomerization (polymerization): This reaction occurs rapidly during standby and frying periods. The molecule is rearranged and the double bonds can often end up closer together making the fat more unstable and more sensitive to oxidation (Warner, 1985)

b. Pyrolysis: It results in the extensive breakdown of the chemical structure of the fat resulting in the formation of lower molecular weight compounds (Warner, 1985).

c. Hydrolysis: It is the major chemical reaction taking place during frying caused by the water in the food. It results in the formation of free fatty acids. The smoked point is reduced and the oil and food develop off-flavors. Baking powder and moisture in the food promote hydrolysis (Warner, 1985).

Table 2.1 Changes in the oil during frying

Type of alternation	Causing agent	Resulting compounds
Hydrolic	Moisture	Fatty acid
		-monoglycerides
		Diglycerides
		Glycerol
Oxidative	Air	oxidized monomers
		oxidative dimmers and polymers
		non polar dimmers and polymers
		volatiles compounds (hydrocarbons, aldehydes and ketones)
Thermal temperature	Temperature	cyclic monomers
		dimers and monomers
Solubilization	Food	colored compounds
		food lipids

(Source: Stanley, 1995)

Fig 2.2: Changes during deep fat frying

2.2.4 Deep-Fat Fryers

The processes used to fry food products can be divided into two broad categories: those that are static and smaller, classified as batch fryers used in the catering restaurants, those that fry large amount of products in a moving bed, used in the food industry, classified as continuous fryers (R.D.*et.al*., 2000).

2.2.4.1 Batch Frying Systems

Batch fryers should be of the appropriate size and installed in the proper number. Other factors such as (a) fuel source (b) speed of temperature recovery, and (c) safety should be taken into consideration when selecting a frying apparatus (R.D.*et.al*., 2000)..

Different types of batch fryers include: gas-fired, electric, and pressure fryers

2.2.4.2 High-Capacity Frying Systems

The industrial fryer consists of several basic components, which are familiar for both continuous and batch systems. In designing a continuous fryer, factors, such as the amount of food, the conveyor system, the food characteristics, and the handling system after the frying are important to effectively produce high quality products. An oversized fryer can be very inefficient, causing severe oil degradation, creating cleanup problems, and resulting in poor quality of the product. It is better to design a fryer for maximum efficiency in producing one product type than multiple products inefficiently (R.D.*et.al*., 2000).

A continuous fryer system consists of at least five independent set of equipment: (1) the kettle or tank containing the frying oil, (2) a heating unit with a control system for generating thermal energy, (3) a conveying system for moving the product into, through, and out of the frying process, (4) a fat system, which pumps and filters the frying oil, and (5) an exhaust system for removing the hot vapors emerging from the product (R.D.*et.al*., 2000).

2.4.5 Calorific value and uses of fats and oils

Fats and oils are widely distributed in foods; almost every natural food has considerable quantities of them and is of great nutritional value. During recent years the percentage calories derived from fat has increasing markedly in the average nutritional diet (Swami Nathan, 1985).They provide fatty acids which are necessary to animal metabolism and represent a concentrated reserve of energy yielding about 37 KJ (9K calories) per gram in comparison

with corresponding values of 16.5KJ (4K calories) and 22.75KJ (5.5K calories) per gram for carbohydrate and protein respectively (Wilson et al, 1965).

The availability (consumption) of fats and oils per person per annum is 11.5Kg, and average person in developing countries are getting only 5.1kg per person per annum whereas in developing countries it is 31.3kg per person per annum (Shremitta, 1982). For an average Nepalese the availability is about 3.3kg per annum per capita. The general features of fats and oils according to Nepal Standard are given in table 2 of appendix-1.

Fats in the diet is consumed in two forms, the visible fats like butter, margarine and invisible fats which occurs in foods like milk, egg, dry bean, fruits and vegetables etc (Fox et al , 1982).

In addition to the source of energy, fats and oils supplies essential fatty acids (PUFA) (Burr and Burr, 1930); carry fats soluble vitamins (A, D, E, K), control blood lipid, protect body, serve as source of deposited energy in adipose tissue, improve the palatability and give a satiety value and thus used for preparing variety of foods or diets. (Swaminathan, 1991).

Although having a wide range of used and great nutritional and calorific value, the fate of fats and oils is that it is very prove to oxidation therefore stabilization or preservation of such a valuable food component, from the onset of rancidity (Oxidation) is a must, which can be easily achieved by the use of tomato seed powder/oil as natural antioxidant even at home scale and commercial scale.

2.3.6 Composition of Palm oil

Palm oil is one of the 17 major oils and fats produced and traded worldwide. Palm oil accounts for almost 30% of the global vegetable oils production, with 60% of the share represent the overall world export (10). Due to this fact, Palm oil has become one of the most important commodities, especially in the food industry. As their thermal stability is concerned, palm oil and its fractions, mainly pal, olein (single fractionated), double –fractionated palm olein, red palm oil/olein and palm stearin are widely utilized in frying activities, including continuous frying. These products demonstrate inherent frying properties, which is often regarded as a heavy duty frying oil with stronger resistance towards thermo-oxidation than most of the other vegetable oils and fats. Apart from that, the availability of both palm oil and pal fractions at

competitive prices also contributes to the wide usages of these products for frying purpose (Source: http://cogeneration.net/refined_palm_oil.htm).

Crude palm oil is refined, bleached and deodorized to obtain palm oil with the iodine value (IV) of 50 to 55. Palm olein is the liquid fraction obtains via fractionation of palm oil. These are mainly two grades of palm olein mainly of single fractionated palm olein and double fractionated palm olein. Single- fractionated palm olein, which comprises the bulk of refined palm olein, has an IV of 56 to 59 while the IV of double-fractionated palm olein is ranged from 60 up to 67. The unbleached palm oil/olein is known as red palm oil/olein. Palm stearin, which is a co-product of palm olein is more solid with a wide range of IV between 27 to 45. Palm based shortening is generally contained 30 to 40% of palm oil and its fractions in the formulation (http://cogeneration.net/refined_palm_oil.htm).

The role of frying oil is essentially to provide an efficient heat transfer medium. The oil transmits the heat rapidly and uniformly to the surface of the foods. At the same time, some of the oil/fat absorbed by the fried foods becomes a source of flavor and nutrition. However, the quality of oil/fat is difficult to control as degradation takes place during the frying process. It involves a complex pattern of both thermodynamic and oxidative reactions, which are very much dependent on the type of oil/fat used and the matrix (composition) of fried foods. Oxidations of oil, which is one of the major deterioration reaction during heating and frying, includes a significant loss of quality, where it leads to changes in functional, sensory, nutritional values and safety of the fried foods. Oxidation will further promote the formation of new compounds such as diacyl glycerol, monoglycerols, monomers, polymers, free fatty acids and other oxidative substances, which are harmful to human. This leads Firestone and colleagues to review legislation and regulation concerning the quality of frying oils/fats in the USA and some European countries. Others researches also reported recommendations on the criteria for evaluating the fry-life of the frying (http://en.wikipedia.org/wiki/Deep_frying)

Currently, there are only few research works that investigate thermal properties of oils and fats, and most of them focused on the effect of antioxidants or additives. Nevertheless, studies on the stability of palm products upon prolonged heating have not reported. The physio-chemical analysis of palm oil is shown in appendix 3.

2.3. General properties of oils (fat)

In this section the physical and chemical properties related to these work only has been described.

2.3.1. Physical characteristics

2.3.1.1. Colour:

Most crude oils are coloured, usually with and orange hue due to the presence of dissolved carotenoids. Occasionally brown shades can be present usually as a result of oxidation while the green tints of some olive, rapeseed, and soybean one generally due to presence of chlorophyll (Sleeter, 1983). The most widely technique of colour measurement is the Lovibond Tintometer method, in which the colour of light transmitted.

2.3.1.2. Specific gravity

Specific gravity of the fats and oils is less than one (about 0.86) and therefore they floats on the water surface. Solid fats are lighter than liquid fats. Length of fatty acids tents to increasing the specific gravity (Bloor, 1943)

2.3.1.3. Refractive index:

The refractive index of an oil is defined as the ratio of the speed of light in vacuum (but for practical purpose scale of instruments normally indicates refractive indices with respect to air rather than vacuum) to the speed of light in the oil under examination (Bailey,1982). The refractive index of oil is characteristic within certain limits for each type of oil. It is related to the degree of saturation (i.e. iodine value), to the ratio of cis/trans damage to the oil (Rossel, 1986).The refractive index of fats and oils increase with increase in chain length of fatty acids in triglycerides (Dugan, 1976). The RI of oil may be determined more commonly by means of and abbe type refractometer. The standard value for the oil used is supplied in appendix-6.

2.3.1.4. Moisture and Volatile matter

Crude oils may have levels of moisture upto 2-3% (Rossel, 1986). Moisture content is determine by standard air over method and is expressed as percentage of water and volatile content together. Moisture content is the most important factor which affect the storage

quality, since moisture activates lipase causing an increase in the free fatty acids content in oil (Bhatnager et al, 1974)

2.3.2. Chemical characteristics

2.3.2.1. Acid (Titer value)

Acid value is the number of milligram of KOH required to neutralize the free fatty acid present in 1 gm of fat or oil. The acid number thus, tells us the quantity of free fatty acids present in fats and oils (Jain, 1979). The standard values of oils are of appendix-6.

For the most part, natural oils and fats are in the triglycerides form when freshly extracted from the source. With prolonged storage, however the triglycerides begin to break down giving rise to FFA. This hydrolysis is brought about by a variety of agents: presence of moisture oil, elevated temperature and most important of all lipase (enzyme) coming from the source or containing microorganism. Consequently, the neutral oil became a mixture of tri, di, mono glycerides, FFA and glycerol. Some fats/oils are retaining stable but others such as crude rice bran oil are notoriously susceptible to hydrolysis. Whenever the oil presence of excess free fatty acid is a sure indicator of unnatural state of oil. The presence of FFA in large excess, though not a health hazard is desirable for several reasons i.e.

- The oil is no longer the same as virgin oil.
- The oil tends to smoke during deep fat frying.
- The oil is susceptible to rancidity
- The product prepared from such oil turn rancid very soon

2.3.2.2 Peroxide value (PV)

The most common cause of oil deterioration is rancidity-the most common cause of oil deterioration is oxidation. It is generally accepted that the first product formed by oxidation of oil is hydroperoxides. The usual method of assessment is by determination of the peroxide value (PV), which is reported in units of milli-equivalents of oxygen per kg of fat. It is the very sensitive indicator of the early stages of oxidative deterioration of fats and oils. PV therefore provides a means of predicting the risk of the development of flavor rancidity. PV is the good guide to the quality of fat and a freshly refined fat should have PV of less than 1 unit. Fats that have been stored for some period of time after refining may be found to have PV of

upto 10 units before undue flavor problems are encountered. This is due to the fact that the peroxide themselves shave no flavour. They do, however, decomposes into aldehydes and ketones, many of which have pronounced off flavour (Hamilton, 1986).

2.3.2.3. Saponification value (S.V)

The Saponification value (S.V) of a fat or oil is defined as the number of milligram of Potassium hydroxide (KOH) required saponifying one gram of the fat or oil (IUPAC, 1979). The Saponification number thus provides information on the average chain length of the fatty acids in the fat or oils. It varies inversely with the chain length of the fatty acids. The shorter the average chain length of the fatty acids, the higher is the Saponification value (Jain, 1979). It is the valuable test for the determination of adulteration. Oxidation of unsaturated fatty acid raises this value. The standard values for different oils are tabled on appendix 6.

2.3.2.4 Unsaponofiable matter:

The unsaponofiable matter content is equal to the total quantity of substances dissolved in the fat which after saponifiable are insoluble in aqueous solution but which are soluble in the organic solvent used in the determination (ISO, 1983). The unsaponofiable matter content is helpful to reveal the oxidation of oil to some extent. The badly oxidized oils polymers, the polymerized oil fatty acids are extracted with the unsaponofiable matter (Hamilton et.al., 1986). The standard UM values for the oils are presented in table of Appendix 6.

2.3.2.5 Iodine value

Iodine value is defined as the percentage of iodine absorbed by the oil or fat under the test conditions Wij's method is recognized for the test (ISO, 1979). The iodine value of a fat is a measure of its degree of unsaturation (Meyer, 1960). The standard iodine values for the oils are given in table of Appendix 6.

2.3.2.6 Fatty Acid Composition

The classical method for identification of fats and oils has been replaced by fatty-acid composition analysis determined by GLC patterns. The classic method was based on the identification of a specific fat or oil by a combination of its iodine value, relative density, refractive index, and saponification value. The advantages of the GLC procedure are that it permits identification of source oils that cannot be identified by the classical methods, plus it offers the ability to identify the source oil proportions in a blended product. Further, because

the fatty acid composition requires only one analysis, it can be made more rapidly and applies equally well to refined and unrefined oils, thus requiring only one set of standards. Gas chromatography includes those chromatograph techniques in which the mobile phase is a moving gas. In general, the procedure involves passing the methyl esters, or transesterified triglycerides, to be analyzed through a heated column by means of a carrier gas such as helium or nitrogen. The components of the mixture are eluted with the gas and detected and measured at the exit end of the column by a suitable means. The retention time is the time required for a given compound to pass through the column. The fatty acid esters exit in the order of saturation. The retention line is indicated on the horizontal axis of the chart and is a qualitative index of the substance, and the area under the curve is in each case a quantitative measure of the component. The fatty acid analysis provides a rapid and accurate means of determining the fatty acid distribution of fats and oils products. This information is beneficial for all aspects of product development, process control, and marketing because the physical, chemical, and nutritional characteristics of fats and oils are influenced by the kinds and proportions of the component fatty acids and their position on the glycerol radical. Fatty acids are classified by their degree of saturation (Lawson, 1997)

• Saturated fatty acids*:* Fatty acids in which all carbon atoms in the chain contain two hydrogen atoms and therefore have no double bonds

• Monounsaturated fatty acids*:* Fatty acids that have only one double bond in the carbon chain

• Polyunsaturated fatty acids*:* Fatty acids that have two or more double bonds in the carbon chain

Each fatty acid has an individual melting point. The melting points of saturated fatty acids increase with chain length and decrease as the fatty acids become more unsaturated; for a given fatty acid chain length, the saturated fatty acid will have a higher melting point than the unsaturated fatty acid. Capric (C-10:0) and longer chain saturated fatty acids are solids at room temperature. The unsaturated fatty acids are chemically more active than the saturates because of the double bonds, and this reactivity increases as the number of double bonds increase. The double bonds are subject to oxidation, polymerization, hydrogenation, and isomerization. The physical characteristics of a fat or oil are dependent upon the degree of unsaturation, the

carbon chain length, the isomeric fatty acid forms, and the molecular configuration. Usually, fats are liquid at room temperature when the level of unsaturated is high and solid when the level of unsaturated is low; however, this generalization can be complicated by *trans*-isomers that have different melting characteristics than the *cis*-isomer of the unsaturated fatty acid. (Swern, 1979) The fatty acid composition of palm oil is shown on Appendix 7.

2.4. Rancidity in fats and oils

Rancidity, simply can be said to be the subjective organoleptic appraisal of the off flavour quality of food; rancid of flavour are concerned with the changes that result from reaction with atmospheric oxygen i.e. result from reaction with atmospheric oxygen i.e. oxidative rancidity, or by hydrolytic reactions catalyzed by lipase from food or from microorganisms (Lundburg, 1961). The off flavors can also be caused by taints arising by absorption on contamination, in which case the lipid acts as good reservoirs. The effects of hydrolytic reactions and absorption can be minimized by cold storage, good transportation, careful packaging and sterilization (Galliard, T;Burdon, T.A;Davies, A.C; Frampton, A;Allen, J.C.;Young, C.C.; 1983). However, since oxidative rancidity or autoxidation is a chemical process (reaction) with a low activation energy 4-5 kcal/mole for the first step and 6-14 kcal/mole for second step, the rate of this reaction is not significantly diminished by lowering the temperature of food storage (Bailey, 1979).

2.4.1. Mechanism of autoxidation

Lundburg (1962), described that the unsaturated bonds present in all fats and oils represent active centers which among other things, may react with oxygen. This reaction leads to the formation of primary, secondary, tertiary oxidation products which may make the fat or fat containing foods undesirable for consumption. Farmer and sundralingam (1942) and farmer et al (1943) showed that hydroperoxides are formed during usual autoxidation of fats. It is well noted that hydroperoxides by themselves do not contribute materially to unwanted odours and flavour but are caused by secondary substances formed during the various reactions and possibly through further oxidation of the peroxides and their degradation product. (Lee, 1975)

A fatty acid (RH) is converted to a free radical by abstraction of a proton at the cis-double bond which is invented to the trans-configuration while moving into conjugation with adjacent double bond. This reaction is connected mainly with unsaturated acyl group. (Lee, 1975), with

alpha position relative to the double bond. A shift of the double bond may or may not be involved in the formation of hydroperoxide. The fatty acid free radical (R) takes of oxygen to produce a peroxy free radical (RO2) which reacts with another fatty acid (RH) to form a hydroperoxide (ROOH) and a new free radical (R) thus setting off a cyclic chain reaction (Uri, 1961 A) and proceeds as –

Initiation reaction:

Activation energy 4.5kcal/mole

$$RH \longrightarrow R' + H'$$

$$RH + O_2 \longrightarrow ROO' + H'$$

Propagation reaction:

$$ROO' + RH \longrightarrow ROOH + R$$

$$R' + O_2 \longrightarrow ROO'$$

Termination reaction:

$$ROO' + R' \longrightarrow ROOR$$

$$R' + R' \longrightarrow R - R$$

$$ROO' + ROO' \longrightarrow ROOR + O_2$$

Secondary initiation reaction:

Activation energy 6-14kcal/mole

$$ROOH \longrightarrow RO' + 'OH$$

$$ROOH \longrightarrow ROO' + H'$$

Fig 2. 3. Free radical mechanism of autoxidation of a lipid molecule (RH) (Girdon, 1986)

In the presence of metal ion chain branching or free radical multiplication occur (Arwitting, 1974) as:

$$ROOH + M^{2+} \longrightarrow RO_2 + OH + M^{3+}$$

$$ROOH + M^{3+} \longrightarrow RO_2 + H^{+} + M^{2+}$$

The rate of an autocatalytic reactions increase with time because products which are formed during the reactions tend to catalyse the reaction. Hence, as the reaction proceeds the rate of hydroperoxide accumulation increases (De man, 1976). The rate of autoxidation increases exponentially with increasing unsaturation in the usual fatty acid and saturated fatty derivatives autoxidize very slowly to form hydroperoxide. The cis-isomer of the unsaturated fatty acids are more succeptible to autoxidation than the trans. (Farmer et al, 1943).

2.4.2. Measurement of Autoxidation

It is generally accepted that the first product formed by oxidation of an oil or fat is a hydroperoxide.The most cannon method of assessment is therefore by determination of the peroxide value (P.V.) which is reported is units of milliequivalent of per gram of fat. The validity of PV to monitor rancidity has been questioned on many grounds and argued that PV as a stand and for rancidity argued of all fats and oils with a single limit value is without scientific basis.

(Arumughan eta al, 1984)However, several workers have compared various chemical parameters for their suitability to justify rancidity of fats and oils with varing degree of success labuze(1971)has reviewed the kinetics of lipid oxidation. Gray (1983) and lea (1931) has accepted and prescribed the peroxide value measurement as usual method. There are oils prescribed by various national and international agencies based on peroxide value which also support the facts.

There are numerous analytical procedures for measurement of peroxide value. In all cases the result and accuracy of the test depends on the experimental condition as the method is highly empirical (Rossell, 1983). The most common method are those based on the idometric titration originally reported by Lea (1931) and Wheeler (1932) which measures the iodine procedure from potassium iodide by peroxide source of error in these methods are the

absorption of iodine at saturated bonds in the fatty acids and the liberation of iodine from potassium iodide by oxygen present in the solution to the titrated. Other type of errors which can arise (Gray, 1978) include variation in the weight of sample used, variation in the reaction condition such as time and temperature and constitution and reactivity of the peroxide present in oil.

2.4.3. Toxic products of Rancidity

Oxidative rancidity leads to the formation of both undesirable and toxic compounds and thus nutritionally undesirable (Sanders, 1983). Their different classes of substance peroxidized fatty acid and their subsequent in product, polymeric material and oxidized sterols occurring in oxidized fat have been shown to have toxic effects (Artman, 1969; Wilson, 1976). Hydroxy acids, ketoacids and aldehydes, found in oxidized fat are responsible for the off flavour and odours characteristic to rancid of food (Swemitt, 1982) which degrade the quality of fats and oils.

Agduhr (1926), Whipple (1932), Hass (1938) carried out the early work on the toxicity of rancid lipid in different experimental animals observed the syndromes as-(a)Diarrhoea (b)Poor rate of growth (c)Cardiomyopathy (d)Hepatorneghy (e)Steatitis or yellow fat diseases (f)Haemolytic anaemia (g)Secondary deficiencies of vitamins E and A.

Recently it has been postulated that lipid peroxidation may play an important role in causation of artheroscletotic disease and carcinogenic effect (Wilson, 1976 and Kummerow, 1979).

2.5 Factors affecting the oxidation

2.5.1 Light and radiations

Visible light seems to split up the decomposition of hydroperoxides may be due to absorption of the light by peroxide or other compounds. The action of ultraviolet light has a greater effect. The amount of hydroperoxide produced is directly proportional to the total amount of light absorbed (Lundburg, 1949). Beta, gama and high energy radiation catalyze autoxidation by catalysis of peroxide decomposition (Lee, 1983).

2.5.2 Heavy metals:

Heavy metal especially Fe, Cu, Co, Mn, Ni increase the maximum rate of oxidation. The metal ions initiate a free radical chain by the electron transfer which was introduced by Harber and Willstaltes (1931). So presence of traces of heavy metal is fats and oils without doubt one of the important reason for their oxidative deterioration (Pyke, 1976).

2.5.3 Degree of unsaturation

The polyunsaturated fatty oxides and other highly unsaturated fatty compounds are particularly susceptible to oxidative rancidity. So presence of unsaturated influences the rancidity (Allen Hamilten, 1983).

2.5.4 Moisture

Hydroxide rancidity is generally caused by microorganism and moisture. The factor tends to reduce the quality of oil whether by microbial growth or by accelerating the chemical reaction (Hull, 1980)

2.5.5 Oxygen pressure

Bolland (1946) found thatr the rate of oxidation varied with the oxygenpressure. At very low oxygen pressure the rate of oxidation is approximately proportional to the pressure; but at higher the rate was independent of this factor. As long as the oxygen is present in the limited quantity, the rate of autoxidation increases with increasing oxygen pressure until a constant oxidation rate is reached beyond the given pressure (Berk, 1976). The formation of volatile compounds or hydroxide in oil during storage can be minimized by lowering the oxygen content (Min and Wen, 1983)

2.5.6 Temperature

The rate of reaction is accelerated by increasing the temperature. The activation energy strongly depends upon the temperature under condition of normal initiation temperature, a rise in temperature affects the rate of autocatalytic autoxidation more than the majority of chemical reaction (Lee, 1931). The rise in temperature influnces by two ways- it speeds the chain propagation reaction and also the decomposition of peroxide resulting in an increase in the

conceration of free radicals available for the start spread of reaction chain (Vorbeck et.al., 1961)

2.5.7 Catalyst

Biological catalyst including oxidative enzyme if present can present accelerate the rate considerably (Lee, 1976)

2.5.8 Presence or absence of Antioxidants

Antioxidant is capable of retarding or preventing the development of rancidity or other flavour deterioration due to oxidation (Allen and Hamittor, 1983). So presence or absence of the natural synthetic antioxidant influences the rancidity.

2.6 Antioxidants and their role in fats and oil

2.6.1 Definition of antioxidant

Antioxidant is any substance that works within the body to reduce oxidative damage. Highly reactive chemicals within the body, such as free radicals, can damage the normal tissues of the body. Antioxidants work to counteract this damage (Bhattarai B. 2009, a class seminar on Natural antioxidants and their impact on human health p.1)

Any substance which is capable of delaying or preventing the development of the rancidity or other flavor deterioration due to oxidation is called antioxidant (Coppen, 1983). Antioxidants are often said to inhibit lipid peroxidation. Antioxidants do not prevent lipid peroxidant, they merely interrupt and terminate the chain reaction and thus decrease the quantity of lipid peroxidized per free radical initiation (Lundburg, 1961).

Antioxidants are widely used as ingredients in dietary supplements used for health purposes such as attempting to prevent cancer and heart disease. Studies have suggested antioxidant supplements has benefits for health, but several large clinical trials did not demonstrate a definite benefit for the formulations tested, and excess supplementation may even be harmful (www.wikipedia.org).

2.6.2. Necessity of use of antioxidants

Although there is also vacuum packaging or packing under inert gas to exclude oxygen and refrigeration/freezing, which greatly reduce the rate of autoxidation unfortunately they are not always applicable. Furthermore, it is a fact that little oxygen is needed to initiate and maintain oxidative process and it is quite impossible or expensive to remove the least traces of air from a food product. Also, antioxidants are generally effective, easily applied and inexpensive. Other justification for need of an antioxidant use are an antioxidant can extend shelf life of food, reducing wastage and complaints it can reduce nutritional losses (oil soluble vitamin) and more important it can widen the range of fats which can be used (Allen and Hamilton, 1883)

2.6.3 Mechanism of action

The antioxidants are active in lengthening the induction period in the process oxidation of fats, probably due to the absorption of the activation energy of chain reaction that result in the oxidation of antioxidants (Filler et.al., 1994; Lowley et.al., 1969; Mohan and Chapman, 1953). An antioxidants act by reducing with free radicals fatty acids free radicals or peroxide radicals as they formed, converting them to the original substrate and thereby terminate the chain propagation (or initiation). Free radicals of the antioxidants molecule are formed in this process, but the structure of an antioxidant is such that they are relatively stable and donot have enough energy to react with the fat to form further free radicals (Coppen, 1983). The process scheme is as follows.

An antioxidant (AH) apparently reacts with radicals in following manner-

$R\cdot + AH \longrightarrow RH + A\cdot$

$RO\cdot + AH \longrightarrow ROH + A$

$ROO\cdot + A\cdot \longrightarrow ROOH + A$

$R\cdot + A\cdot \longrightarrow RA$

$RO\cdot + A\cdot \longrightarrow ROA$

$A\cdot + ROO\cdot \longrightarrow$ Non radical product

$A\cdot + A\cdot \longrightarrow$ Non radical product

At high concentrations the antioxidant may have a peroxide effect (Pokorny, 1971) and one of the reactions may be as follows-

$A \cdot + RH \longrightarrow AH+R\cdot$

(Lundburg, 1961 and Deman 1976)

The mode of action of all should be added to the fat as early as if possible in its life to produce the maximum effect. It follows rthat an antioxidants will oSnly really be effect if it is added during the initiation period (Coppen, 1983).

2.6.4 Type of antioxidants

2.6.4.1 Synthetic (Artificial) Antioxidant

Numerous compounds have been developed synthetically used to inhibit oxidation (Meyer, 1960). Most of them are phenolic compound among which Butylated hydroxyl toluene (BHT) and Butylated hydroxyanisole (BHA) (Stuckey, 1962) are common in use. A quantitative tolerance limit for these synthetic antioxidants in Federal Regulations are limited not exceed total content of 0.02% of fat or oils either of fats or oils either alone or combination (Bauernfeind et. Al.,1974).

a. Tertiary Butyl Hydroquinone (Food Grade)

Tertiary Butyl Hydroquinone (TBHQ) is a Synthetic food grade antioxidant that is used to stabilize foods, fats and vegetable oils against oxidative deterioration and thus extending their storage life. Basically in simple terms TBHQ can be called a chemical that is used as preservative. It helps in preserving food products. There is a natural antioxidant called Tocopherol that is present in vegetable oils that is insufficient for oxidative stability, even if external Tocopherol is added to the vegetable oil it does not increase the stability hence a synthetic antioxidant is required. TBHQ is certified as safe for human consumption. In many major developing organizations like FDA (Food and Drug Administration), FSIS (Food Safety and Inspection Service) and others permit the use of TBHQ or combinations with BHA (Butylated Hydroxy Anisole) or BHT (Butylated Hydroxy Toluene) at concentrations upto 0.02% by weight of the fat or oil content of the food (http://www.crystalquinone.com/TBHQ.htm).

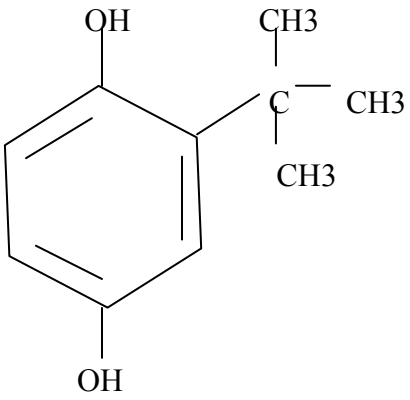

Fig 2.4**.** Chemical structure of TBHQ

b. Butylated Hydroxyanisole(BHA)

Butylated hydroxyanisole (BHA) is a mixture of two isomeric organic compounds, 2- tert-butyl- 4 – hydroxyanisole and 3 – tert – butyl – 4 – hydroxyanisole. It is prepared from 4-methoxyphenol and isobutylene. It is a white or pale yellow solid (Crystal or Flake) with a faint aromatic odour (http://www.crystalquinone.com/BHA.htm).

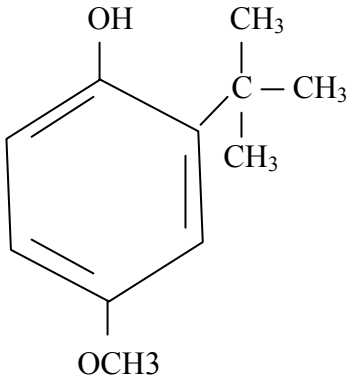

Fig 2. 5. Chemical structure of BHA

(Source: http://www.crystalquinone.com/BHA.htm)

c. Butylated Hydroxy Toluene (BHT)

Butlyated Hydroxyanisole is a synthetic antioxidant that is a commonly used fat soluble food preservative since 1947, with broad biological activities. It prevents spoilage by reacting with oxygen. It slows development of off-flavours, odours and colour changes caused by oxidation. It protects animal's aganist radiation and the acute toxicity of various xenobiotics and mutagens. Butylated hydroxyanisole (BHA) is a mixture of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole. It is prepared from 4-

methoxyphenol and isobutylene. It is normally insoluble in water, but for commercial applications, it can be converted to a soluble form. BHT was first used as an antioxidant food additive in 1954. An antioxidant is a substance that prevents the oxidation of materials with which it occurs. BHT, therefore, prevents the spoilage of food to which it is added. BHT has grown to be very popular. Among food processors and is now used in a great range of products that include breakfast cereals, chewing gum, dried potato flakes, enriched rice, potato chips, candy, sausages, freeze-dried meats, and other foods containing fats and oils. BHT is sometimes used in conjunction with a related compound, Butylated hydroxyanisole (BHA) as a food additive.BHT does have other commercial uses, as in animal feeds and in the manufacture of synthetic rubber and plastics, where it also acts as an antioxidant (http://www.crystalquinone.com/BHT.htm).

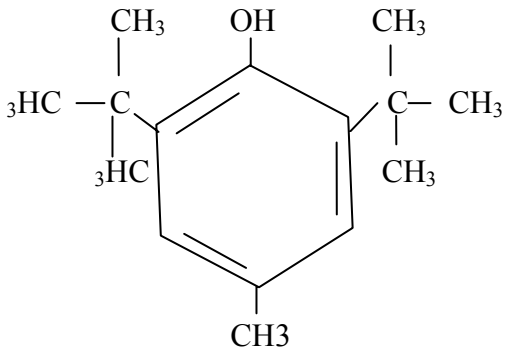

Fig 2.**6**: Chemical structure of Butylated Hydroxy Toluene
(Source: http://www.crystalquinone.com/BHT.htm)

d. Fate of synthetic antioxidants

In recent years the possible toxicity of synthetic antioxidants has been investigated by several workers.

Butylated hydroxyanisole(BHA) has been banned. Most antioxidants are phenolic in structure and there by donation of a hydrogen atom to acyl or peroxide radicals, with the formation of relatively stable radicals nonradical products.

Japan following research by (Imaida et. Al.,1983) which showed that the antioxidant prompted carcinogenesis in rats. Butylated hydroxotoluene has also been implicated as a

promoter of tumors (Djurhuus et. Al., 1982). These studies have encouraged the development and use of naturally occurring antioxidants.

2.6.4.2 Natural Antioxidants

Modern lipid science discovered that antioxidants are widely distributed in nature (Bracco et al, 1981) Natural antioxidants seems to be more adequate for protection against oxidation and have many inherent qualities unsuppressed by the synthetic antioxidants (Loliger, 1983). It imparts no adverse effect in its long run of use; natural antioxidants do not have a quantitative tolerance limit in federal regulations (Bauernfeind et al, 1974). Natural antioxidants can be used in a number of applications even where there is no choice for anything else, because of company policy or food legislations and public pressure group. There are some scientific evidences which alone is sufficient to support for using natural antioxidants.

The antioxidants activity from natural sources has been demonstrated in spices (Chang et al, 1950); vegetable extracts (Pralt et al, 1964) and plant protein and their hydrolyzates (Bishov et al 1975, 1972). None have achieved commercial importance.

The tocopherols are an important group of natural antioxidants. Chipault (1962) and Aoes Jorgensen (1962) cover some its early work on tocopherol in vitro animal fat oxidation study showed that tocopherol have antioxidant activity at levels as low as (0.008%or 0.01%). Mixed with citric or ascorbyl palmate, tocopherol are efficient as BHT. In absence of oxygen, they are relatively heat and light stable (Bauerinfeind et al, 1974)

The tocopherols are slightly viscous pale yellow liquids freely soluble in most organic solvents; insoluble in water. To retard the development of oxidative rancidity and tocopherols are used in foods as antioxidants (Johnson jand Peterson, 1974). Tocopherols have a molecular wt of 30-69 and boiling point 200-220 c (0.1mm). In addition to food uses vitamin is used in food supplement and pharmaceutical dosage formulation. (HASNRC food and Nutrition Board, 1967).

Tocopherols are readily oxidized and consequent protects the fat from oxidation (Meyer, 1960). Mode of action of natural antioxidants are not different from dose of similar synthetic phenols and polyphenols are proton donors which terminate free radical chins(Bishov et al, 1974).

2.6.4.3. Synergestic Antioxidants

The preventive antioxidants which act by reducing the rate of chain initiation is called synergistic antioxidants although they have no effect as protectants when used along with fats (Lee,1975). These compounds helps to increase (improve) the ability of the phenolic antioxidants to retard rancidity. Furia (1968)has presented an excellent review of the use of sequestrates (metal inactivators) in foods. Many components exhibit metal deactivating properties in edible triglyceride oils as evidenced by improvement in oxidative and/or flavour stability. Among these most important is citric acid (Dutton et al, 1947); and Dutton et al(1949).

All metal inactivating compounds have free hydroxyl for carboxyl groups that co-ordinate readily with metal forms salts readily (Cowan, 1962). Schwab et al (1953) proposed the metal inactivators, in effect compleses with peroxidant metal and hold them in a chelat or ring structure held winter co-ordination complexis, where the metal can no longer function as peroxidant.

2.6.5 Antioxidants in oils and fats

Antioxidants are the substances that generally prevent, delay or retard the onset of rancidity (off-flavor) in food products due to oxidation of the unsaturated fatty acids incorporated in food products. The use of antioxidants helps extend the shelf-life of a food, minimizes waste and nutritional losses, and extends the scope of use of various fats/oils. Antioxidants are available in both natural and synthetic forms. However, the use of synthetic antioxidants in food items has been almost abandoned on account of health and legal issues. In facts, there is immense interest in the use of natural antioxidants (Bhattacharyya D.K., 2003)

Tocopherols are the most well known antioxidants *in vitro* and *in vivo* and the relative antioxidants activities of the four tocopherol isomers, namely, β, γ and δ are recorded that show the the four isomers react differently depending on the reaction conditions and the natural of the substrate. There are also four corresponding tocotrienols. The tocopherols are tocotrienols are together termed ‘Tocols’ contribute to vitamin E actively, and serve to quench biological free radicals and prevent their destructive effects. They also suppress cholesterol synthesis in the liver. Both tocopherols and tocotronols reduce platelet aggregation and

modulate the synthesis of prostanoids. In fact, they prevents heart diseases, reduce the risk of cancer, and reducing or delaying cataracts (Bhattacharyya D.K., 2003).

Among the synthetic antioxidants, four common synthesis foods grade antioxidants, namely, Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Propyl gallate(PG)and Tertiary butyl hydroxyquinone (TBHQ) are well known for their use as effective antioxidants in some common fats an oils. BHA, BHT and Propyl gallate are most classical synthetics while TBHQ being the recent discovery. TBHQ is approved in the United states, in many parts of Asia and South America, and in some Eastern European countries. TBHQ is not approved in the European union or Japan (Bhattacharyya D.K., 2003).

Antioxidant have enormous demands for our health and nutrition and their use in food products is extremely important as the provision for direct consumption for biological role and preserving food products containing oils and fats. It is necessary to make proper selection of an individual antioxidants for a particular application in a particular food. New kind of antioxidants such as conjugated Linoleic acids (CLA) and conjugated Linoleic acids (CL_nA)as occurring in some specific fats/oils have been examined. Studies conducted over the past two decades under in vivo condition have numerous health benefits reducing carcinogenesis, antheroscalerosis, on set of diabetes, and body fat mass. The beneficial implication regarding dietary CLA and CL_nA for human health is apparent (Bhattacharyya D.K., 2003).

It is imperative to make extensive investigation on the vast multitude of oil-bearing materials to ascertain the extent of occurrences and the nature of antioxidants and their activity as well in the immense importance of antioxidants in biological and technical applications. (Bhattacharyya D.K., 2003)

2.7 Health benefits of antioxidants

Antioxidants are a diverse group of chemical that can naturally found in vegetables, fruits and plants in general. Antioxidants are also synthesized in our body. Example of dietary antioxidants is Vitamin E, C, A, phenolic acids, selenium, chlorophyll and chlorophyll derivatives, carotenoids. Flavonoids, glutathione, coenzyme Q 10, melatonin, and lycopene. Synthetic dietary antioxidants include Butylated hydroxyanisole (BHA), and Butylated hydroxytoluene (BHT). Chemicals with antioxidant activity produced in human body include

uric acid, high density lipo-protein (HDL), and amino acid such as arginine (Bhattarai B., 2009

There are certain conditions like poor diet, cigarette smoking, environmental pollutants, and exposure to ultraviolet radiations which can lead to increased free radicals in the body leading to a condition known as oxidative stress. Due to high concentration of free radicals certain diseases like arthritis, sinusitis, vision problems, may occur. It has been seen that high antioxidants intake prevents the risk of various cancer of stomach, prostate, colon bladder, and breast throughout person's life. Antioxidants, if taken properly also diminish the effects of aging. It plays a role in minimizing wrinkling of skin and loss of the muscles elasticity. Antioxidant also prevents memory failure and reduction in immunity (Timalsina A.2007, a class seminar on antioxidants and their role in human health p.7)

Antioxidants combination can be safely used during pregnancy or when breast feeding. Antioxidants normally are not given with empty stomach to any person. If given empty stomach, it can cause milk nausea or may upset stomach. Antioxidants may be available in tablet form, soft gel form, or capsule form. New researchers have shown that purple berry due to their dark color is rich source of antioxidants. Moreover it is advantageous that the food high in antioxidants does not taste bed. So, the best preventive medicine is right in front of our eyes in the form of fruits and vegetables containing antioxidants (www. Diabetes Mellitus-information.com).

2.7.1. Disadvantages of antioxidants

2.7.7.1 The antioxidant paradox

There is a growing interest in antioxidants, both as nutritional supplement and as adjunct therapy to cure degenerative diseases. Antioxidants contribute an important defence system against a variety of diseases and environmental stress. People who consume diet rich in fruits and vegetables are expected to live a disease free healthy life, because they they consume significant amount of polyphenolic antioxidants present in fruits and vegetables. Many intracellular antioxidants such as ascorbic acid, α-tocopherol becomes reduced when cells are attacked by diseases. Surprisingly, supplementation of these naturalmantioxidants to the body seldom fills up the deficiencies and cures the disease. Much clinical trial with Vitamin E was

not successful indicationg that antioxidants may not always be beneficial even if circumstantial evidence shows its deficiency giving to Antioxidant Paradox.

The paradoxical role of antioxidants is directly related to recently described Redox Signaling of the antioxidants. The functionalrole of many antioxidants depends on redox cycling. For example, the best described intracellular Vitamin E supplementation in the face of infracted myocardium exerted pro-oxidant effects resulting in the ropture of plaques. The same was true for Vitamin C which also can induce in the cells at the earlier stages of the diseases. When a cell is attacked by environmental stress, the cell's defence is lowered because of massive generation of the reactive oxygen species. The cell immediately responds to the stress by upregulating its antioxidants defence that includes Mn-SOD, GSHPx-1, HO-1, and many other inducible proteins such as Bcl-2. During the induction process, reactive oxygen species function as signaling molecules. It should be easily understood that in these pathophysiological conditions, even though the antioxidants are lowered and supplementation of the antioxidants are warrented, the antioxidants should be harmful because they will prevent the function of the reactive oxygen species to perform signal transduction to induce intracellular antioxidants again leading to the term Antioxidant Paradox. In summary, even though antioxidants are essential nutrients for thr body, and the consumption of the antioxidants should be beneficial in many casas, their use may also by harmful if used wrongly. Antioxidant therapy should be undertaken as commonly done for other drug therapy after careful evaluation of the disease process and only if such therapy is warranted. (Maulik N. and Das D.K.,2003)

2.7.7.2 Adverse effects

Relatively strong reducing acids can have anti-nutritional effects by binding to dietary minerals in the gastrointestinal tract and preventing them from being absorbed. Notable examples are oxalic acid and phytic acid, which are high in plant-based diets. Some tannin also has this negative characteristic. Calcium and iron deficiencies are not uncommon in mideastern diets where there is high consumption of phytic acid present in beans and unleavened bread (http://en.wikipedia.org/wiki/Antioxidant).

Other extremely powerful nonpolar antioxidants such as eugenol also happen to have toxicity limits that can easily be exceeded with the misuse of essential oils.

While antioxidants supplementation is widely hypothesized to prevent the development of cancer, antioxidants may, paradoxically, interfere with cancer treatments. One explanation for this effect is that the growth-promoting environment of cancer cells leads to high levels of redox stress under baseline conditions, and this makes cancer cells more susceptible than normal cells to the further stress of chemotherapy or radiation therapy. So by reducing the redox stress in cancer cells, antioxidant supplements could decrease the effectiveness of the therapy designed to kill them (Schumacker P., 2006)

2.8. Importance of peroxide value and acid value in assessing food quality and food safety

Fats and oils in foods oxidized during processing, circulation and preservation. This reaction causes deterioration in taste, flavor, odor, color, texture and appearance, and a decrease in the nutritional value of foods (Frankel E.N., lipid oxidation. The oily Dundee.1988). Furthermore, the reaction can induce food poisoning. Therefore, from a food quality and food safety perspective, this oxidation reaction must be suppressed. Instant noodles are a fried food, and therefore instant noodles content a lot of fat and oils. In 1964 and 1965, Japan had a food poisoning epidemic caused by the degradation of the fat and oil in instant noodles (Inagaki, N., 1966). Many people who ate the degraded instant noodles develop acute symptoms such as diarrhea, nausea, abdominal pain, fatigue, and headache, but fortunately, no one die. After the incidence the ministry of health and welfare, currently the ministry, Labor and welfare, in Japan set standards for instant noodles in the food sanitation Law to protect against food poisoning and to control the quality of instant noodles. In that law, peroxide value (PV) and acid value (AV) were chosen as useful indices to control food safety and quality, and the standard values of PV and AV were set at no more than 30 mequiv/kg and 3 respectively. These values were chosen because they indicate stage of fat and oil deterioration. After setting these values, there has been no reported cases food poisoning caused by instant noodles in Japan.

At the initial stage of fat and oil deterioration, the reasons for measuring PV and AV are very different because of the different mechanism underlying the formation of hydroperoxides

and FFA from fat and oil. Hydroperoxides is formed by the oxidation of fats and oils. Whereas FFA is formed by the hydrolysis of fat and oil. PV is an index to quantity the amount of hydroperoxides in fat and oil. Several studies have reported that secondary oxidized oil product is generally toxic. (Artman.N.R. 1969) Also, weakly oxidized fats and oil at levels of only 100 mequiv/kg of PV are neurotoxic.Therefore, the formation of hydroperoxides, the primary oxidized product of fat and oil, must be suppressed to protect against the oxidation of fat and oil and the formation of secondary oxidized products from both food quality and food safety perspectives. Meanwhile, AV is an index to measure the amount of FFA. The FFA themselves are not toxic; however, the presence of FFA affects food quality. Consequently, measuring both indices is indispensable to control food quality and safely.

There is currently a movement worldwide to use only AV to control food quality and safety. For example, the 36th session of the Codex on Food Additives and Contaminants help at Rotterdam. The Netherlands, in 2004 expressed the opinion that PV is not safety factor As a result; the 28th session of the Codex Committee on Method of Analysis and Sampling held at Budapest, Hungary, in 2005 determined that only AV is the useful as an index of fat and oil deterioration to control the quality and safety of instant noodles. PV was not recommended as an index in this standard (ALINIRM 05/28/23, 2005,) this is very dangerous, because there is evidence that the oxidized products of fat and oil formed from deteriorated fat and oil are the real cause of food toxicity(Artman.N.R.1969). Furthermore, it is impossible to predict the magnitude of the PV from the AV because the underlying mechanisms of formation are completely different.

During the storage, many kinds of reactions, such as oxidation, hydrolysis, polymerization, cyclization , and β-scission can occur in the fats and oils. It is very difficult to determine how the individual reaction interacts to form toxic compounds. Almost all of these reactions, however, relate to oxidation and proceed *via* the formation of lipid hydroperoxides. Consequently, protecting against the formation of lipid hydroperoxides is the best way to maintain food safety and quality. In the Food sanitation Law of Japan, PV is set to no more than 30 mequiv/kg because deteriorate instant noodles with a PV as low as 100 mequiv/kg have caused food poisoning in Japan (Inagaki, N.,1966). A PV value of 100 mequiv./kg might not be very high, but animal studies reveal that this level of deteriorated fat and oil is

neurotoxic. During the oxidation of fat and oil, sudden oxidation is the propagation period, occurs after the induction period. More the antioxidants are consumed during the induction period. Although 30 mequiv/kg is much less than 100 mequiv/kg. once the sudden oxidation starts during the propagation period the 100 mequiv/kg level would be reached soon after the 30mequiv/kg level. Furthermore, this initial stage of PV alteration cannot be estimated by changes in AV because the two, indices do not increase simultaneously. Consequently, setting a criterion of 30meqiuv/kg for PV in instant noodles is important to control food safety.

It is not sufficient to monitor food deterioration with AV alone to maintain food quality and food safety. We conclude that PV must be adapted as an index in the Codex standard for instant noodles.

2.9. Relationship between PV and AV in Instant Noodle

The concept for measuring PV and AV are completely different. It is now accepted that the secondary oil oxidized products such as polymerized oil, cyclic fatty acid, hydroperoxy alkenal and hydroxyl alkenal are main cause of toxicity in oxidized oil. Therefore, the formation of lipid hydroperoxide, the primary oil oxidized product, must be suppressed to prevent the formation of the secondary oil oxidized products in Instant Noodle. In Japan, PV is hired to monitor the formation of the primary oil oxidized product, namely lipid hydroperoxid. On the contrary, AV is measured to keep the food quality. During the food processing and storage, free fatty acids are formed in the noodle by the hydrolysis of the oil. Free fatty acid itself is not a very toxic compound; however, it becomes a cause of the reduction of flavor and taste. The purpose of measuring AV is to check the free fatty acid level in Instant Noodle (F.M *et.al.,* 1951)

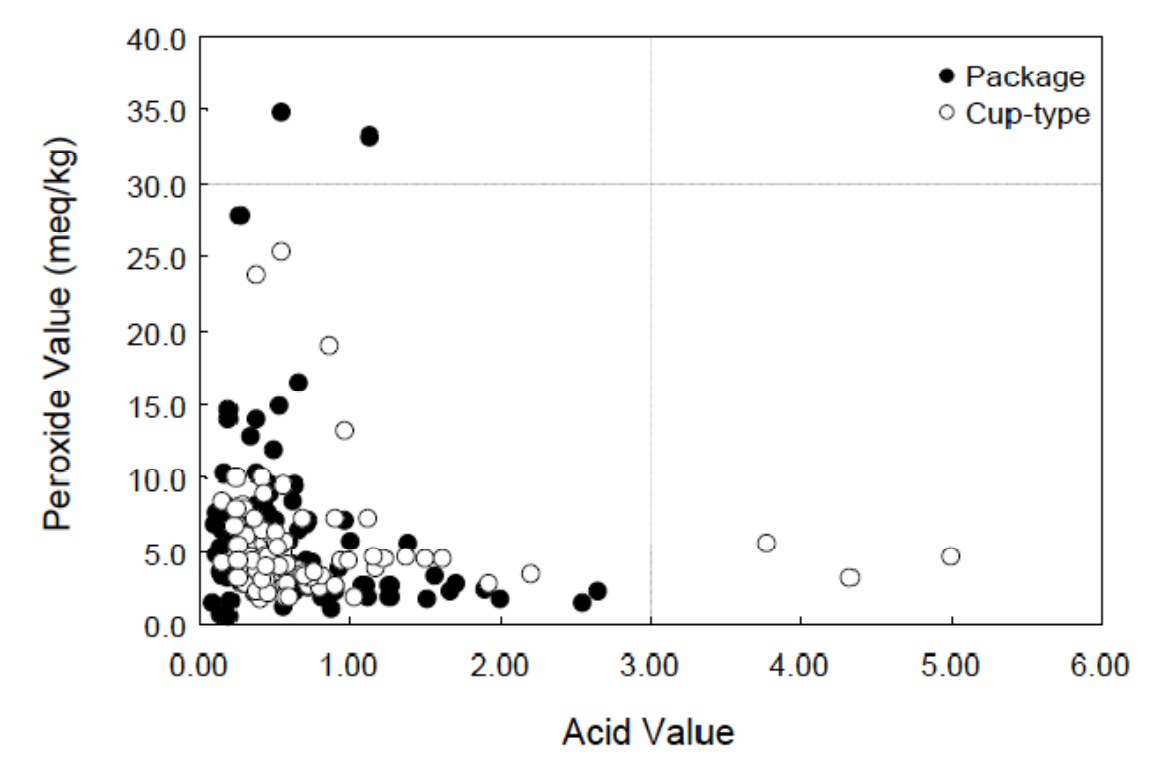

Fig. 2.7 Acid value (A.V.) as Peroxide value (P.V) in 218 instant noodles collected from all over the world

Japan proposed the food standard of Instant Noodle that contains PV and AV to the Codex Regional Coordinating Committee for Asia to make it international standard. However, several representatives of Asian countries did not accept the proposal from Japan. Particularly, including PV in the standard was opposed because they recognized that the PV and AV would increase together during the deterioration of Instant Noodle and measuring AV is enough to keep the food safety and quality. As mentioned above, the concepts for measuring PV and AV are completely different and PV is an essential item to keep the food safety. Consequently, 218 kinds of fried type Instant Noodles were collected from commercial base of all over the world and measured PV and AV of them to grasp the deteriorated situation of Instant Noodles sold in the market. Furthermore, the relationship between PV and AV values was investigated to confirm the truth of other countries opinions. All the measured values on PV and AV are plotted in Figure 1. These results show that the both values are spread to wide range and some of them exceed the criteria (PV: 30 meq/kg and AV: 3) established in Food Sanitation Law in

Japan. Since almost all samples were sold in cool condition, the samples exceeding 30 in PV might be exhibited under strong light for a long period. On the other hand, the samples exceeding 3 in AV might be stored under high humidity. Light and moisture strongly affect the degradation of oils, made a deteriorated Instant Noodle, which is as same as deteriorated Instant Noodle caused food poisoning in 1964, with sunlight and high temperature and succeeded in reappearing the food poisoning with the sample. Consequently, it would be said that cutting light or sun light is the most important way to preserve Instant Noodle even the material of the package film can suppress the UV and water transmission. In Figure 8, if the both PV and AV increase simultaneously during storage, the approximating curve against these plots must become ever-increasing curve. However, the plots are not scattered like that. The coefficient of correlation for PV and AV was calculated with Pearson's product-moment coefficient of correlation and the result was -0.1083. This value means that the plots are scattered in the downward-sloping and the correlation between PV and AV is poor because the coefficient of correlation is lower than zero and the absolute value is near zero. Consequently, the coefficient of correlation reveals that PV and AV do not form simultaneously in the oil of Instant Noodle during the deterioration. Furthermore, the *P* value was also calculated and the value was 0.1106. This value also explains that the relation between PV and AV is not significant because the value was bigger than 0.05. Therefore, analyzing only AV cannot grasp any deteriorated situation of the oil in Instant Noodle and analyzing both PV and AV has a strong and significant meaning. We conclude that PV is also an indispensable factor to keep the food safety and quality of Instant Noodle (F.M *et.al.,* 1951).

2.10. Increase PV in the oil of Instant Noodle

A great number of studies concerning the oxidation or heating of the oil have been carried out so far. These studies are mainly separated to three types of studies. (1) the most popular study is that the oil is heated at more than 250 ^{0}C under oxygen omitted circumstances such as under nitrogen, carbon dioxide, etc. This kind of heating forms polymerized oil and cyclic fatty acid without containing oxygen molecular in the structure. These compounds are very toxic; however, these compounds are not oxidized compound. Furthermore, it must be said that these study conditions are not realistic. Consequently, these results are not available when the food toxicity of the oil is discussed.

(2) The oxidations of the oil under atmospheric condition are also carried out. In this degradation, the oxidation of the oil proceeds by radical chain reaction via lipid peroxyl radical (Figure 9) Therefore, the compound formed in this reaction contains oxygen molecular in it. These studies are separated to two types of studies. One is heating the oil over 100 ^{0}C and the other is less than that. Taking account of the accumulation of lipid hydroperoxide (PV) in the system, the heating temperature is important point. For instance, the temperature usually hired for deep-fry and stir-fry is around 180°C and the lipid hydroperoxideis decomposed easily under this condition. As the result, the PV of the oil does not increase it and the secondary oil oxidized products are formed instead of that. However, it has been reported that the polymerized oil and cyclic fatty acid are not accumulated much in the system. Frankel et al. measured the cyclic fatty acid level in the oil used at fast foods restaurant and found that 0.1-0.5% of total fatty acid was changed to cyclic fatty acid23). The oxidation heated at less than 100 ^{0}C accumulates lipid hydroperoxide in the system because the rate of the formation of lipid hydroperoxide is faster than the rate of the decomposition of that. Normally, this kind of oxidation is called “autoxidation”. The autoxidation also proceeds under atmospheric condition and accumulates the lipid hydroperoxide (PV) in the system at first (Figure 10). The amount of the lipid hydroperoxide finally reaches to the top, after that, it starts to decrement because the rate of the formation of lipid hydroperoxide becomes slower than the rate of the decomposition of that. The reaching level depends on the kinds of fatty acids consisting of the oil, heating temperature, etc. The decomposed lipid hydroperoxide forms aldehyde, ketone, alcohol, alkane, etc. It is now accepted that the hydroxyl alkenal and hydroperoxyl alkenal formed in autoxidation is very strong toxic compounds. Therefore, the autoxidized oils are also toxic (Inagaki, N., 1966).

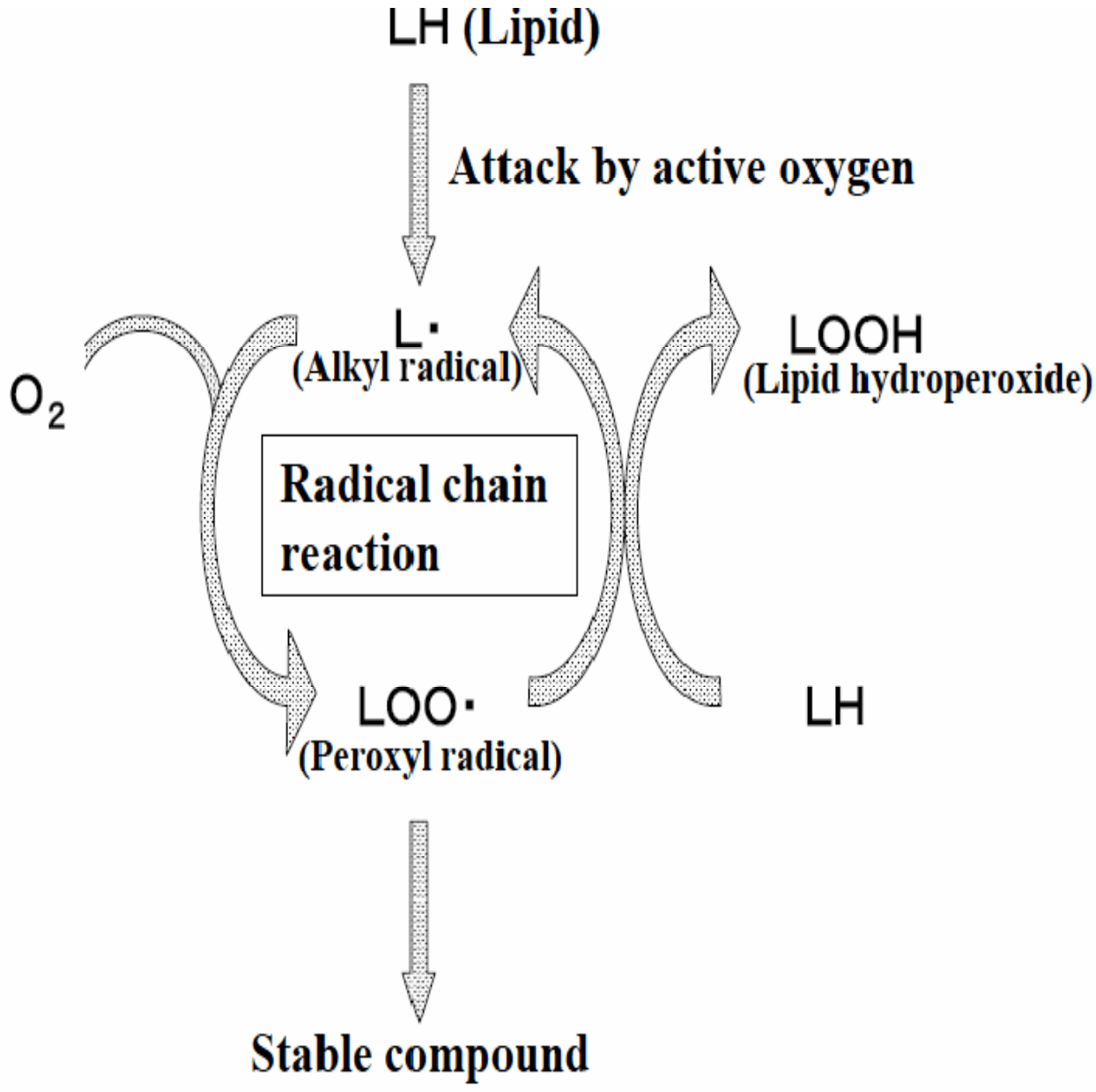

Fig. 2.8 Radical chain reaction on lipid oxidation.

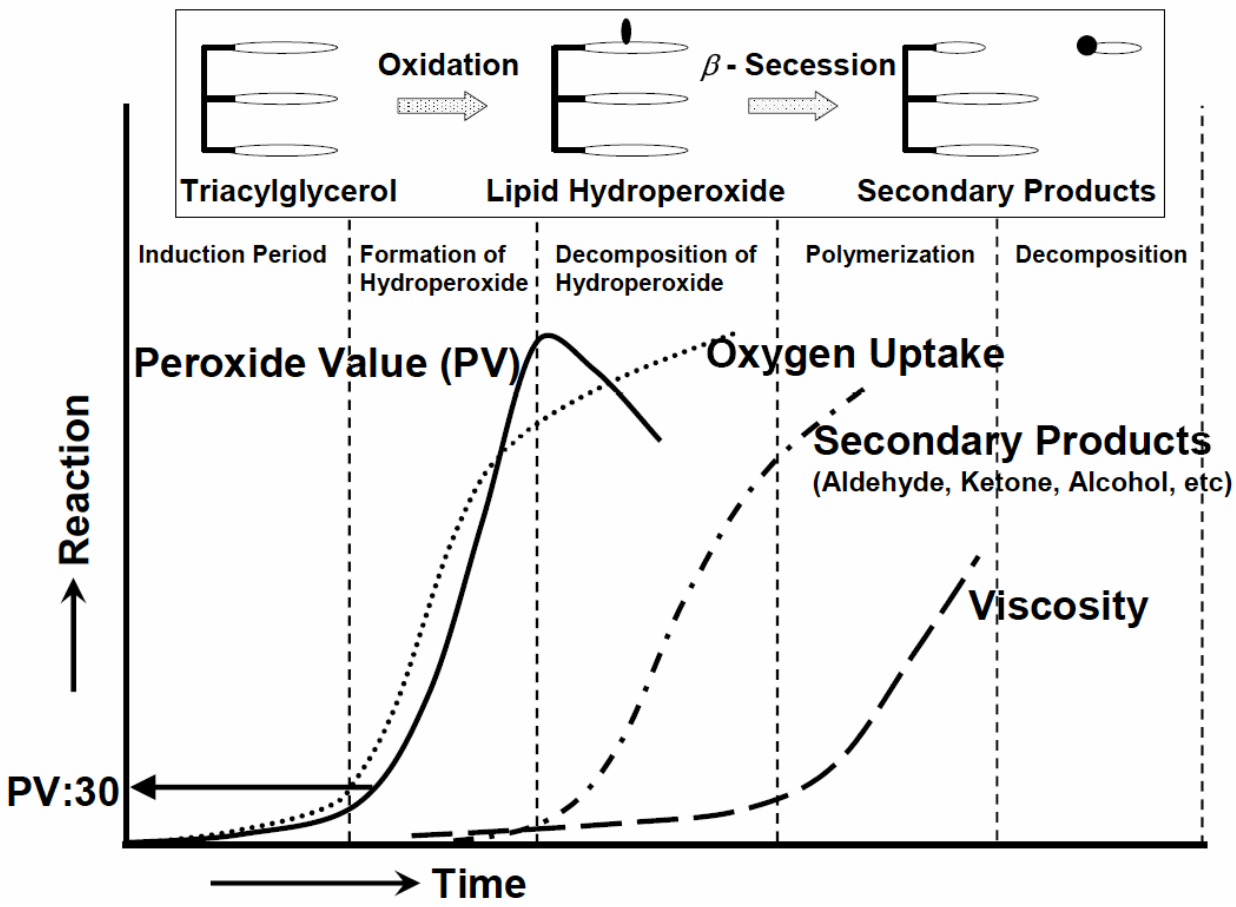

Fig. 2.9 Each stage of autoxidation of the lipids.

(3) The oil degraded by heat and light is also a big problem. This degradation is mixture of autoxidation and photoxidation. Photoxidation is not radical reaction, but ene reaction. Therefore, the reaction mechanism is different from radical chain reaction to form the different kinds of degraded oil compounds. The oxidation of the oil in Instant Noodle that caused the food toxicity was developed by these reactions as already mentioned in previous section. It is very difficult to understand how the individual reaction intertwine with each other and finally cause the toxic compounds in this oxidation (Inagaki, N., 1966).

Almost all of the oxidation reactions proceed via formation of lipid hydroperoxides. Therefore, to prevent the formation of lipid hydroperoxides is the best way to keep the food safety and quality. The oil in Noodle is also no exception. In fact there are a few studies which measured how increased PV during the degradation of the oil. Therefore, oxidation of the

Instant Noodle was carried out to grasp how increase PV in Instant Noodle. As the results, PV increases slowly till the PV reaches to 50 meq/kg, however, after beyond the 50 meq/kg26), explosive increment of PV started in the case of the Instant Noodle is Stored at 60 ^{0}C. In Food Sanitation Law of Japan, PV is set in 30 meq/kg or less than that. This would not be a high value. The toxicity of lipid hydroperoxide was investigated by Tovar et al. and the LD50 was 12,760 mg/kg mice. On the contrary, LD50 of 4-hydroperoxy-2-nonenal, strong toxic compound formed in oil deterioration, is 77.5 mg/kg mice14). Though the deteriorated oil in Instant Noodle which PV is 30 meq/kg does not show any toxicity, the concept of the setting PV in 30 meq/kg is to prevent the oxidation to proceed to the next stage reaction via lipid hydroperoxide and the formation of toxic compounds. Therefore, setting PV in a low value possesses great significance to obtain the quality and safety. Particularly, the low standard value has a great meaning in the case of the oxidation pathway is complicated like the mixture of autoxidation and photoxidation (Inagaki, N., 1966)

Part III

Material and Method

3.1 Material

3.1.1 Cut cake (noodles)

Cut cake (60-62gm) was taken as frying sample from Yoxian Machinary Plant (YM Plant) from CG Foods (Nepal) Pvt. Ltd., Nawalparasi.

3.1.2 Palm oil

Refined Bleached Deodorized (RBD) palm oil of Acid Value (AV) and Peroxide Value (PV) less than 0.3 and 3 respectively was selected for frying purpose, obtained from CG Foods (Nepal) Pvt. Ltd. (which was supplied by Shree Shiva Shakti Ghee Udhyog Pvt. Ltd.

3.1.3 Synthetic Antioxidants

a. Tertiary butylated hydroxyl quinine (TBHQ): TBHQ of laboratory grade was used.

b. Butylated hydroxyl anisole (BHA): BHA of laboratory grade was used.

c. Butylated hydroxyl toulene (BHT): BHT of laboratory grade was used.

3.1.4 Sample plan and coding

The sample for frying was taken fresh from YM Plant. Four cut cakes were fired in every hour then frying oil analyzed for its Acid Value, Peroxide Value, Saponification Value and Specific Gravity. The proportion of different antioxidants like TBHQ, BHA, and BHT in each case was varied as 100ppm, 150ppm and 200ppm. Oil, using no antioxidants for frying was taken as control sample and analyzed.

The fried sample was coded as $TBHQ_n$, BHT_n and BHA_n

n stands for 100ppm, 150ppm and 200ppm.

In similar manner control sample used only palm oil.

3.2 Methods (Analytical)

3.2.1 Estimated of Peroxide Value

The frying oil was analyzed for Peroxide Value at interval of one hour followed the procedure by AOAC, 2005 (41.1.16).

3.2.2 Estimation of Acid Value

Acid Value of frying oil was determined by titration method according to AOAC, 2005 official method (41.1.23)

3.2.3 Estimation of Iodine Value

Iodine Value of RBD palm oil was determined by Wij's method and the procedure followed was same as described by AOAC, 2005 official method (41.1.15).

3.2.4 Estimation of specific gravity

Specific gravity of palm oil was determined by pycnometer followed by AOAC, 2005 (41.1.05)

3.2.5 Estimation of moisture content

Moisture content in RBD palm oil was determined by Hot air oven method (Rangana, 2008)

3.2.6 Estimation of Saponification Value

The Saponification value of frying oil was determined by the procedure of Rangana, 2008 .

3.2.7 Estimation of Unsaponofiable matter

The Unsaponofiable matter of RBD palm oil sample was estimated according to Rangana, 2008.

3.2.8 Data Analysis

Data analysis was done by SigmaXL, a statistical add in for excel 2007.

Methodology:

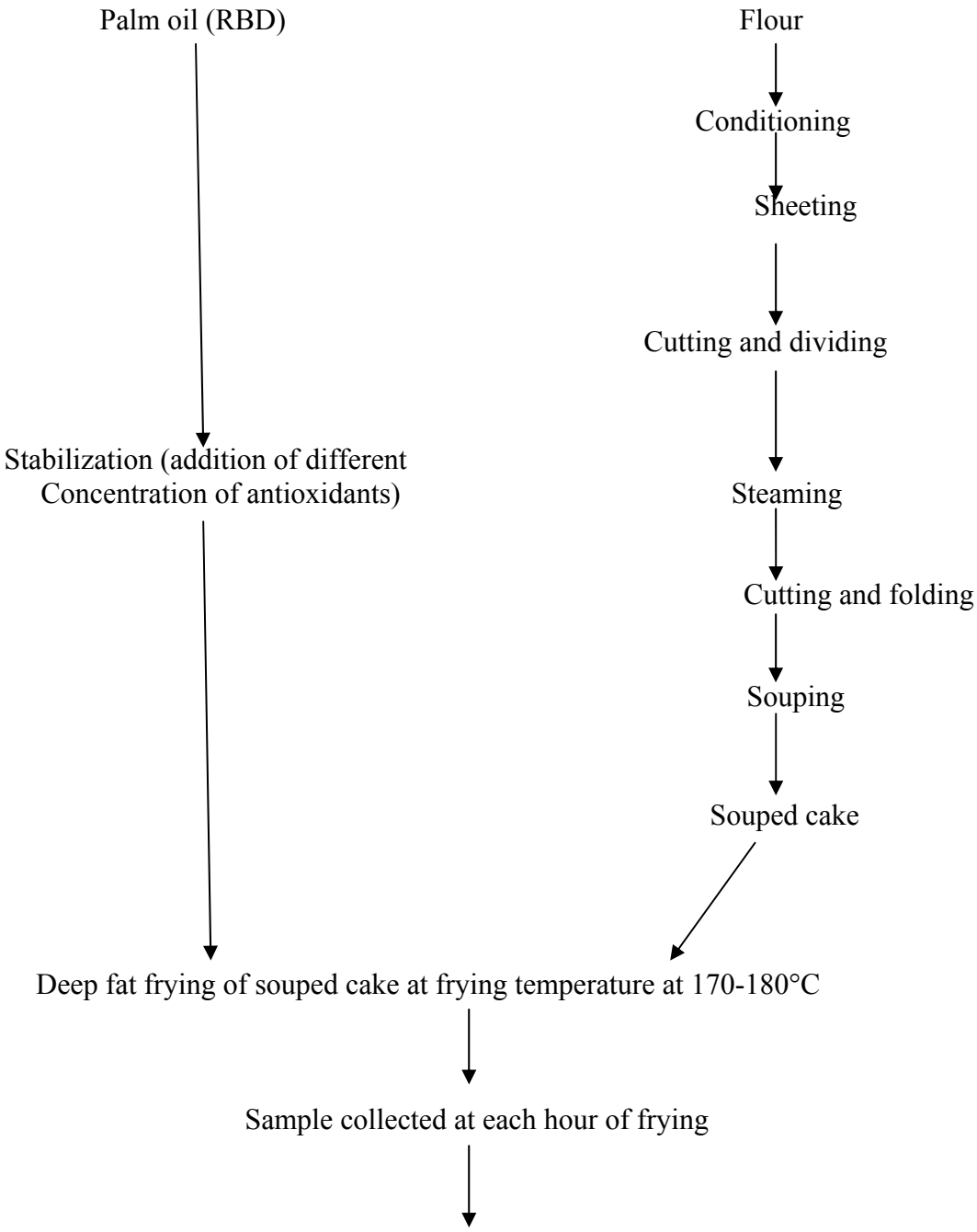

Fig 3.1 Flow chart of frying process

Part IV

Results and Discussion

4.1 Effect of different concentration of TBHQ on Acid Value

The acid values measured at regular interval of time (1 hr) of different concentration of TBHQ are shown in figure 4.1 listed in Appendix 8 (table 1)

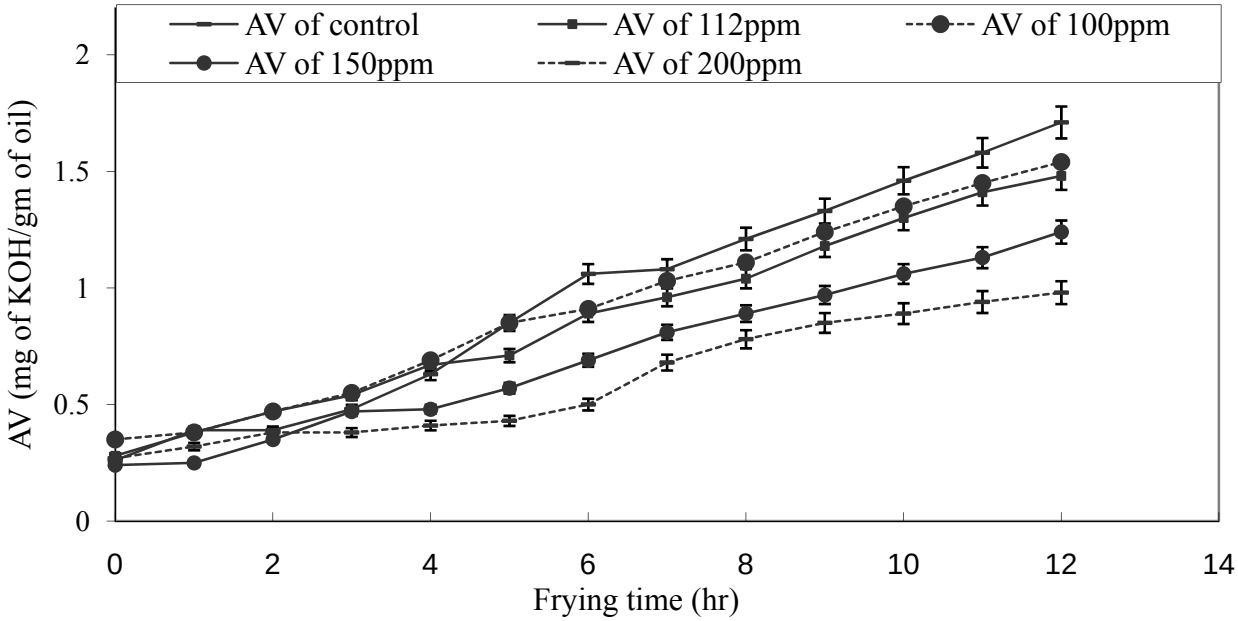

Fig 4.1 Change in acid value with respect to frying time (hr) of oil using different TBHQ concentration

In figure 4.1, it was found that acid value (AV) increased with increased in frying time whether there was used of TBHQ or not and found significance difference. There was inversely proportion between acid value and antioxidants proportion (upto 200ppm). NS recommends the AV of oil upto 1mg of KOH/gm of oil; it was found that TBHQ of 200ppm was more stable for rise in AV which extended the stability of oil upto 12hr while that of control (without using TBHQ) extended upto 6hr but the correlation coefficient(r) became decreased while increase in concentration of TBHQ upto 200ppm i.e. 0.99750, 0.99628 and 0.97518 of 100ppm, 150ppm and 200ppm respectively. All the concentration are statistically significance at 95% of confidence level where r □ 6PE (probability error) refers Appendix 1 (table 1)

4.2 Effect of different concentration of BHA on Acid Value

The acid values measured at regular interval of time (1 hr) of using 100ppm, 150ppm and 200ppm of BHA are listed in Appendix 8 (table 2) and represented graphically in figure 4.2

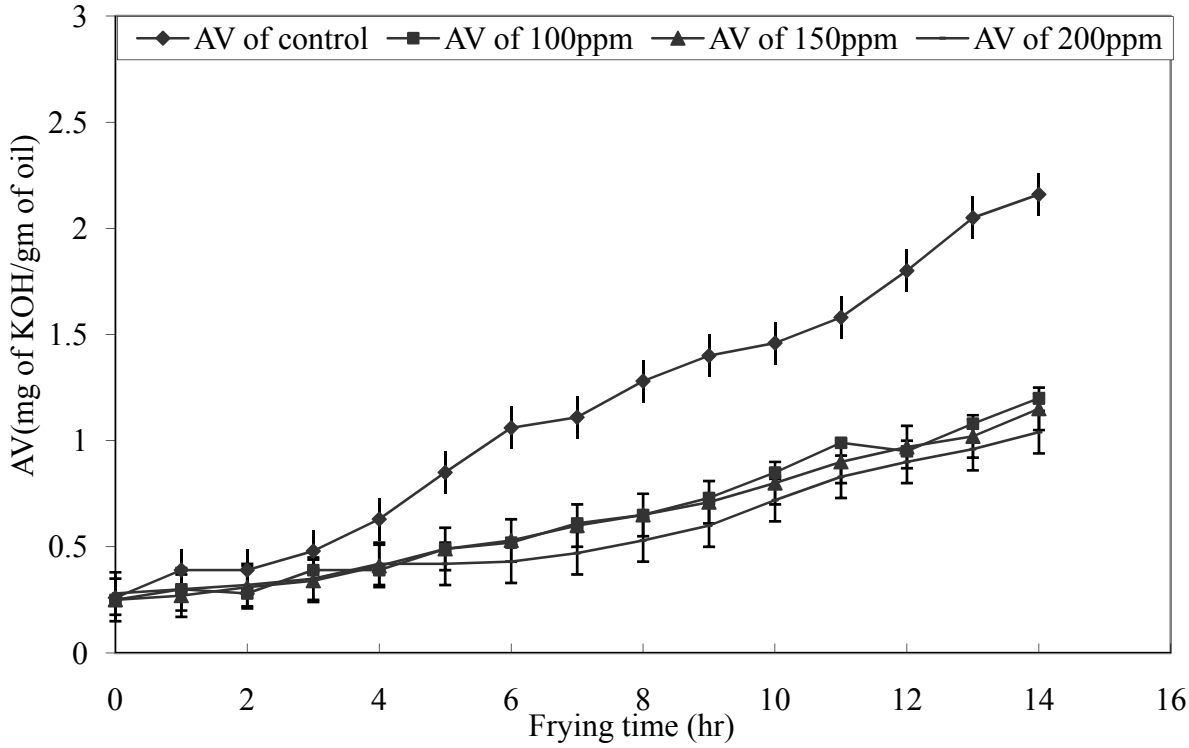

Fig 4.2 Change in acid value with respect to frying time (hr) of oil on different concentration of BHA

While using BHA of different concentration it was found that decrease in AV with increase in concentration of BHA up to 200ppm. The concentration of 200ppm shows more stable than others concentration it increased the stability of frying oil up to 14hr while that of control (without using BHA) was 6hr and which was 2hr more that of TBHQ (using 200 ppm) but the correlation becomes decrease with increase in concentration up to 200ppm, it shows the strong positive correlation on 150ppm concentration of BHA, correlation coefficient was found to be 0.98533, 0.99163 and 0.96483 of 100ppm, 150ppm and 200ppm respectively. All concentration are positively statistical significance at 95% level of confidence where r>6PE refers to appendix 1 of correlation table 5

4.3 Effect of different concentration of BHT on Acid value

The acid value measured at regular interval of time (1 hr) using 100, 150 and 200ppm of BHT are listed Appendix 8(table 3) and represented graphically in figure 4.3

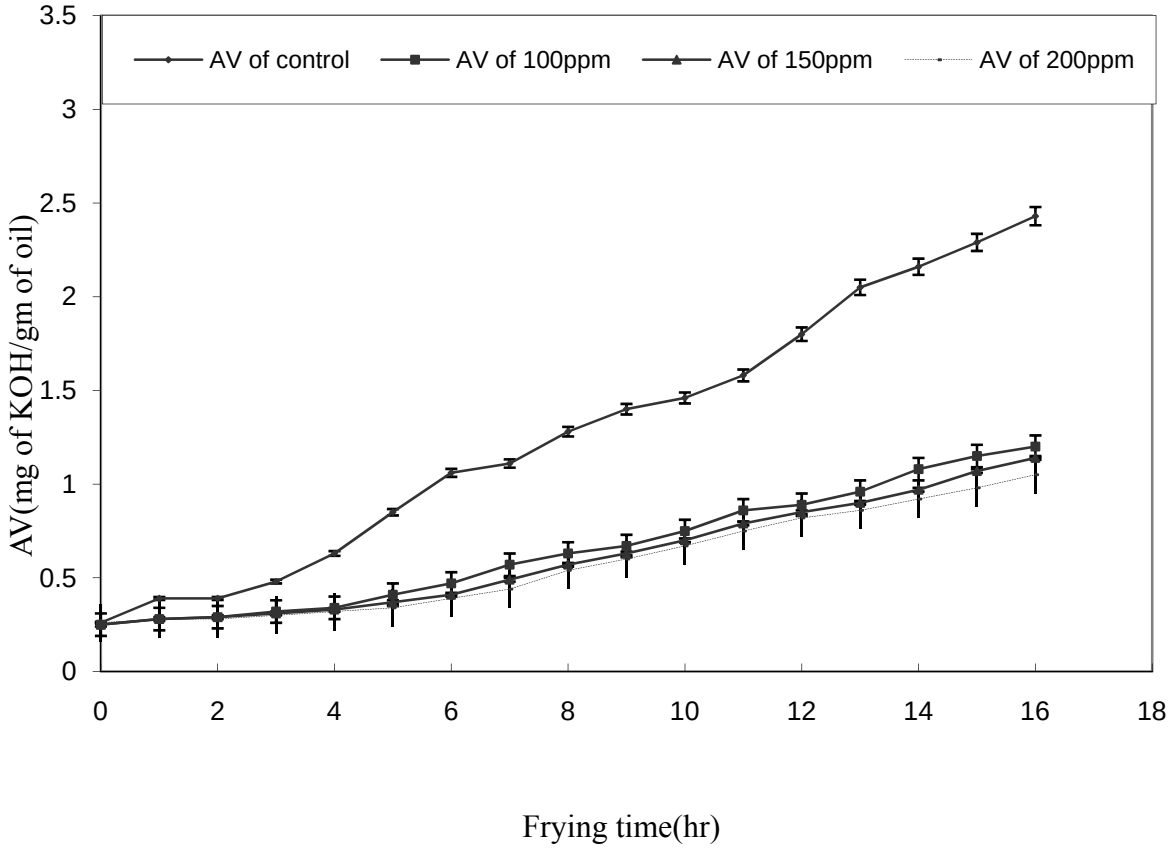

Fig 4.3 change in acid value with respect to frying time (hr) of **oil** on different concentration of BHT

It was found that AV reached to its limit within 16hr while using BHT of 200ppm and that of control (without using BHT) reached within 6hr.

BHT was more significance than TBHQ and BHA on the basis of AV. It increases the AV around 1mg of KOH/gm of oil (Nepal Standard) within 16hr while 200ppm concentration BHT was used. And also same result obtained as above mention two antioxidants but 100ppm BHT was positively strong significance than 150 and 200ppm having correlation coefficient 0.98518, 0.97619 and 0.96641 respectively where r> 6PE is for all cases refer to Appendix 1 correlation table 9. It also found that by increased antioxidants concentration increased the

stability time of frying oil, it is strongly support from the slope table refers to Appendix 2 of table 1, 2 and 3

In any frying situation there is obvious rise in acid value with increase in frying time. Although there is no any supporting literature regarding the correlation between release of free fatty acid and concentration of antioxidants which was evident during dissertation work and found strong positive relation for control and weak positive correlation for the oil of 200ppm. Free fatty acid itself is not a very toxic compound; however it impairs flavor and taste of itself and the product. The purpose of measuring AV is not significant in aspect of public health but it is regarded as the quality parameter of oil. Here result obtained shows the positive correlation between the decreases in rise in AV against increase in antioxidants concentration.

4.4 Effect of different concentration of TBHQ on Peroxide Value

The Peroxide value measured at regular interval of time (1 hr) using TBHQ of 112ppm, 100ppm, 150ppm and 200ppm different are listed in Appendix 8(table 4.) and represented graphically in figure 4.4

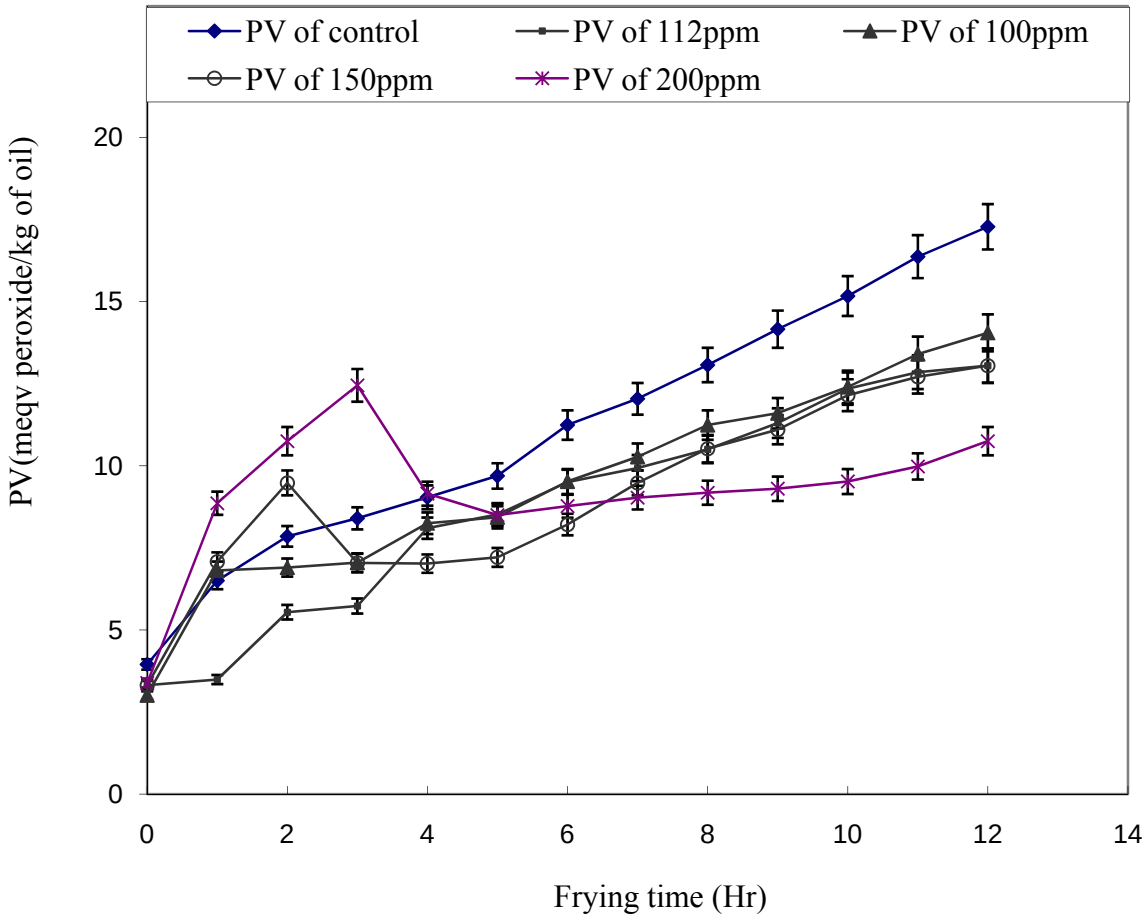

Fig 4.4 Change in peroxide value with respect to frying time (hr) of oil using different TBHQ concentration

As shown in graph above, it was found that rise in peroxide value (PV) became decrease with increase in TBHQ concentration. PV increases abruptly at first hour of heating and reaches its maximum peak on 200ppm concentration then decrease down. NS recommended the PV of fat and oil upto 10mequiv of peroxide/kg of oil. It increases the stability of oil on the basis of PV up to 11hr while that of control reaches to its standard limit within 5 hr. But 150ppm TBHQ is statistically significance than 112, 100 and 200ppm and correlation coefficient was found to be 0.90723, 0.095214, 0.97246 and 0.86014 that of 112, 100, 150 and 200ppm respectively where r>6PE refers to Appendix 1 of correlation table 2

It obviously says that the PV of frying oil increases with increase in frying time. It can also be said that while using antioxidants suppress the PV of frying oil. PV is an index to quantity of hydro peroxide in fats and oil. The oxygen is the main cause of rise in PV of frying oil. In the degradation, the oxidation of the oil proceeds by the radical free chain reaction via lipid peroxyl radical as shown in figure 9. Therefore, the compound formed in this reaction contains oxygen molecule in it. Heating temperature is the important point. During the deep fat frying around 180°C the lipid peroxide decomposed easily as a result PV of the oil does not increase and the secondary oil oxidized product are formed. During first hour of heating the rate of formation of lipid peroxide is faster than the rate of decomposition. Normally, this kind of oxidation is called 'Autoxidation'. The autoxidation also proceed under atmospheric condition and accumulates the lipid hydroperoxide (PV) in the system at first (which is support by the figure 10 in literature review). The amount of the lipid hydroperoxide finally reaches to the top, after that, it starts to decrement because the rate of the formation of lipid hydroperoxide becomes slower then the rate of the decomposition starts. The decomposition of lipid hydroperoxide forms aldehyde, ketone, alcohol, alkane, etc. The oil also degraded by heat and light so degraded factors are mixture of autoxidation and photoxidation. Increase the antioxidants concentration faster the formation of lipid peroxide and also it reaches at the peak where PV become higher then it slows down the formation after that the rate of decomposition of lipid peroxide is faster and again increases the peroxide with increasing frying time.

4.5 Effect of different concentration of BHA on Peroxide value

The Peroxide value measured at regular interval of time (1 hr) of 100, 150, 200ppm concentration of BHA and control (without using BHA) are listed in Appendix 8 (table 5) and represented graphically in figure 4.5

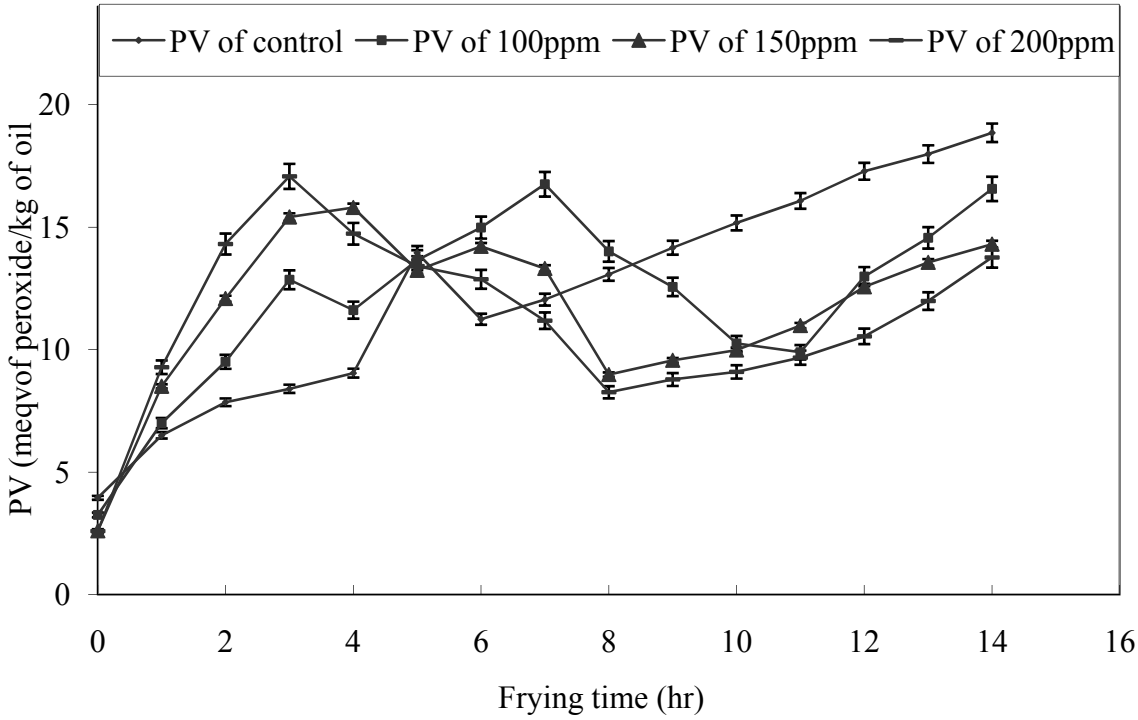

Fig 4.5 Change in peroxide value with respect to frying time (hr) of oil using different BHA concentration

While using BHA of different concentration it did not extend the stability of frying oil in comparison with TBHQ. It also increased the stability upto 11hr which is same as TBHQ but rise in peroxide value become decrease while increase the antioxidant concentration(compare with control) on the basis of statistically analysis there is no statistically significance in any concentration of BHA where r<6PE refer to Appendix 1 of correlation table 6.

4.6 Effect of different concentration of BHT on Peroxide value

The Peroxide value measured at regular interval of time (1 hr) using 100, 150, 200ppm concentration of BHT and control (without using BHT) are listed Appendix 8 (table 6) and represented graphically in figure 4.6

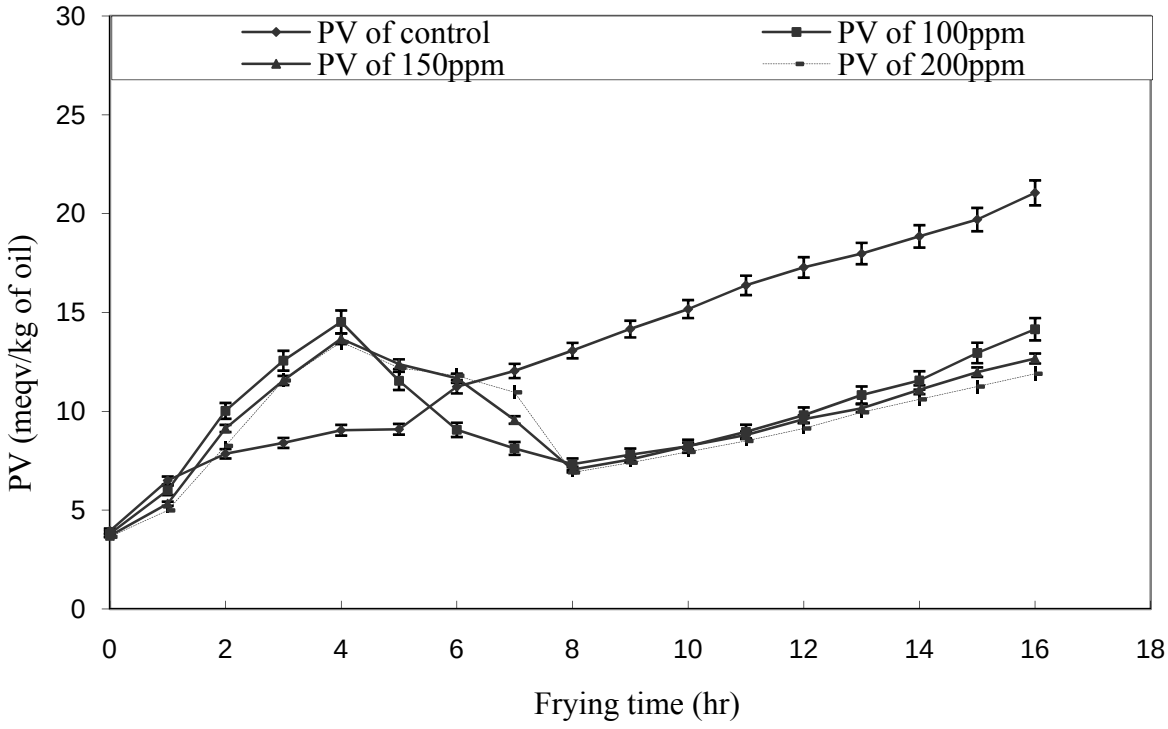

Fig 4.6 Change in peroxide value with respect to frying time (hr) of oil using different concentration of BHT

BHT extends the stability of frying oil upto 13hr which was more significance than TBHQ and BHA. But on the basis of statistically analysis it was not significance where r<6PE . correlation coefficient (r) was found to be 0.44372, 0.40017 and 0.34972 respectively that of 100, 150 and 200ppmand 6PE was found to be 0.81253, 0.84972 and 1.00004 respectively.

TBHQ of 150ppm concentration having correlation coefficient 0.904012 was more significance than that of BHA and BHT. Almost all to the oxidation reactions proceed via formation of lipid peroxides. Therefore, to prevent the formation of lipid hydroperoxide is the best way to keep the food safety and quality. It also shown that the increasing concentration of

antioxidants increasing the stability time of frying oil, it is strongly support from the slope table Appendix 2 of table 4, 5 and 6.

4.7 Effect of different concentration of TBHQ on Specific gravity

The specific gravity measured at regular interval of time (1 hr) of using 112, 100, 150 1nd 200ppm concentration of TBHQ are listed in Appendix 8 (table 7) and represented graphically in figure 4.7

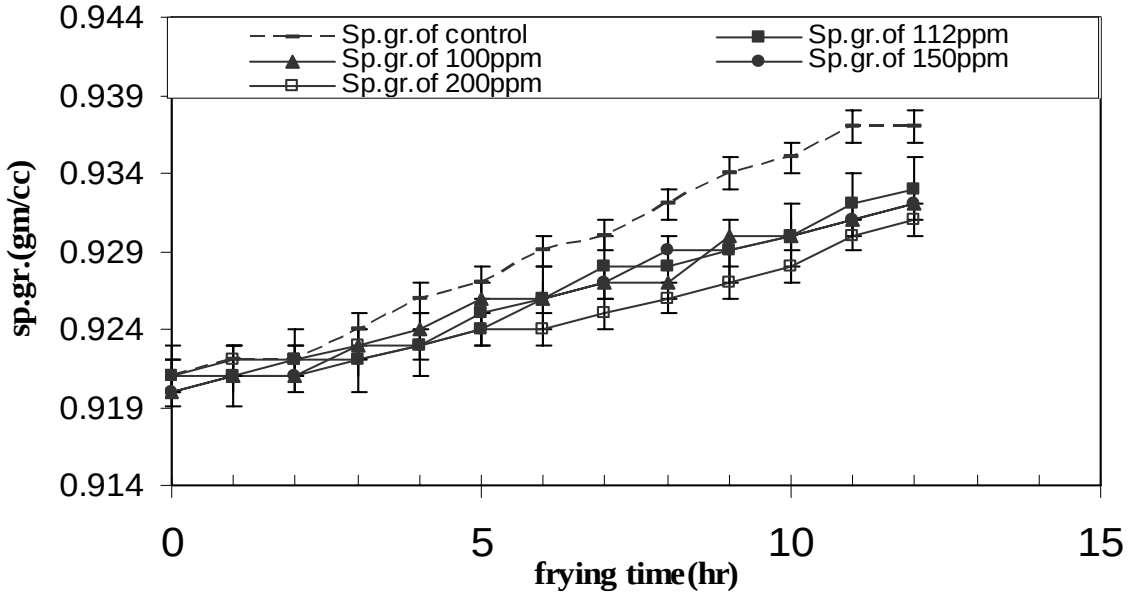

Fig 4. 7 Rise in specific gravity against frying time (hr) using different concentration of BHT

It was found that rise of specific gravity became decrease with increase in TBHQ concentration whether there used of antioxidants or not but it was significantly different. It was found that correlation coefficient (r) 0.98897, 0.99204, 0.99572 and 0.972002 respectively that of 112ppm, 100ppm, 150ppm and 200ppm, which all are greater than 6PE (r□6PE). And 150ppm of TBHQ was found to be strongly significance over other concentration.

4.8 **Effect of different concentration of BHA on Specific gravity**

The specific gravity measured at regular interval of time (1 hr) using BHA of 100, 150 and 200ppm are listed in Appendix8 (table 1) and represented graphically in figure 4.8

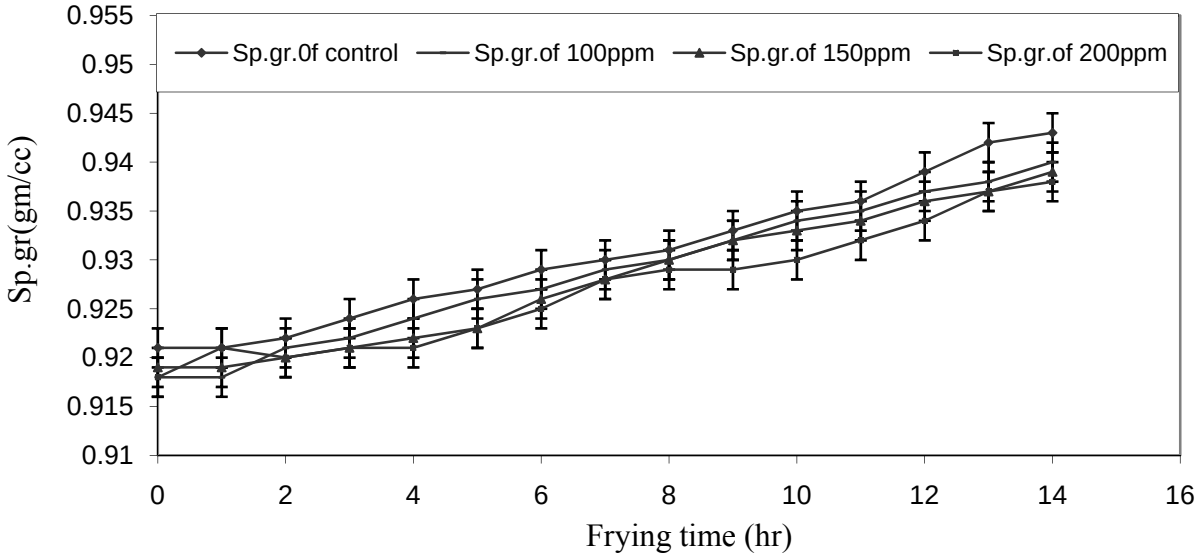

Fig 4. 8 Change in specific gravity with respect to frying time(hr) using different concentration of BHA

It was found that rise in specific gravity became decrease while increase in BHA concentration upto 200ppm. From the correlation coefficient (r) Appendix 2 (table 8), it was found that 150ppm of BHA was found to be statistically significance over 100 and 200ppm of BHA where r □ 6 PE but all concentration are within the limit of r□6PE.

4.9 Effect of different concentration of BHT on Specific gravity

The specific gravity measured at regular interval of time (1 hr) using different concentration of BHT are listed in Appendix 8 (table 9) and represented graphically in figure 4.9

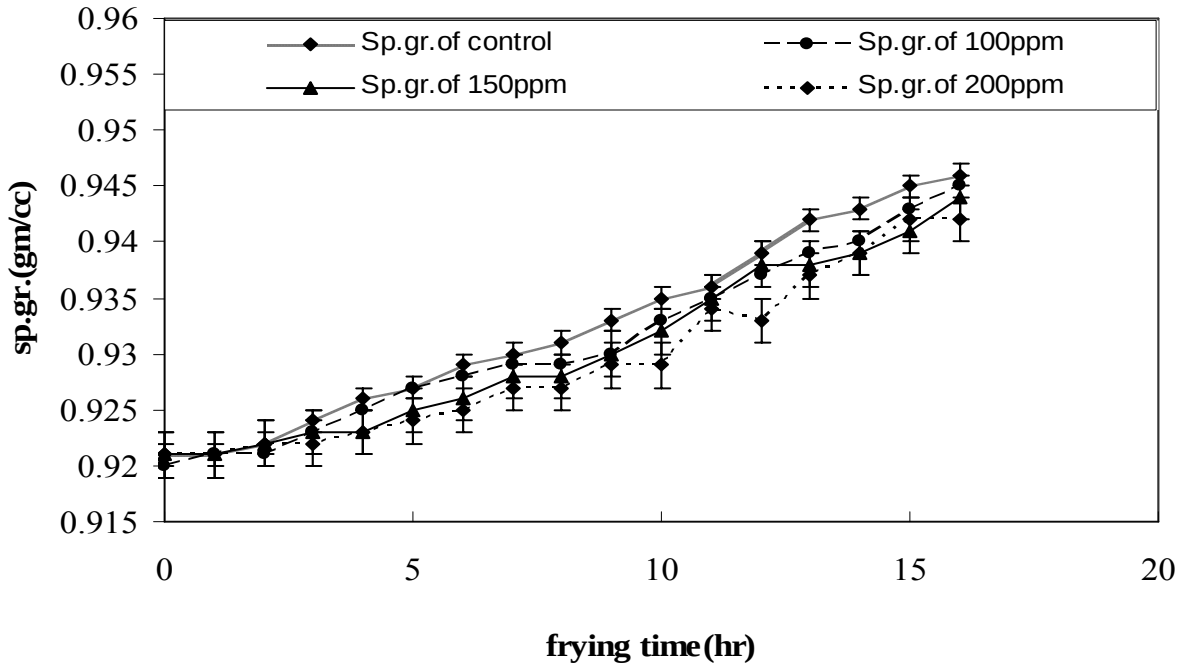

Fig 4.9 Change in specific gravity against frying time (hr) using different concentration of BHT

The correlation coefficient of BHT was found to be 0.97200, 0.98292 and 0.96956 respectively of 100, 150 and 200ppm which statistically significance r>6PE refer to the Appendix 1 of correlation table12 respectively It is well known fact that the specific gravity of oil increase with increase in frying time while frying the noodles, it increases the moisture content in oil which increases the specific gravity of oil. While using the different antioxidants the specific gravity of oil slowly increase with increase in antioxidants concentration. It is found that BHA is more statically significance than TBHQ and BHT.. It also found that 150 ppm of three antioxidants are more significance than 100 and 200ppm which is also shown on the Appedix 1 of correlation table 4, 8 and 12.It also indicates that the increasing concentration of antioxidants increasing the stability time of frying oil, it is strongly support from the slope table refer Appendix 2 of table 7, 8 and 9.

4.10 Effect of different concentration of TBHQ and frying palm oil on Saponification value

The Saponification value measured at regular interval of time (1 hr) of using 112, 100, 150 and 200ppm concentration of TBHQ are listed in Appendix 8 (table10) and represented graphically in figure 4. 10

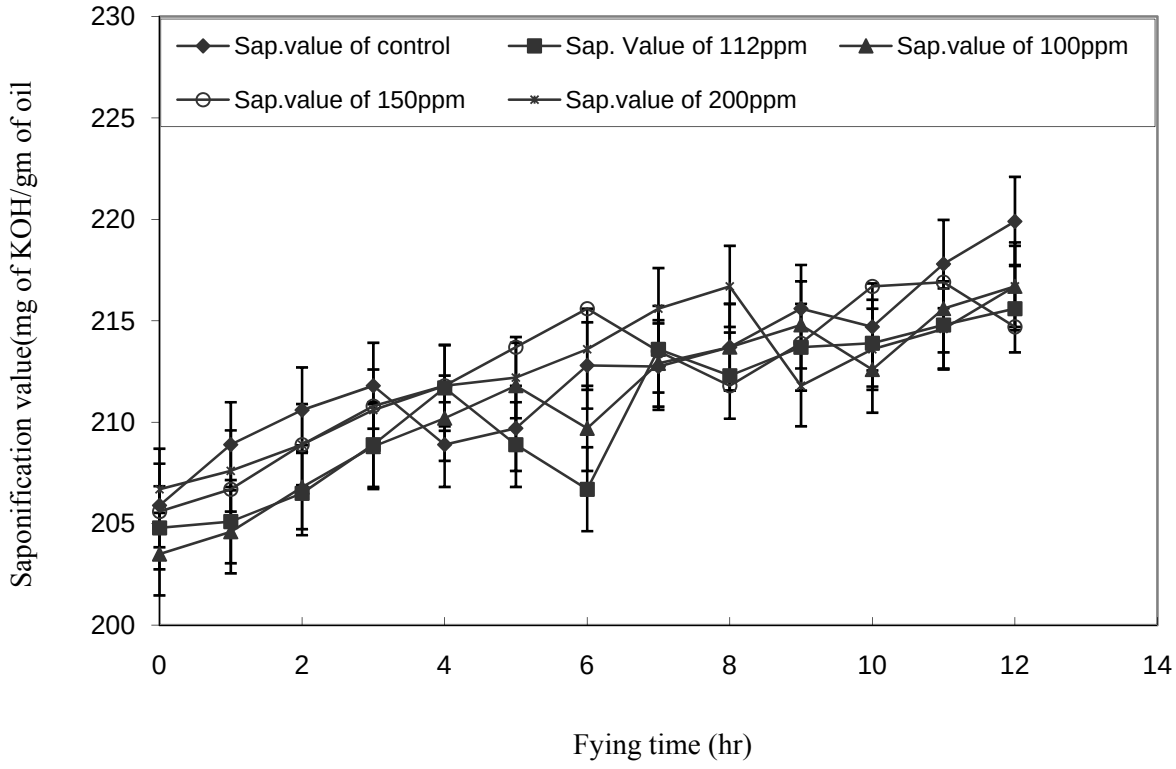

Fig 4. 10 Change in saponification value with respect to frying time (hr) using different concentration of TBHQ

It was found that saponification value became fluctuate with frying time. The correlation coefficient (r) was found to be 0.90723, 0.95213, 0.87246 and 0.86014 that of 112, 100, 150 and 200 ppm respectively where r□6PE for all concentration but found statistically more significance on 100ppm concentration.

4.11 Effect of different concentration of BHA and frying palm oil on Saponification value

The Saponification value measured at regular interval of time (1 hr) using 100, 150 1nd 200ppm concentration of BHA are listed in Appendix 8 (table 110 and represented graphically in figure 4. 11

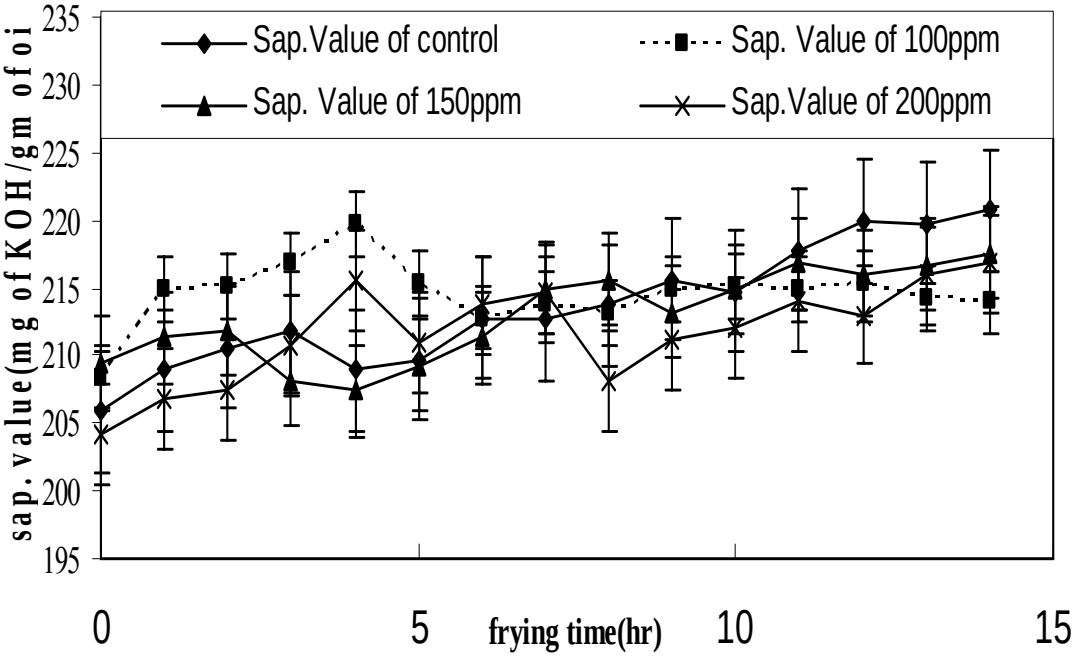

Fig 4. 11 Change in saponification value with respect to frying time (hr) using different concentration of BHA

It was found that saponification value became fluctuate with increase frying time with increase in antioxidant concentration. It was found that 150ppm of BHA was found to be statistical significance over 200ppm where r□6PE. Correlation coefficient (r) was found to be 0.10407, 0.84415 and 0.72665 where r□ 6PE for 100 ppm.

4.12 Effect of different concentration of BHT and frying palm oil on Saponification value

The specific gravity measured at regular interval of time (1 hr) of using 100, 150 and 200ppm concentration of BHT is listed in Appendix 8 (table 12) and represented graphically in figure 4.12

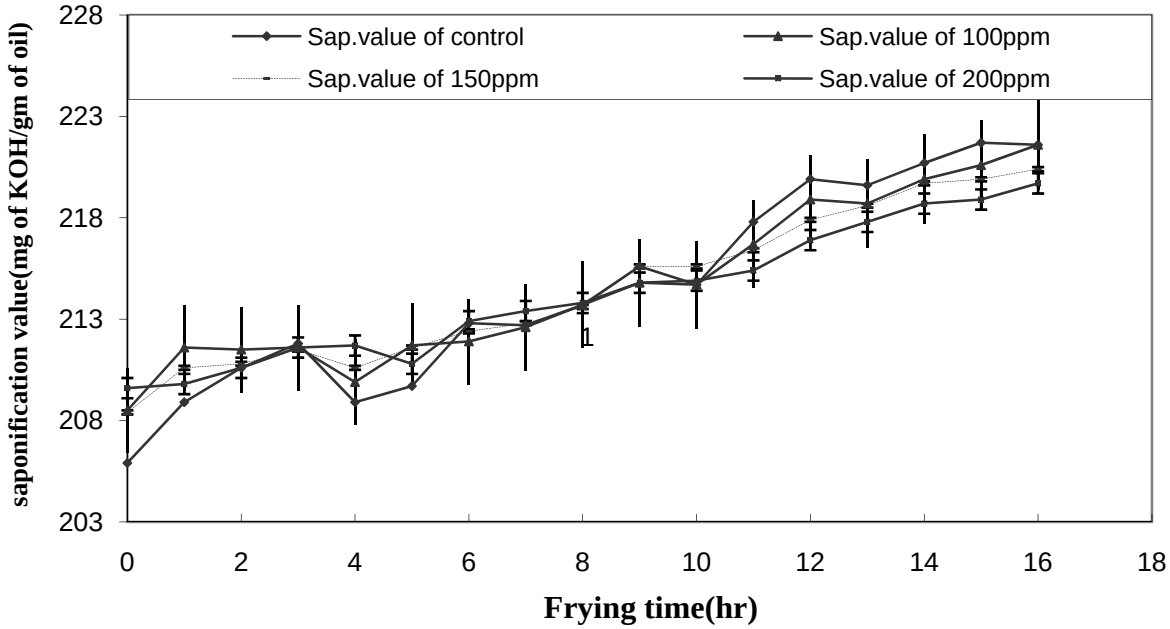

Fig 4. 12 Change in saponification value against frying time (hr) using BHT

It was found that 200ppm of BHT was found to be significance over 100 and 150 ppm wher in all case r□6PE.

Saponification value was determined to check the adulteration of oil. In the dissertation, it was found that the fluctuation of saponification value during frying, it is due to the fact that while frying the noodles there increases the moisture content which increase the saponification value and also broken burnt noodles were removed out from the frying oil in mean time, at that time saponification value became decrease. It was also found that 100ppm of TBHQ, 150ppm of BHA and 200ppm of BHT is more significance than others concentration and correlation coefficient was found to be 0.95213, 0.84400 and 0.98648 respectively refers to the Appendix 1 of correlation table 3, 7 and 11 respectively. So 200ppm of BHT is more significance than that of TBHQ and BHA.

4.13 Comparison of acid value and specific gravity using different concentration of TBHQ

The acid value and specific gravity measured at regular interval of time (1 hr) of different concentration of TBHQ are listed in Appendix 8 (table 13) and represented graphically in figure 4.13

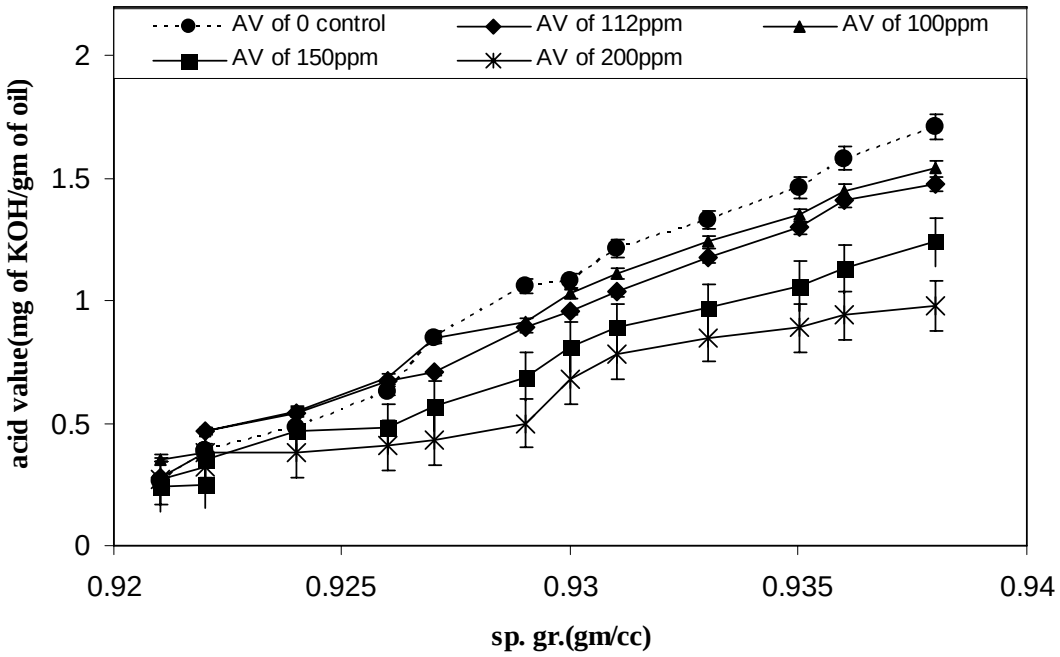

Fig 4. 13 Change in acid value against specific gravity of frying palm oil using TBHQ of different concentration at 170 to 180°C

It was found that acid value became increase with increase in specific gravity with increase in frying time. Refers to Appendix 1 (table 16) it was indicated that 150 ppm of TBHQ was found to be statistically significance over 112, 100 and 200ppm concentration. The r value was found as 0.98871, 0.993183, 0.99650 and 0.96503 where r□6PE for all cases.

4.14 Comparison of acid value and specific gravity using different concentration of BHA

The acid value and specific gravity measured at regular interval of time (1 hr) of different concentration of BHA are listed in Appendix 8(table 14) and represented graphically in figure 4.14

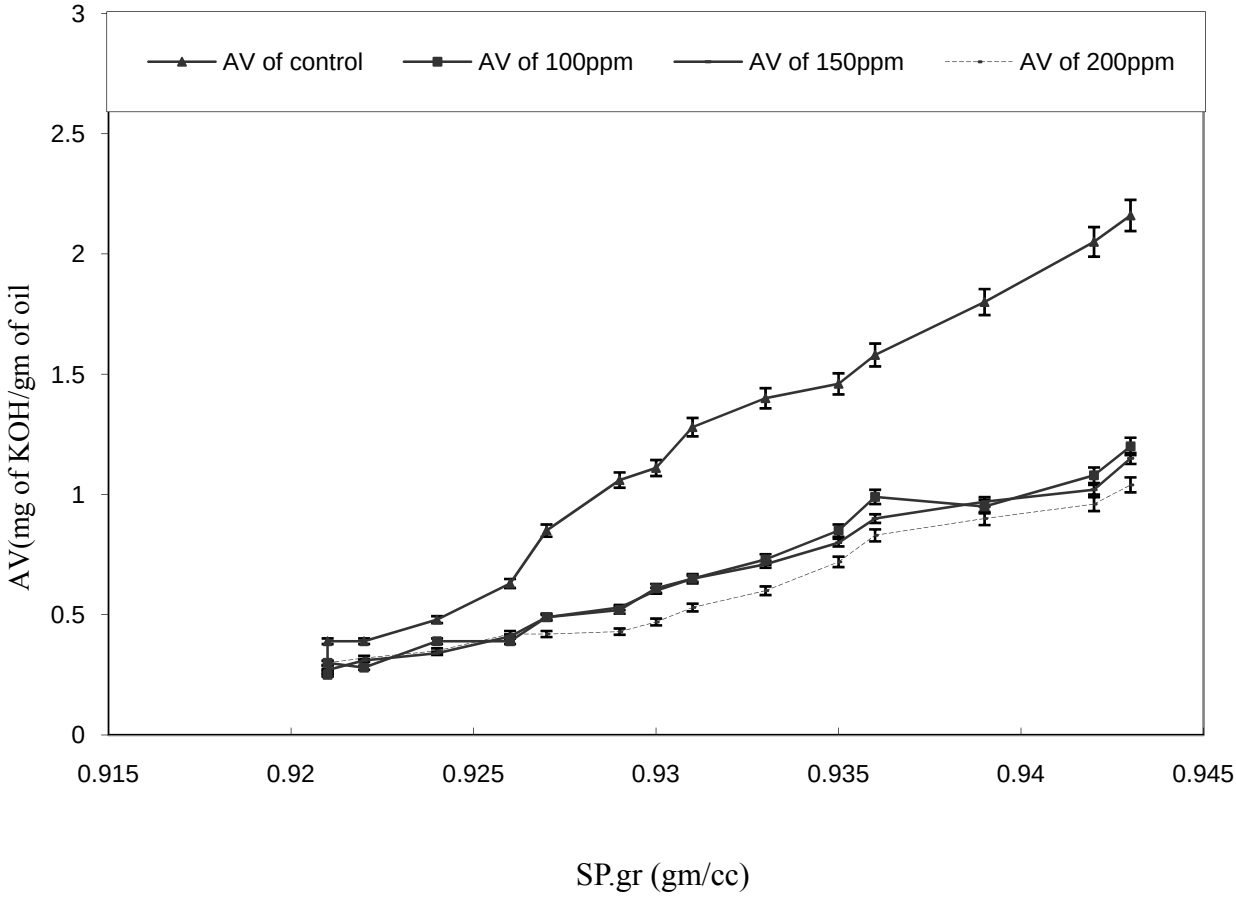

Fig 4. 14 Change in acid value against specific gravity of frying palm oil using BHA of different concentration at 170 to 180°C

It was clear from the graph shown above that acid value increase with increase in specific gravity of oil. There is positive relation between these two parameter and found that 150 ppm of BHA was found to be statistical significance over 100 and 200ppm r value was found as 0.99244, 0.98964 and 0.96538 respectively that of 100, 150 and 200ppm respectively where r□6PE for all cases.

4.15 Comparison of acid value and specific gravity using different concentration of BHT

The acid value and specific gravity measured at regular interval of time (1 hr) using 100, 150 and 200ppm of BHT are listed in Appendix 8 (table 15) and represented graphically in figure 4.15

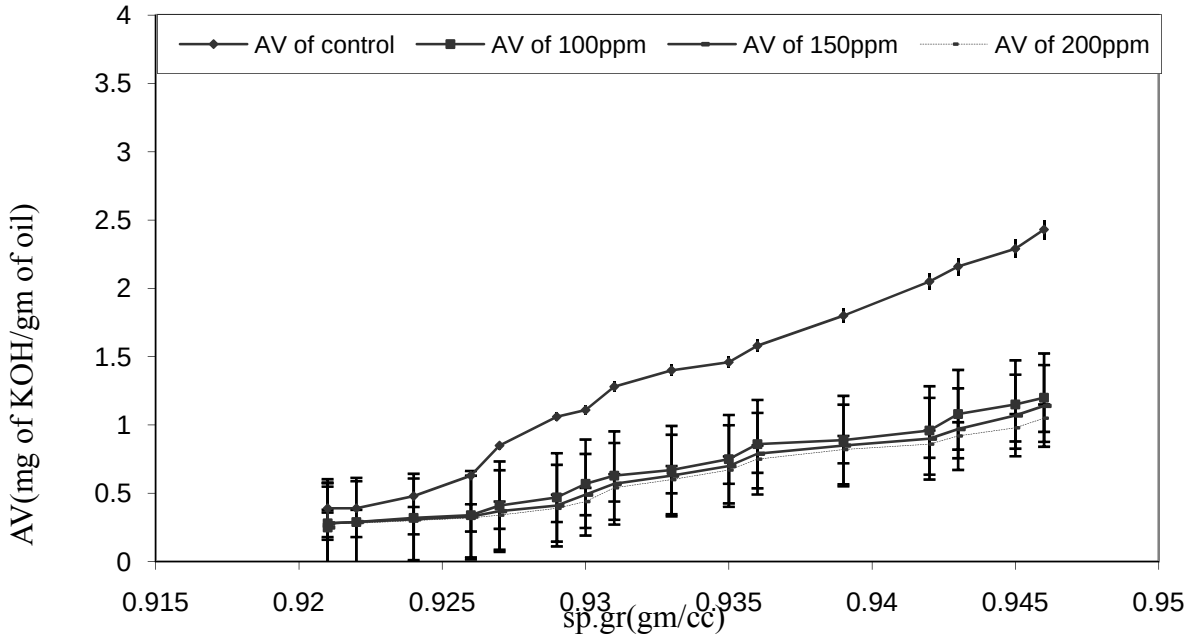

Fig 4. 15 Change in acid value against specific gravity of frying palm oil using BHT of different concentration at 170 to 180°C

It was found that 150ppm of BHT was found to be statistical significance over 100 and 200ppm BHT where r□6PE for all cases.

It is found that 150ppm TBHQ is more statically significance than 150ppm of BHA and BHT. It is well known fact that the specific gravity of oil increase with increase in frying time while frying the noodles, it increases the moisture content in oil which increases the specific gravity of oil. The specific gravity of oil slowly increases with increase in antioxidants concentration it is strongly support from the slope table refer Appendix 2 of table 7,8 and 9

4.16 Comparison of acid value and peroxide value using different concentration of TBHQ

The acid value and peroxide value measured at regular interval of time (1 hr) of different concentration of TBHQ are listed in Appendix 8 table (16) and correlation coefficient is shown in Appendix 2 of table 1.

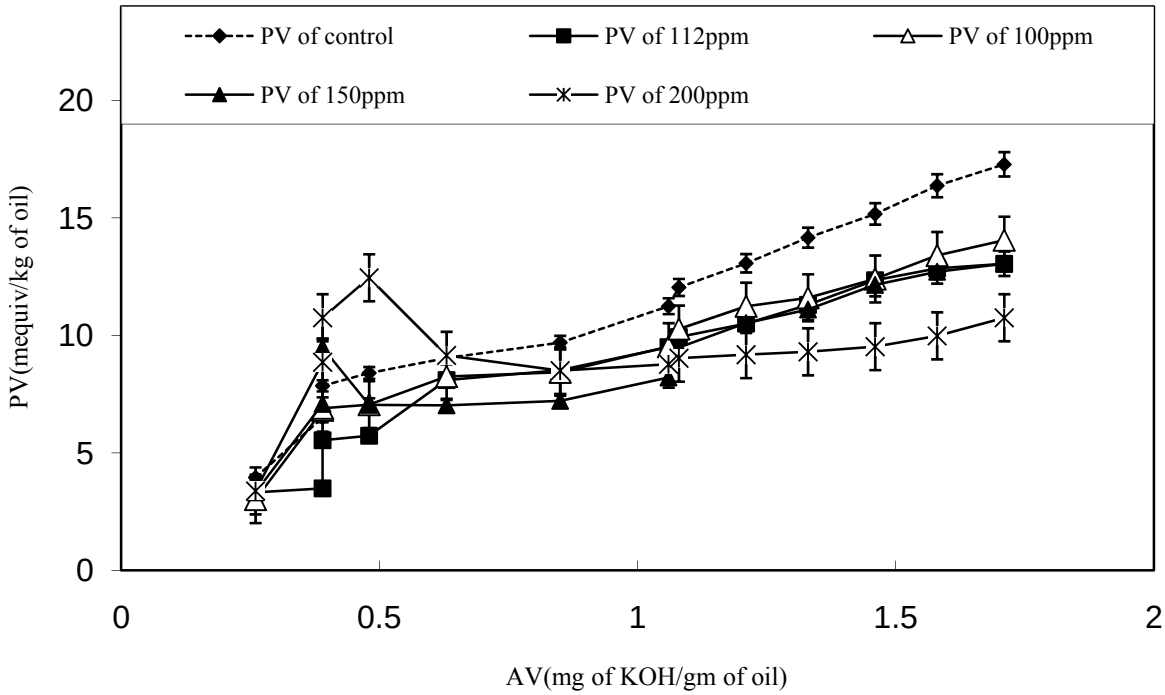

Fig 4. 16 Relation of AV against PV on different concentration of TBHQ

It was found that acid value increased with increased in peroxide value with frying time in certain limit. Peroxide value increased with increase in acid value in first hour of heating then peroxide value became decreased then again increased it was the beginning of secondary oxidation. There was not perfect relation between these two parameters. But correlation coefficient was found to be 0.98871, 0.99650, 0.99318 and 0.96503 respectively that of 112, 100, 150 and 200 ppm where r□6PE for all cases and at 100ppm it was found more statistically significance than other concentration of THHQ.

4.17 Comparison of acid value and peroxide value using different concentration of BHA

The acid value and peroxide value measured at regular interval of time (1 hr) of different concentration of BHA are listed in table **4.**17 and correlation coefficient is shown in Appendix 2 of table 2.

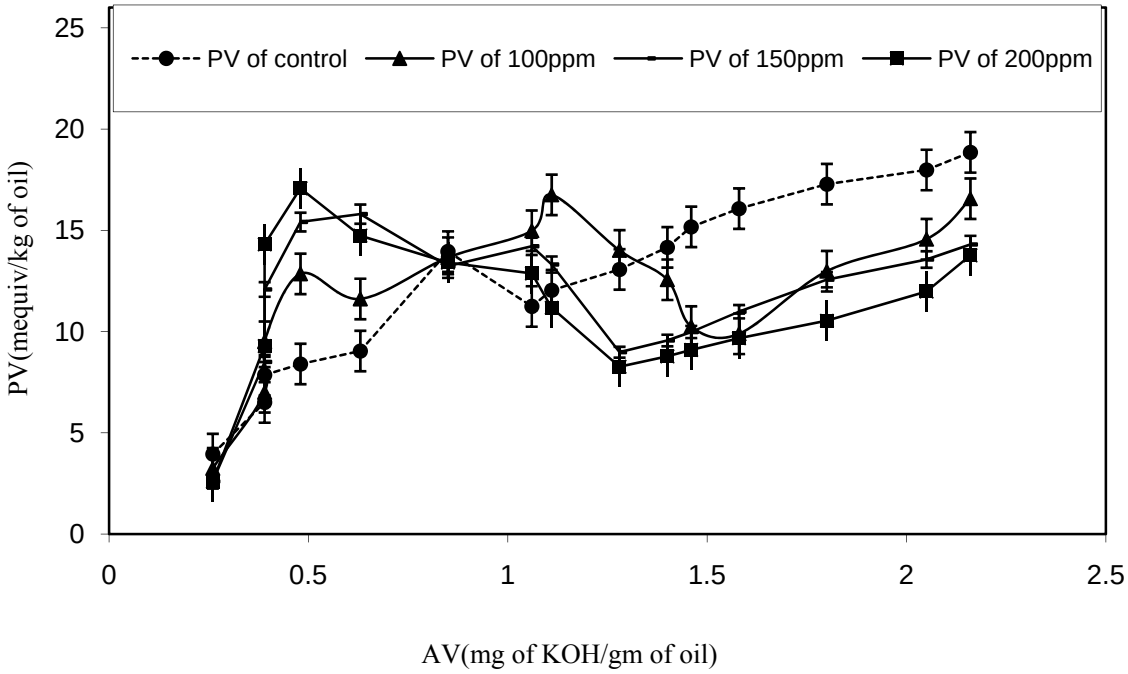

Fig 4. 17 Relation of AV against PV on different concentration of BHA

It was found that PV increased with increased in AV upto certain limit then PV became decreased then peroxide value became increased, the beginning of secondary oxidation. The correlation coefficient was found that 0.99008, 0.99493 and 0.98466 for 100, 150 and 200ppm respectively where r□ 6PE for all cases and 150ppm of BHA was found to be positive statistically significance over 100ppm and 200ppm.

4.18 Comparison of acid value and peroxide value using different concentration of BHT

The acid value and peroxide value measured at regular interval of time (1 hr) using 100, 150 and 200ppm concentration of BHT are listed in Appendix 8 (table18) and correlation coefficient is shown in Appendix 2 of table 3.

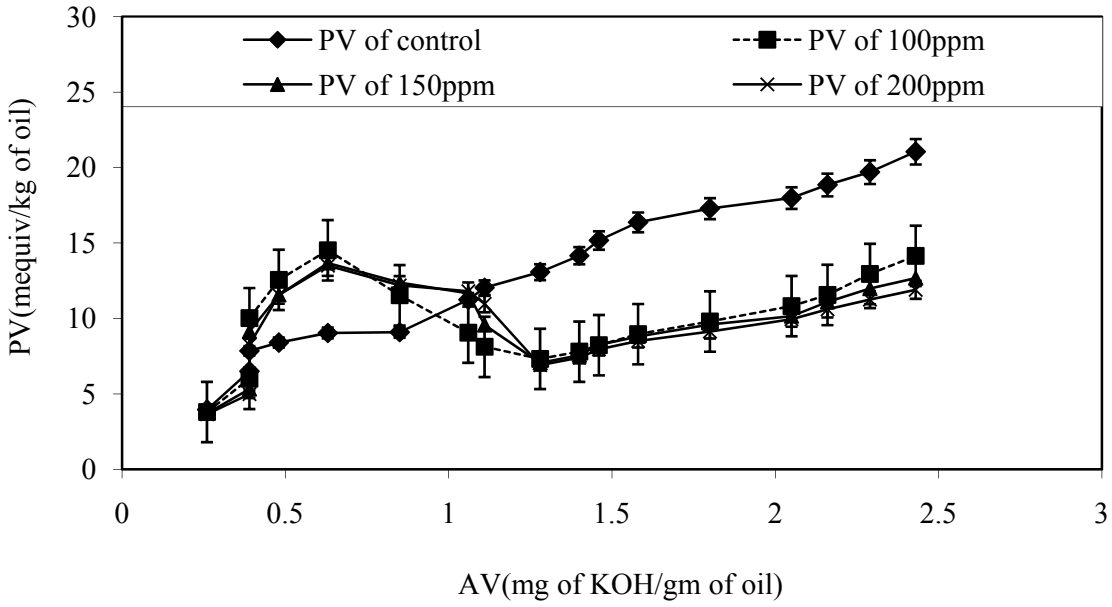

Fig 4. 18 Relation of AV against PV on different concentration of BHA

It was found that 150ppm of BHT was statistically significance over 100 and 200ppm where r□6PE. PV increased with increased in AV upto certain limit then PV decreased and again increased.

The concept for measuring PV and AV are completely different. It is more accepted that the secondary oil oxidized products such as polymerized oil, cyclic fatty acid, hydroxyl alkenal are main cause of toxicity in oxidized oil. Therefore, the formation of lipid hydroperoxide, the primary oil oxidized product, must be suppressed to prevent the formation of secondary oil oxidized product in Instant Noodles. It is already discussed above in literature review, in Japan; there did an experiment where 218 different noodles sample collected from different countries and analyzed AV and PV. There showed no positive relation between AV and PV at that condition (in literature review) both values were spread wide range and some of

them exceed the criteria but in this dissertation there found positive relation between AV and PV at frying condition (at 170 to 180°C) to some extent. The result shows increase in AV with frying time with increase in PV but this is not well known fact. According to graphical representation shown on graph 16, 17 and 18, it shows that AV increase with increase with PV. On the statistical representation it shows that Pearson's correlation coefficient(r) between AV and PV decrease with increase in concentration of antioxidants, this finding accept for all three antioxidants. But on the statistical analysis TBHQ of 150ppm concentration is only statistical significant at 95% confidence level where r>6PE, 0.89664> 0.22614 refer to the Appendix 1 of correlation table 13 and also for other two antioxidant r > 6PE which is also statistically significant refers to Appendix 1 of correlation table 14 and 15. But it couldn't be said the positive relation between AV and PV with time in all cases, there is not any supporting literature to accept this result.

According to result obtained, it is concluded that oxidation of the oil is very apprehensive phenomenon for food safety. It involves the formation of lipid hydroperoxide (indicated by PV) and the secondary oil oxidation product. This change can be measured only by PV, not only AV, because PV and AV donot increase simultaneously. Consequently measuring PV in oil is very important to grasp the deterioration level of oil in food from the food safety point of view. The formation of lipid peroxides low at first, however it increases at an explosive pace after induction period. To prevent this explosive increase of lipid hydroperoxide, keeping PV at low level.

Conclusion and recommendation

5.1 Conclusion

From the result and discussion the following conclusions were made-

1. Higher the concentration of antioxidants delayed the rise in PV, AV and specific gravity of frying oil to some extent.
2. BHT was found to be statistically significant over TBHQ and BHA to stable the PV of frying oil
3. TBHQ was found to be statistically significant over BHA and BHT to stable both AV and PV of the frying oil.
4. Concentration of 150ppm TBHQ was found to be the most significance over BHA and BHT to stable the frying oil which extended the stability upto 12hr on the basis of acid value.
5. Concentration of 200ppm BHT was found to be statistically significant over TBHQ and BHA which extend the stability of frying oil upto 16hr, according to Codex.
6. While using the TBHQ of 150ppm it increased the stability of oil 6hr more than that of control (without using TBHQ) and while using BHT it increased the stability 9hr more than that of control (without using BHT).
7. It is recommended to use 150ppm of TBHQ to the industry to control oxidation of oil.

5.2 Recommendation

Fried foods are popular among a wide range of consumers, especially the younger generation all over the world. It is imperative to use frying oils with less harmful effects in long term consumption of fried foods.

Though natural antioxidants are superior to synthetic ones, it is recommended to use synthetic antioxidants upto 200ppm because of scarcity of natural antioxidants as it is required in large amount for industrial purpose. Furthermore, the work can be extended as:

● Deep fat frying stability of palm oil using synergistic antioxidants and chelating agents like citric acid, ascorbic acid, etc.

● Storage stability of fried product using different natural and synthetic antioxidants on different packaging materials.

● Furthermore, study on an alternative to deep fat frying such as baking can also be done.

Summary

Frying is the process of cooking and drying through contact with hot oil and it involves simultaneously heat and mass transfer. Deep fat frying is the most complex edible fat and oil application. During deep fat frying, thermal, oxidative, and hydrolytic reactions take place resulting in physical and chemical changes in the oil and the formation of new compounds. The fried sample, souped noodles contain maximum amount of water which help the oil to rancid.

Generally in the industries TBHQ used as synthetic antioxidant but in the dissertation three different types of antioxidants TBHQ, BHA and BHT of lab grade were used for study purpose. First physiochemical parameters of palm oil was analyzed (Appendix 3) then samples (souped cake) were fired in the oil using different concentration antioxidants. It is well known fact that AV increase with increase in temperature where frying temperature for all experiment was 170 to 180°C**.** When TBHQ of 100ppm, 112ppm, 150ppm and 200ppm used; acid value raises upto standard value of oil (NS standard of oil for AV is 1mg of KOH/ gm of oil) after 7hr, 8hr, 10hr and 12hr respectively while that of control(without using antioxidants) exceed its limit after 6 hr of frying. Similarly while using BHA and BHT of 100ppm, 150ppm and 200ppm the value raised within 12hr, 13hr and 14hr and 14hr, 15hr and 16hr respectively. From statistical analysis Pearson's correlation coefficients(r) between different concentration of TBHQ of AV and frying time (hr) were found to be greater than 6PE (probable error) where (r>6PE) (Appendix 1, table 16). Similarly while using BHA and BHT similar result was found as TBHQ (appendix 1, table 17 and 18) where (r>6PE). The slope between AV and frying time (Appendix 2), for all concentration signified that by increasing the antioxidant concentration increase the stability of frying oil on the basis of AV. Though, still there is not any supportive literature about the finding, this result also shows by increasing the concentration of antioxidants it delay the rise in AV. In codex only AV mentions as quality parameter of fats/oils BHT of 200ppm is recommended as most significant over TBHQ and BHA which increased the stability of oil upto 16hr.

The analysis between PV and frying time (hr) when TBHQ of 100ppm, 112ppm, 150ppm and 200ppm used the stability exceed its limit (NS for fats/oils is 10 mequiv of peroxide/kg of oil) after 6hr, 7hr, 7hr and 11hr of frying and that of control reached to its limit within 5hr.

Similarly in case of BHA and BHT while using the concentration of 100ppm, 150ppm and 200ppm the value reached to the standard within 11hr, 10hr and 11hr and 12hr, 12hr and 13hr respectively. By the statistical analysis while using TBHQ the Pearson's correlation coefficient(r) between PV and frying time was found to be greater than 6PE for 100 and 150ppm concentration which were positively statistical significance but for 200ppm it was found 6PE>r which was not statistically significance. Similarly Person's correlation coefficients (r) between PV and frying time for BHA and BHT were found to be smaller than 6PE (6PE>r) except control which is not statistically significance. So most significant result was obtained by using TBHQ of 150ppm. But from the slope between PV and frying time, it was found that by increasing the concentration of antioxidants delay the retards of rancidity which is universal truth (Appendix 2, table 4, 5, 6).

From specific gravity of frying oil, it was found that by increasing the antioxidants concentration delaying the rise in specific gravity of oil. It was found that 150ppm concentration of TBHQ, BHA and BHT strongly significance. The Pearson's correlation coefficient(r) between specific gravity and frying time (hr) of three different antioxidants were greater than 6PE found as (r>6 PE). Comparatively 150ppm of BHA is more significance than TBHQ and BHT. The fact was also support from the slope between specific gravity and frying time (hr) and found that by increasing the concentration of antioxidants delaying the rise in specific gravity of frying oil with frying time. The graphs shown between acid value and specific gravity of different concentration of different antioxidants showed positive relation. Pearson's correlation coefficient(r) between acid value and specific gravity strongly correlated with each other and statistically positive significant where r>6PE refers to Appendix 1 of table 16, 17, 18 respectively.

There is not any perfect relation between saponification value and frying time (hr), which fluctuated with time. TBHQ of 100ppm concentration is statistically significant where r equal (r > PE) but 150ppm of TBHQ is also statistically significant.

According to NS, TBHQ of 150ppm concentration is statically significant and with stability over 12hr others two antioxidants of different concentration. But according to codex, BHT of 200ppm concentration is statistically significance over other two antioxidants which extends the stability of frying oil over 16hr.

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Appendices

Appendix 1

Correlation table 1:

Correlation between rise in AV against frying time in hour

Statistical output	Concentration of TBHQ				
	control	112ppm	100ppm	150ppm	200ppm
r	0.993474	0.997896	0.996281	0.997508	0.975181
PE	0.002533	0.000818	0.000969	0.001445	0.009545
6PE	0.015198	0.004908	0.005814	0.00867	0.05727

Correlation table 2:

Correlation between rise in PV against frying time(hr)

Statistical Output	Concentration of TBHQ				
	control	112 ppm	100 ppm	150 ppm	200 ppm
r	0.993955	0.98441	0.976983	0.904012	0.374878
PE	0.006024	0.006024	0.00886	0.035586	0.167348
6 PE	0.036143	0.036143	0.053161	0.213516	1.004087

Correlation table 3:

Correlation between rises in saponification value against frying time (hr)

statistical output	Concentration of TBHQ				
	control	112ppm	100ppm	150ppm	200ppm
r	0.932659	0.907232	0.952139	0.872469	0.86014
PE	0.025341	0.03445	0.018192	0.046497	0.050656
6PE	0.152047	0.206701	0.109154	0.27898	0.303935

Correlation table 4:

Correlation between rises in specific gravity against frying time (hr)

Stats output	Concentration of TBHQ				
	control	112ppm	100ppm	150ppm	200ppm
r	0.996676	0.98897	0.992043	0.995729	0.972002
PE	0.001292	0.004271	0.005749	0.010691	0.037241
6PE	0.007753	0.02563	0.034497	0.064148	0.223447

Correlation table 5:
Correlation between rises in Acid value against frying time (hr)

Statistical output	concentration of BHA			
	control	100ppm	150ppm	200ppm
r	0.993286	0.98533	0.99163	0.96483
PE	0.002413	0.00525	0.00301	0.01246
6PE	0.014475	0.03151	0.01803	0.07474

Correlation table 6:
Correlation between rises in peroxide value against frying time (hr)

Statistical output	concentration of BHA			
	control	100ppm	150ppm	200ppm
r	0.9713755	0.604576	0.314743	0.069245
PE	0.010172	0.114377	0.16241	0.179403
6PE	0.061032	0.686262	0.97446	1.076418

Correlation table 7:
Correlation between rises in saponification value against frying time (hr)

Statistical output	concentration of BHA			
	control	100ppm	150ppm	200ppm
r	0.9564679	0.104077	0.844157	0.726656
PE	0.015302	0.178319	0.051856	0.085081
6PE	0.091812	1.069914	0.311136	0.510486

Correlation table 8:
Correlation between rises in specific gravity against frying time (hr)

Statistical output	concentration of BHA			
	control	100ppm	150ppm	200ppm
r	0.9922982	0.991866	0.998282	0.982926
PE	0.002766	0.000524	0.002882	0.006103
6PE	0.016596	0.003144	0.017292	0.03662

Correlation table 9:
Correlation between rises in Acid value against frying time (hr)

Statistical output	concentration of BHT			
	control	100ppm	150ppm	200ppm
r	0.9922	0.9762	0.9852	0.9664
PE	0.002607	0.00496	0.007934	0.011136
6PE	0.015642	0.02976	0.047604	0.066814

Correlation table 10:
Correlation between rises in peroxide value against frying time (hr)

Statistical output	concentration of BHT			
	control	100ppm	150ppm	200ppm
r	0.9957578	0.44372934	0.400177	0.349724
PE	0.001427	0.135423	0.141621	0.166747
6PE	0.008562	0.812538	0.849726	1.000483

Correlation table 11:
Correlation between rises in saponification value against frying time (hr)

Statistical output	concentration of BHT			
	control	100ppm	150ppm	200ppm
r	0.968551	0.9590294	0.98367	0.98648
PE	0.010439	0.013534	0.00546	0.00453
6PE	0.062634	0.081204	0.03278	0.02717

Correlation table 12:
Correlation between rises in specific gravity against frying time (hr)

Statistical output	concentration of BHT			
	control	100ppm	150ppm	200ppm
r	0.99419	0.98372	0.989967	0.96956
PE	0.001953	0.003366	0.00039	0.01011
6PE	0.011718	0.020196	0.00232	0.06065

Correlation table 13:
Correlation between peroxide values against acid value

Statistical output		concentration of TBHQ control	112ppm	100ppm	150ppm	200ppm
	r	0.994937	0.988715	0.996501	0.993183	0.965034
	PE	0.00196	0.00437	0.001361	0.002648	0.013394
	6PE	0.01811	0.02625	0.00817	0.01589	0.080366

Correlation table 14:
Correlation between peroxide values against acid value

Statistical output		concentration of BHA control	100ppm	150ppm	200ppm
	r	0.9918	0.9965	0.99318	0.96503
	PE	0.00197	0.00578	0.0035	0.01227
	6PE	0.01184	0.03467	0.02097	0.07363

Correlation table 15:
Correlation between peroxide values against acid value

Stats output		concentration of BHT control	100ppm	150ppm	200ppm
	r	0.99621	0.99008	0.99493	0.98466
	PE	0.00127	0.00333	0.00171	0.00513
	6PE	0.00765	0.01997	0.01024	0.0308

Correlation table 16:
Correlation between rises in specific gravity against acid value

Stats output		concentration of TBHQ control	112ppm	100ppm	150ppm	200ppm
	r	0.994937	0.988715	0.996501	0.993183	0.965034
	PE	0.00196	0.00437	0.001361	0.002648	0.013394
	6PE	0.01811	0.02625	0.00817	0.01589	0.080366

Correlation table 17:

Correlation between rises in specific gravity against acid value

Stats output	concentration of BHA			
	control	100ppm	150ppm	200ppm
r	0.994514	0.982441	0.989646	0.96538
PE	0.001973	0.005778	0.003495	0.012271
6PE	0.01184	0.034669	0.020972	0.073626

Correlation table 18:

Correlation between rises in specific gravity against acid value

Stats output	concentration of BHT			
	control	100ppm	150ppm	200ppm
r	0.996214	0.990083	0.994928	0.984661
PE	0.001274	0.003327	0.001706	0.005133
6PE	0.007646	0.019967	0.010237	0.0308

Appendix 2

Slope of different concentration of antioxidants

Slope 1: AV vs. frying time using different concentration of TBHQ

slope	concentration
0.125659	control
0.102087	112
0.104945	100
0.868131	150
0.0648351	200

Slope 2: AV vs. frying time using different concentration of BHA

slope	concentration
0.137571	control
0.068107	100
0.064536	150
0.055214	200

Slope 3: AV vs. frying time using different concentration of BHT

slope	concentration
0.139926	control
0.063333	100
0.058186	150
0.053456	200

Slope 4: PV vs. frying time using different concentration of TBHQ

slope	concentration
1.023626	control
0.853516	112
0.783846	100
0.651978	150
0.198022	200

Slope 5: PV vs. frying time (hr) using different concentration of BHA

slope	Concentration
0.970179	control
0.488786	100
0.238964	150
0.054107	200

Slope 6: PV vs. frying time (hr) using different concentration of BHT

slope	Concentration
0.999706	Control
0.253407	100
0.213799	150
0.18652	200

Slope 7: Specific Gravity vs. Frying time (hr) using different concentration of TBHQ

slope	concentration
0.001433	Control
0.001088	112
0.00108	100
0.001087	150
0.000786	200

Slope 8: Specific Gravity vs. Frying time (hr) using different concentration of BHA

slope	concentration
0.001621	Control
0.001641	100
0.001564	150
0.001404	200

Slope 9: Specific Gravity vs. Frying time (hr) using different concentration of BHT

slope	concentration
0.001653	Control
0.001547	100
0.001483	150
0.001402	200

Slope 10: Specific Gravity vs. Acid value using different concentration of TBHQ

slope	concentration
0.928257	Control
0.896703	112
1.008242	100
0.801648	150
0.717582	200

Slope 11: Specific Gravity vs. Acid value using different concentration of BHA

slope	concentration
0.962857	Control
0.055054	100
0.635957	150
0.57156	200

Slope 12: Specific Gravity vs. Acid value using different concentration of BHT

slope	concentration
0.955392	Control
0.765931	100
0.742157	150
0.647304	200

Appendix 3

Physio-chemical characteristics of RBD palm oil

RBD palm oil was estimated for its important physio-chemical property and the values so obtained are given in table- 4.1

Parameter	Palm oil
Moisture content (%)	0.03
Acid value (mg of KOH/gm of oil)	0.21
Peroxide value (meqv/kg oil)	1.54
Saponification value	198
Unsaponifiable matter(gm/kg of oil)	5
Density(gm/cc)	0.899
Iodine value	50

Appendix 4

The results of analysis of fried noodles are shown below

Determination	Calculated value
Moisture content(%)	1.00
Total ash(%)	3.76
Acid insoluble ash (%)	0.085
Protein(%)	10.56
Extracted fat	
A. Acid value	0.76 mg of KOH/gm of oil
B. Peroxide value	5.89 milliequivalent of peroxide/ kg of oil

Appendix 5

Physio-chemical properties of different antioxidants

Chemical Name: TERTIARY BUTYL HYDROQUINONE

Chemical formula: $C_{10}H_{14}O_2$

Molecular Weight: 166.24

Trade Name: TBHQ

PARAMETER	NORMS	ANALYSIS METHOD
Appearance	White Crystalline Powder	Visual
Purity by HPLC	99.00% Min	HPLC
Melting Point	126.5°C to 128°C	Capillary Melting Point
Tert. Butyl-p-Benzo Quinone	0.20% Max	HPLC
2, 5- Di-tert.butyl Hydroquinone % by Mass	0.20% Max	HPLC
Arsenic (as AS)	3 ppm Max	AAS
Hydroquinone, % by mass	0.10% Max	HPLC
Toluene	25 ppm Max	HPLC
Heavy Metals (as Pb)	10 ppm Max	AAS
U.V.Absorbance (Polynuclear Hydrocarbon)	PASSES TEST	Spectra U.V.

Common Name	**BHA**
Molecular Weight	180.25
Empirical Formula	$C_{11}H_{16}O_2$
CAS Number	25013-16-5
Description	A white or pale yellow solid (Crystal or Flake) faint aromatic odour.
Assay (As C11H16O2)	
Chromatographic	Min. 98.50%
3-Tert Butyl 4-Hydroxyanisole (3-BHA)	Min. 95.00 %
Melting Range	Between 48°C to 63°C
Heavy Metals (As Pb)	Max. 2 mg/kg.
Sulphated Ash	Max. 0.01% m/m

Chemical Name:	**Butylate hydroxy toulene**
Trade Name:	BHT
Description	White crystals
Odour	No obnoxious odour
Colour (APHA) of solution	10 haze max.
Moisture content	0.1% max
Freezing point	69.2° C min
Purity(% wt.)	99% min.
Residue on ignition	0.002% Max
Heavy metal (as Lead)	Max. 10 ppm

(Source: www.antioxidant.com)

Appendix 6

Nepal standards of differend vegetable oils

Oil	Refractive Index (40°C)	Melting Point	Saponification Value	Unsaponifiable matter (gm/kg of oil)	Iodine Value	Peroxide Value (mequiv of O_2 /kg of oil)	Acid Value (mg of KOH /gm of oil)	Phosphorous content (%)	Hexabromide test
Palm Oil	1.4491 - 1.4552	<37°C	195-205	<12	45-56	<10	<6		
Palmolein Oil	1.4550 - 1.4610		195-205	<12	54-62	<10	<6		
Palm Kernel Oil	1.4490 - 1.4520		237-255	<12	10-23	<10	<10		
Rapeseed Oil	1.465-1.469		168-193	<20	94-126	<10	< 0.5		-ve
Soybean Oil	1.4650 - 1.4710		189-195	<15	120-140	<10	< 2.5	< 0.02	

(Source: Nepal Standard)

Appendix 7

Palm Oil Composition and Physical Characteristics

Characteristic	Typical	Range
Specific gravity, 50°C	—	0.888 to 0.889
Refractive index, 50°C	—	1.455 to 1.456
Iodine value	53.	0 46.0 to 56.0
Saponification number	196	190 to 202
Unsaponifiable number	0.5	0.15 to 0.99
Titer (°C)	46.3	40.7 to 49.0
Melting point (°C) (MDP)	37.5	35.5 to 45.0
Solidification point (°C)	—	35.0 to 42.0
Cold test (hours)	none	—
Carotene content (mg/kg)	—	500 to 700
AOM stability (hours)	54.0	53 to 60
Tocopherol content (ppm):		
α-Tocopherol	172	129 to 215
β-Tocopherol	30	22 to 37
γ-Tocopherol	26	19 to 32
δ-Tocopherol	13	10 to 16
Tocotrienol content (ppm):		
α-Tocotrienol	59	44 to 73
β-Tocotrienol	59	44 to 73
γ-Tocotrienol	350	262 to 437
δ-Tocotrienol	94	70 to 117
Fatty acid composition (%):		
Lauric(C-12:0)	0.2	0.1 to 1.0
Myristic (C-14:0)	1.1	0.9 to 1.5
Palmitic (C-16:0)	44.0	41.8 to 46.8
Palmitoleic (C-16:1)	0.12	0.1 to 0.3
Stearic (C-18:0)	4.5	4.5 to 5.1

Oleic (C-18:1)	39.2	37.3 to 40.8
Linoleic (C-18:2)	10.1	9.1 to 11.0
Linolenic (C-18:3)	0.4	0.4 to 0.6
Arachidic (C-20:0)	0.4	0.2 to 0.7
Triglyceride composition (%):		
Trisaturated(GS3)	10.2	4.0 to 10.5
Disaturated (GS2U)	48.0	41.0 to 59.0
Monosaturated (GSU2)	34.6	32.0 to 54.0
Triunsaturated (GU3)	6.8	3.0 to 12.0
Crystal habit	β′	—
Solids fat index (%) at:		
10.0°C	34.5	30.0 to 39.0
21.1°C	14.0	11.5 to 17.0
26.7°C	11.0	8.0 to 14.0
33.3°C	7.4	4.0 to 11.0
37.8°C	5.6	2.5 to 9.0
40.0°C	4.7	2.0 to 7.0

(Source: www.palmoil.com)

Note: G = glycerides; S = saturated; U = unsaturated; MDP = Mettler droping point
AOM = active oxygen method.

Appendix 8

Table no 1 Analysis of frying palm oil using TBHQ of different concentration. The frying temperature is 170 to 180˚C.

Time(hr)	AV of control	AV of 112ppm	AV of 100ppm	AV of 150ppm	AV of 200ppm
0	0.26±0.001	0.28±0.002	0.35±0.001	0.24±0.004	0.27±0.001
1	0.39±0.002	0.38±0.003	0.38±0.001	0.25±0.001	0.32±0.001
2	0.39±0.001	0.47±0.001	0.47±0.002	0.35±0.003	0.38±0.003
3	0.48±0.002	0.54±0.001	0.55±0.002	0.47±0.001	0.38±0.001
4	0.63±0.003	0.67±0.001	0.69±0.001	0.48±0.001	0.41±0.004
5	0.85±0.001	0.71±0.003	0.85±0.001	0.57±0.002	0.43±0.002
6	1.06±0.001	0.89±0.001	0.91±0.001	0.69±0.001	0.5±0.003
7	1.08±0.001	0.96±0.001	1.03±0.003	0.81±0.001	0.68±0.001
8	1.21±0.002	1.04±0.002	1.11±0.003	0.89±0.003	0.78±0.003
9	1.33±0.002	1.18±0.002	1.24±0.001	0.97±0.001	0.85±0.001
10	1.46±0.003	1.3±0.001	1.35±0.004	1.06±0.001	0.89±0.002
11	1.58±0.001	1.41±0.001	1.45±0.001	1.13±0.002	0.94±0.001
12	1.71±0.001	1.48±0.002	1.54±0.004	1.24±0.001	0.98±0.003

* control sample is without TBHQ

Table no 2 Analysis of frying oil using BHA of different concentration. The frying temperature is 170 to 180°C.

Time(hr)	AV of control	AV of 100ppm	AV of 150ppm	AV of 200ppm
0	0.26±0.001	0.25±0.001	0.25±0.001	0.28±0.002
1	0.39±0.002	0.3±0.002	0.27±0.001	0.3±0.002
2	0.39±0.001	0.28±0.001	0.31±0.002	0.32±0.002
3	0.48±0.002	0.39±0.002	0.34±0.001	0.35±0.001
4	0.63±0.003	0.39±0.001	0.41±0.003	0.42±0.001
5	0.85±0.001	0.49±0.002	0.49±0.004	0.42±0.001
6	1.06±0.001	0.52±0.001	0.53±0.002	0.43±0.004
7	1.11±0.001	0.61±0.001	0.6±0.001	0.47±0.001
8	1.21±0.002	0.65±0.003	0.65±0.001	0.53±0.001
9	1.33±0.002	0.73±0.001	0.71±0.002	0.6±0.002
10	1.46±0.003	0.85±0.001	0.8±0.001	0.72±0.001
11	1.58±0.001	0.99±0.003	0.9±0.001	0.83±0.003
12	1.71±0.001	0.95±0.001	0.97±0.001	0.9±0.001
13	2.05±0.001	1.08±0.001	1.02±0.003	0.96±0.001
14	2.16±0.001	1.2±0.003	1.15±0.001	1.04±0.003

*control sample is without BHA

Table No 3 Analysis of frying using BHT of different concentration. The frying temperature is 170 to 180˚C.

Time(hr)	AV of control	AV of 100ppm	AV of 150ppm	AV of 200ppm
0	0.26±0.001	0.25±0.002	0.25±0.001	0.26±0.001
1	0.39±0.002	0.280.001	0.28±0.002	0.28±0.001
2	0.39±0.001	0.290.002	0.29±0.001	0.28±0.002
3	0.48±0.002	0.32±0.001	0.31±0.003	0.3±0.002
4	0.63±0.003	0.34±0.003	0.33±0.001	0.32±0.001
5	0.85±0.001	0.410.003	0.37±0.004	0.34±0.002
6	1.06±0.001	0.470.004	0.41±0.002	0.39±0.001
7	1.11±0.001	0.570.001	0.49±0.001	0.44±0.002
8	1.21±0.002	0.63±0.001	0.57±0.001	0.54±0.001
9	1.33±0.002	0.67±0.003	0.63±0.002	0.6±0.002
10	1.46±0.003	0.75±0.001	0.70. ±001	0.67±0.001
11	1.58±0.001	0.860.004	0.79±0.002	0.75±0.004
12	1.71±0.001	0.89±0.003	0.85±0.001	0.82±0.003
13	2.05±0.001	0.960.001	0.9±0.001	0.86±0.002
14	2.16±0.001	1.08±0.001	0.97±0.003	0.92±0.001
15	2.290.001	1.150.001	1.07±0.003	0.98±0.002
16	2.430.001	1.2±0.003	1.14±0.001	1.05±0.001

*control sample is without BHT

Table no 4 Analysis of frying oil using TBHQ of different concentration. The frying temperature is 170 to 180˚C

Time hr	PV of control	PV of 112ppm	PV of 100ppm	PV of 150ppm	PV of 200ppm
0	3.95±0.001	3.32±0.002	3.01±0.002	3.32±0.003	3.38±0.001
1	6.5±0.001	3.49±0.001	6.81±0.001	7.08±0.001	8.86±0.001
2	7.85±0.002	5.54±0.002	6.9±0.003	9.48±0.003	10.75±0.003
3	8.4±0.001	5.73±0.001	7.05±0.001	7.04±0.002	12.45±0.001
4	9.04±0.003	8.1±0.003	8.25±0.002	7.02±0.001	9.15±0.002
5	9.69±0.001	8.52±0.001	8.43±0.002	7.21±0.003	8.5±0.001
6	11.24±0.001	9.5±0.001	9.52±0.001	8.21±0.001	8.77±0.002
7	12.04±0.001	9.93±0.002	10.27±0.002	9.48±0.004	9.03±0.001
8	13.07±0.003	10.5±0.001	11.24±0.001	10.52±0.001	9.18±0.003
9	14.16±0.004	11.3±0.001	11.6±0.001	11.1±0.001	9.3±0.001
10	15.17±0.002	12.35±0.002	12.4±0.002	12.15±0.003	9.52±0.003
11	16.37±0.001	12.85±0.001	13.4±0.001	12.71±0.001	9.98±0.003
12	17.28±0.002	13.05±0.002	14.05±0.002	13.05±0.002	10.75±0.001

*control sample is without TBHQ

Table no 5 Analysis of frying oil using BHA of different concentration. The frying temperature is 170 to 180˚C

Time(hr)	PV of control	PV of 100ppm	PV of 150ppm	PV of 200ppm
0	3.95±0.001	3.25±0.001	2.6±0.001	2.59±0.001
1	6.5±0.001	7±0.001	8.5±0.003	9.28±0.001
2	7.85±0.002	9.5±0.003	12.08±0.001	14.31±0.003
3	8.4±0.001	12.85±0.001	15.41±0.003	17.07±0.001
4	9.04±0.003	11.61±0.001	15.8±0.001	14.73±0.003
5	9.69±0.001	13.65±0.001	13.23±0.004	13.41±0.001
6	11.24±0.001	14.98±0.001	14.21±0.001	12.87±0.002
7	12.04±0.001	16.75±0.001	13.31±0.003	11.18±0.001
8	13.07±0.003	14.01±0.003	8.98±0.002	8.26±0.002
9	14.16±0.004	12.56±0.001	9.56±0.002	8.78±0.001
10	15.17±0.002	10.25±0.004	9.98±0.001	9.09±0.004
11	16.37±0.001	9.89±0.001	10.98±0.003	9.67±0.001
12	17.28±0.002	12.98±0.003	12.56±0.001	10.54±0.003
13	17.98±0.001	14.56±0.001	13.56±0.003	11.98±0.001
14	18.85±0.001	16.56±0.002	14.30±0.002	13.76±0.002

*control sample is without BHA

Table No 6 Analysis of frying oil using BHT of different of different concentration. The frying temperature is 170 to 180˚C

Time(hr)	PV of contr	PV of 100ppm	PV of 150ppm	PV of 200ppm
0	3.95±0.001	3.8±0.001	3.7±0.003	3.65±0.001
1	6.5±0.001	6±0.001	5.32±0.001	4.98±0.003
2	7.85±0.002	10.02±0.002	9.13±0.001	8.25±0.001
3	8.4±0.001	12.56±0.001	11.56±0.003	11.56±0.003
4	9.04±0.003	14.52±0.003	13.67±0.001	13.5±0.001
5	9.69±0.001	11.54±0.001	12.38±0.004	12.2±0.002
6	11.24±0.001	9.06±0.003	11.67±0.001	11.8±0.001
7	12.04±0.001	8.12±0.001	9.56±0.002	10.96±0.004
8	13.07±0.003	7.32±0.003	7.05±0.002	6.9±0.001
9	14.16±0.004	7.8±0.001	7.56±0.002	7.4±0.002
10	15.17±0.002	8.23±0.002	8.25±0.002	7.95±0.001
11	16.37±0.001	8.96±0.001	8.8±0.001	8.5±0.002
12	17.28±0.002	9.8±0.004	9.6±0.001	9.13±0.001
13	17.98±0.001	10.82±0.001	10.15±0.001	9.95±0.002
14	18.85±0.001	11.56±0.003	11.09±0.002	10.6±0.001
15	19.7±0.003	12.95±0.001	11.98±0.001	11.25±0.002
16	21.05±0.001	14.15±0.002	12.67±0.003	11.9±0.002

Table No. 7 Analysis of frying oil using TBHQ of different concentration. The frying temperature is 170 to 180**°C**

Time (hr)	Sp.gr.of control	Sp.gr.of 112ppm	Sp.gr.of 100ppm	Sp.gr.of 150ppm	Sp.gr.of 200ppm
0	0.921±0.0001	0.921±0.0002	0.92±0.0003	0.92±0.0003	0.921±0.0004
1	0.922±0.0002	0.921±0.0001	0.921±0.0003	0.921±0.0001	0.922±0.0001
2	0.922±0.0001	0.922±0.0003	0.921±0.0001	0.921±0.00034	0.922±0.0002
3	0.924±0.0002	0.922±0.0004	0.923±0.0002	0.922±0.0001	0.923±0.0001
4	0.926±0.0003	0.923±0.0001	0.924±0.0001	0.923±0.0004	0.923±0.0003
5	0.927±0.0002	0.925±0.0002	0.926±0.0002	0.924±0.0001	0.924±0.0001
6	0.929±0.0003	0.926±0.0003	0.926±0.0001	0.926±0.0003	0.924±0.0002
7	0.930±0.0001	0.928±0.0001	0.927±0.0003	0.927±0.0001	0.925±0.0001
8	0.931±0.0001	0.928±0.0003	0.928±0.0001	0.929±0.0002	0.926±0.0003
9	0.933±0.0001	0.929±0.0004	0.93±0.0004	0.929±0.0001	0.927±0.0001
10	0.935±0.0002	0.93±0.0001	0.931±0.0001	0.93±0.0002	0.928±0.0001
11	0.936±0.0001	0.932±0.0003	0.932±0.0002	0.931±0.0001	0.93±0.0003
12	0.938±0.0003	0.933±0.0002	0.933±0.0003	0.932±0.0003	0.931±0.0002

*control sample is without using TBHQ

Table No.8 Analysis of frying oil using BHA of different concentration. The frying temperature is 170 to 180˚C.

Time(hr)	Sp.gr.0f control	Sp.gr.of 100ppm	Sp.gr.of 150ppm	Sp.gr.of 200ppm
0	0.921±0.0001	0.918±0.0002	0.919±0.0002	0.918±0.0003
1	0.922±0.0002	0.918±0.0001	0.919±0.0001	0.921±0.0001
2	0.922±0.0001	0.921±0.0002	0.92±0.0003	0.920±0.0003
3	0.924±0.0002	0.922±0.0001	0.921±0.0001	0.921±0.0001
4	0.926±0.0003	0.924±0.0001	0.922±0.0001	0.921±0.0002
5	0.927±0.0002	0.926±0.0001	0.923±0.0003	0.923±0.0002
6	0.929±0.0003	0.927±0.0001	0.926±0.0002	0.925±0.0001
7	0.930±0.0001	0.929±0.0003	0.928±0.0002	0.928±0.0004
8	0.931±0.0001	0.93±0.0003	0.93±0.0001	0.929±0.0004
9	0.933±0.0001	0.932±0.0001	0.932±0.0001	0.929±0.0001
10	0.935±0.0002	0.934±0.0003	0.933±0.0001	0.93±0.0002
11	0.936±0.0001	0.935±0.0003	0.934±0.0003	0.932±0.0001
12	0.938±0.0003	0.937±0.0004	0.936±0.0003	0.934±0.0002
13	0.942±0.0001	0.939±0.0002	0.937±0.0001	0.937±0.0002
14	0.943±0.0002	0.940±0.0002	0.939±0.0002	0.938±0.0003

*control sample is without BHA

Table No. 9 Analysis of frying palm oil using different concentration of BHT. The frying temperature is 170 to 180˚C

Time(hr)	Sp.gr.of control	Sp.gr.of 100ppm	Sp.gr.of 150ppm	Sp.gr.of 200ppm
0	0.921±0.0001	0.92±0.0004	0.921±0.0002	0.921±0.0004
1	0.922±0.0002	0.921±0.0001	0.921±0.0001	0.921±0.0001
2	0.922±0.0001	0.921±0.0003	0.922±0.0003	0.922±0.0003
3	0.924±0.0002	0.923±0.0001	0.923±0.0001	0.922±0.0001
4	0.926±0.0003	0.925±0.0002	0.923±0.0003	0.923±0.0003
5	0.927±0.0002	0.927±0.0003	0.925±0.0001	0.924±0.0001
6	0.929±0.0003	0.928±0.0003	0.926±0.0004	0.925±0.0003
7	0.930±0.0001	0.929±0.0001	0.928±0.0001	0.927±0.0001
8	0.931±0.0001	0.929±0.0003	0.928±0.0002	0.927±0.0002
9	0.933±0.0001	0.93±0.0001	0.93±0.0001	0.929±0.0001
10	0.935±0.0002	0.933±0.0002	0.932±0.0003	0.929±0.0004
11	0.936±0.0001	0.935±0.0001	0.935±0.0001	0.934±0.0001
12	0.938±0.0003	0.937±0.0002	0.938±0.0003	0.933±0.0002
13	0.942±0.0001	0.939±0.0002	0.938±0.0002	0.937±0.0003
14	0.943±0.0002	0.94±0.0001	0.939±0.0001	0.939±0.0001
15	0.945±0.0001	0.943±0.0001	0.941±0.0001	0.942±0.0003
16	0.946±0.0001	0.945±0.00013	0.944±0.0003	0.942±0.0001

*control sample is used without BHT

Table No. 10: Analysis of frying oil using TBHQ of different concentration. The frying temperature is 170 to 180˚C

Time(hr)	Sap. Value of control	Sap. Value of 112ppm	Sap. Value of 100ppm	Sap. Value of 150ppm	Sap. Value of 200ppm
0	205.9±0.02	204.8±0.01	203.5±0.02	205.6±0.03	206.7±0.01
1	208.9±0.01	205.1±0.01	204.6±0.01	206.7±0.01	207.6±0.01
2	210.6±0.02	206.5±0.02	206.8±0.03	208.9±0.03	208.9±0.01
3	211.8±0.01	208.9±0.01	208.8±0.01	210.8±0.01	210.6±0.03
4	208.9±0.03	211.7±0.03	210.2±0.02	211.8±0.03	211.8±0.01
5	209.7±0.01	208.9±0.01	211.8±0.03	213.7±0.01	212.2±0.02
6	212.8±0.01	206.7±0.02	209.7±0.02	215.6±0.02	213.6±0.01
7	212.7±0.02	213.6±0.02	212.9±0.01	213.5±0.01	215.6±0.02
8	213.7±0.01	212.3±0.03	213.7±0.03	211.8±0.02	216.7±0.01
9	215.6±0.03	213.7±0.01	214.8±0.01	213.9±0.01	211.8±0.01
10	214.7±0.01	213.9±0.02	212.6±0.03	216.7±0.03	213.6±0.03
11	217.8±0.02	214.8±0.01	215.6±0.01	216.9±0.01	214.6±0.01
12	219.9±0.03	215.6±0.01	216.7±0.02	214.7±0.01	216.7±0.02

*control sample is without TBHQ

Table No. 11 Analysis of frying oil using BHA of different concentration. The frying temperature is 170 to 180˚C.

Time(hr)	Sap. Value of control	Sap. Value of 100ppm	Sap. Value of 150ppm	Sap. Value of 200ppm
0	205.9±0.02	208.33±0.03	209.5±0.03	204.11±0.01
1	208.9±0.01	214.82±0.01	211.3±0.01	206.85±0.01
2	210.6±0.02	215.05±0.02	211.9±0.02	207.52±0.03
3	211.8±0.01	216.75±0.01	208.2±0.01	210.8±0.01
4	208.9±0.03	219.75±0.03	207.4±0.03	215.49±0.02
5	209.7±0.01	215.34±0.01	209.3±0.03	211.03±0.01
6	212.8±0.01	212.78±0.02	211.3±0.01	213.71±0.02
7	212.7±0.02	213.9±0.01	214.9±0.02	214.73±0.01
8	213.7±0.01	213.18±0.02	215.6±0.01	208.16±0.03
9	215.6±0.03	214.85±0.01	213.2±0.02	211.25±0.01
10	214.7±0.01	215.09±0.01	214.9±0.01	212.06±0.03
11	217.8±0.02	214.85±0.01	216.8±0.01	214.09±0.02
12	219.9±0.03	215.35±0.03	215.9±0.02	213.01±0.03
13	219.8±0.03	214.29±0.01	216.74±0.01	215.89±0.02
14	220.7±0.01	213.94±0.02	217.56±0.02	216.76±0.01

*control sample is without BHA

Table No. 12 Analysis o frying palm oil using different concentration of BHT. The frying temperature is 170 to 180°C

Time(hr)	Sap. Value of control	Sap. Value of 100ppm	Sap. Value of 150ppm	Sap. Value of 200ppm
0	205.9±0.02	208.5±0.01	208.4±0.01	209.6±0.01
1	208.9±0.01	211.6±0.01	210.6±0.01	209.8±0.04
2	210.6±0.02	211.5±0.02	210.8±0.03	210.6±0.01
3	211.8±0.01	211.6±0.01	211.5±0.01	211.6±0.03
4	208.9±0.03	209.9±0.03	210.6±0.02	211.7±0.01
5	209.7±0.01	211.7±0.01	211.6±0.01	210.8±0.01
6	212.8±0.01	211.9±0.03	212.4±0.02	212.9±0.02
7	212.7±0.02	212.6±0.01	212.8±0.01	213.4±0.02
8	213.7±0.01	213.7±0.02	213.6±0.03	213.8±0.02
9	215.6±0.03	214.8±0.01	215.6±0.01	214.8±0.01
10	214.7±0.01	214.7±0.03	215.6±0.04	214.9±0.02
11	217.8±0.02	216.7±0.01	216.4±0.01	215.4±0.01
12	219.9±0.03	218.9±0.02	217.9±0.02	216.9±0.03
13	219.8±0.03	218.7±0.02	218.6±0.01	217.8±0.01
14	220.7±0.01	219.9±0.03	219.7±0.02	218.7±0.03
15	221.7±0.01	220.6±0.01	219.9±0.01	218.9±0.01
16	221.6±0.03	221.6±0.01	220.4±0.02	219.7±0.02

*control is without using BHT

*the values are means of triplicate.

*figures in the parenthesis are the standards deviation of the means

Table No 13 Analysis of frying palm oil using TBHQ of different concentration. The frying temperature is 170 to 180˚C

Time(hr)	AV of control	AV of 112ppm	AV of 100ppm	AV of 150ppm	AV of 200ppm	Sp.gr. of control	Sp.gr.of 112ppm	Sp.gr.of 100ppm	Sp.gr.of 150ppm	Sp.gr.of 200ppm
0	0.26	0.28	0.35	0.24	0.27	0.921	0.921	0.92	0.92	0.921
1	0.39	0.38	0.38	0.25	0.32	0.922	0.921	0.921	0.921	0.922
2	0.39	0.47	0.47	0.35	0.38	0.922	0.922	0.921	0.921	0.922
3	0.48	0.54	0.55	0.47	0.38	0.924	0.922	0.923	0.922	0.923
4	0.63	0.67	0.69	0.48	0.41	0.926	0.923	0.924	0.923	0.923
5	0.85	0.71	0.85	0.57	0.43	0.927	0.925	0.926	0.924	0.924
6	1.06	0.89	0.91	0.69	0.5	0.929	0.926	0.926	0.926	0.924
7	1.08	0.96	1.03	0.81	0.68	0.930	0.928	0.927	0.927	0.925
8	1.21	1.04	1.11	0.89	0.78	0.931	0.928	0.928	0.929	0.926
9	1.33	1.18	1.24	0.97	0.85	0.933	0.929	0.93	0.929	0.927
10	1.46	1.3	1.35	1.06	0.89	0.935	0.93	0.931	0.93	0.928
11	1.58	1.41	1.45	1.13	0.94	0.936	0.932	0.932	0.931	0.93
12	1.71	1.48	1.54	1.24	0.98	0.938	0.933	0.933	0.932	0.931

*Control sample is without TBHQ

Table No 14 Analysis of frying palm oil using BHA of different concentration. The frying temperature is 170 to 180˚C

	Sp.gr.of control	Sp.gr.of 100ppm	Sp.gr.of 150ppm	Sp.gr.of 200ppm	AV of control	AV of 100ppm	AV of 150ppm	AV of 200ppm
0	0.921	0.918	0.919	0.918	0.26	0.25	0.25	0.28
1	0.921	0.918	0.919	0.921	0.39	0.3	0.27	0.3
2	0.922	0.921	0.92	0.92	0.39	0.28	0.31	0.32
3	0.924	0.922	0.921	0.921	0.48	0.39	0.34	0.35
4	0.926	0.924	0.922	0.921	0.63	0.39	0.41	0.42
5	0.927	0.926	0.923	0.923	0.85	0.49	0.49	0.42
6	0.929	0.927	0.926	0.925	1.06	0.52	0.53	0.43
7	0.93	0.929	0.928	0.928	1.11	0.61	0.6	0.47
8	0.931	0.93	0.93	0.929	1.28	0.65	0.65	0.53
9	0.933	0.932	0.932	0.929	1.4	0.73	0.71	0.6
10	0.935	0.934	0.933	0.93	1.46	0.85	0.8	0.72
11	0.936	0.935	0.934	0.932	1.58	0.99	0.9	0.83
12	0.939	0.937	0.936	0.934	1.8	0.95	0.97	0.9
13	0.942	0.939	0.937	0.937	2.05	1.08	1.02	0.96
14	0.943	0.940	0.939	0.938	2.16	1.2	1.15	1.04

*control sample is without BHA

Table No. 15 Analysis of frying palm oil using different concentration of BHT. The temperature is 170 to 180°C

Time(hr)	AV of control	AV of 100ppm	AV of 150ppm	AV of 200ppm	Sp.gr.of control	Sp.gr.of 100ppm	Sp.gr.of 150ppm	Sp.gr.of 200ppm
0	0.26	0.25	0.25	0.26	0.921	0.92	0.921	0.921
1	0.39	0.28	0.28	0.28	0.921	0.921	0.921	0.921
2	0.39	0.29	0.29	0.28	0.922	0.921	0.922	0.922
3	0.48	0.32	0.31	0.3	0.924	0.923	0.923	0.922
4	0.63	0.34	0.33	0.32	0.926	0.925	0.923	0.923
5	0.85	0.41	0.37	0.34	0.927	0.927	0.925	0.924
6	1.06	0.47	0.41	0.39	0.929	0.928	0.926	0.925
7	1.11	0.57	0.49	0.44	0.93	0.929	0.928	0.927
8	1.28	0.63	0.57	0.54	0.931	0.929	0.928	0.927
9	1.4	0.67	0.63	0.6	0.933	0.93	0.93	0.929
10	1.46	0.75	0.7	0.67	0.935	0.933	0.932	0.929
11	1.58	0.86	0.79	0.75	0.936	0.935	0.935	0.934
12	1.8	0.89	0.85	0.82	0.939	0.937	0.938	0.933
13	2.05	0.96	0.9	0.86	0.942	0.939	0.938	0.937
14	2.16	1.08	0.97	0.92	0.943	0.94	0.939	0.939
15	2.29	1.15	1.07	0.98	0.945	0.943	0.941	0.942
16	2.43	1.2	1.14	1.05	0.946	0.945	0.944	0.942

*control sample is without BHT

4.16Comparison of acid value and peroxide value using different concentration of TBHQ

Table No. 16 Analysis of frying palm oil using different concentration of TBHQ. The frying temperature is 170 to 180°C

Time(hr)	AV of control	AV of 112ppm	AV of 100ppm	AV of 150ppm	AV of 200ppm	PV of control	PV of 112ppm	PV of 100ppm	PV of 150ppm	PV of 200ppm
0	0.26	0.28	0.35	0.24	0.27	3.95	3.32	3.01	3.32	3.38
1	0.39	0.38	0.38	0.25	0.32	6.5	3.49	6.81	7.08	8.86
2	0.39	0.47	0.47	0.35	0.38	7.85	5.54	6.9	9.48	10.75
3	0.48	0.54	0.55	0.47	0.38	8.4	5.73	7.05	7.04	12.45
4	0.63	0.67	0.69	0.48	0.41	9.04	8.1	8.25	7.02	9.15
5	0.85	0.71	0.85	0.57	0.43	9.69	8.52	8.43	7.21	8.5
6	1.06	0.89	0.91	0.69	0.5	11.24	9.5	9.52	8.21	8.77
7	1.08	0.96	1.03	0.81	0.68	12.04	9.93	10.27	9.48	9.03
8	1.21	1.04	1.11	0.89	0.78	13.07	10.5	11.24	10.52	9.18
9	1.33	1.18	1.24	0.97	0.85	14.16	11.3	11.6	11.1	9.3
10	1.46	1.3	1.35	1.06	0.89	15.17	12.35	12.4	12.15	9.52
11	1.58	1.41	1.45	1.13	0.94	16.37	12.85	13.4	12.71	9.98
12	1.71	1.48	1.54	1.24	0.98	17.28	13.05	14.05	13.05	10.75

***control sample is without TBHQ.**

Table No. 17 Analysis of frying palm oil using different concentration of BHA. The frying temperature is 170 to 180°C

Time(hr)	AV of control	AV of 100ppm	AV of 150ppm	AV of 200ppm	PV of control	PV of 100ppm	PV of 150ppm	PV of 200ppm
0	0.26	0.25	0.25	0.28	3.95	3.25	2.6	2.59
1	0.39	0.3	0.27	0.3	6.5	7	8.5	9.28
2	0.39	0.28	0.31	0.32	7.85	9.5	12.08	14.31
3	0.48	0.39	0.34	0.35	8.4	12.85	15.41	17.07
4	0.63	0.39	0.41	0.42	9.04	11.61	15.8	14.73
5	0.85	0.49	0.49	0.42	13.95	13.65	13.23	13.41
6	1.06	0.52	0.53	0.43	11.24	14.98	14.21	12.87
7	1.11	0.61	0.6	0.47	12.04	16.75	13.31	11.18
8	1.28	0.65	0.65	0.53	13.07	14.01	8.98	8.26
9	1.4	0.73	0.71	0.6	14.16	12.56	9.56	8.78
10	1.46	0.85	0.8	0.72	15.17	10.25	9.98	9.09
11	1.58	0.99	0.9	0.83	16.07	9.89	10.98	9.67
12	1.8	0.95	0.97	0.9	17.28	12.98	12.56	10.54
13	2.05	1.08	1.02	0.96	17.98	14.56	13.56	11.98
14	2.16	1.2	1.15	1.04	18.85	16.56	14.30	13.76

Table No.**18** Analysis of frying palm oil using different concentration of BHT. The frying temperature is 170 to 180°C

Time(hr)	AV of control	AV of 100ppm	AV of 150ppm	AV of 200ppm	PV of control	PV of 100ppm	PV of 150ppm	PV of 200ppm
0	0.26	0.25	0.25	0.26	3.95	3.8	3.7	3.65
1	0.39	0.28	0.28	0.28	6.5	6	5.32	4.98
2	0.39	0.29	0.29	0.28	7.85	10.02	9.13	8.25
3	0.48	0.32	0.31	0.3	8.4	12.56	11.56	11.56
4	0.63	0.34	0.33	0.32	9.04	14.52	13.67	13.5
5	0.85	0.41	0.37	0.34	9.09	11.54	12.38	12.2
6	1.06	0.47	0.41	0.39	11.24	9.06	11.67	11.8
7	1.11	0.57	0.49	0.44	12.04	8.12	9.56	10.96
8	1.28	0.63	0.57	0.54	13.07	7.32	7.05	6.9
9	1.4	0.67	0.63	0.6	14.16	7.8	7.56	7.4
10	1.46	0.75	0.7	0.67	15.17	8.23	8.25	7.95
11	1.58	0.86	0.79	0.75	16.37	8.96	8.8	8.5
12	1.8	0.89	0.85	0.82	17.28	9.8	9.6	9.13
13	2.05	0.96	0.9	0.86	17.98	10.82	10.15	9.95
14	2.16	1.08	0.97	0.92	18.85	11.56	11.09	10.6
15	2.29	1.15	1.07	0.98	19.7	12.95	11.98	11.25
16	2.43	1.2	1.14	1.05	21.05	14.15	12.67	11.9

*control sample is without using BHT