EFFECT OF ANTIOXIDANTS ON PREVENTION OF AUTOXIDATION IN DESICCATED COCONUT MIXED INSTANT RICE PITTU

EFFECT OF ANTIOXIDANTS ON PREVENTION OF AUTOXIDATION IN DESICCATED COCONUT MIXED INSTANT RICE PITTU

by

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DEDICATED TO MY LOVING

PARENTS, SISTER, BROTHER, FRIENDS AND TEACHERS

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ABSTRACT

Instant rice pittu mixtures are prepared by using rice flour, desiccated coconut and salt. As rancidity is the major problem in oil based food products and it is very difficult to overcome under natural conditions, coconut containing rice pittu mixture can be also subjected to spoilage due to autoxidative rancidity. This problem however can be prevented by using antioxidants. By this study, the effect of antioxidants on the prevention of autoxidative rancidity was evaluated.

Ascorbic acid (vitamin C), α tocopherol (vitamin E), butylated hydroxy anisol(BHA), garlic powder and a combination of citric acid and ascorbic acid (vitamin C) were studied in terms of their antioxidant effects. The effect of the antioxidants was evaluated separately for two types of incorporation methods; wet form and dry form. Sensory tests were conducted immediately after the preparation and then monthly upto three months.

The results revealed that BHA was the most effective antioxidant for both forms of incorporation methods. Both garlic powder and α tocopherol kept their antioxidative effect in wet and dry form incorporation methods for three and two months respectively. Ascorbic acid and the combination of citric acid and ascorbic acid had the lowest activity. The antioxidative capabilities of these two compounds in wet and dry forms were kept for two and one months respectively.

The experimental part of this study was undertaken at Harischandra mills Ltd., Matara.

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CHAPTER 1

INTRODUCTION

In recent times Sri-Lanka is gradually developing into an industrialized country. As a result of this, the life style of the people has been remarkably changed and the people spend more time for their day-to-day activities.

Consequently they have only little time to spend for the works connected with cooking and preparation of foods. Hence they naturally tend to consume instant (quick cooking) and partially or completely processed food products.

In developed countries, there is a great demand for instant food products and necessity for this demand was being gradually increased during the last decade. Technologies for most of instant foods were already perfected in the developed world. In contrast, the developing countries like Sri-Lanka are yet to develop the techniques in order to increase the potential for instant food products. But still it remains in an undeveloped level. Hence a great responsibility is laid on Sri-Lankan food manufactures in promoting the production of instant foods.

Instant food products should have several properties; It should keep the nutritional value and a longer shelf life without loosing sensory characteristics until it reaches to the consumer; It should also be easily pre-cooked within few minutes by simple cooking methods; Cost factor is also of primary significance. The quality of an instant food product, after preparation, should match that of the product prepared by conventional methods. Other characteristics of the instant food product should be almost similar with conventionally processed product as far as possible.

Factors enhancing the deterioration of instant food products can be divided into macro (insects, and other organisms) and

micro (microbial, biochemical, chemical, physical) factors. In order to overcome the problems of deterioration, food processors should be able to control these factors.

Acceptance of instant food products mainly depends on the ingredients of the product and the similarity to the foods widely consumed by the nation consumers. As far as Sri-Lankan consumers are concerned they would prefer a rice-based instant food product being rice the staple food in Sri-Lanka.

Among the foods in Sri-Lankan cuisine, pittu is one of the popular traditional food items. Therefore our main objective was to develop a rice-based instant pittu with a long shelf life. The shelf life of the product is mainly determined by microbiological and biochemical factors. Therefore the moisture content of the formula should be kept at an optimal level in order to enhance the shelf life. As the pittu formula consists of oil containing desiccated coconut, our immediate objective was to evaluate the antioxidant effect on suppressing the biochemical reactions leading to this deteriorative process.

CHAPTER 2
REVIEW OF LITERATURE

2.1 RICE

Rice (*Oryza sativa* L.) is the most important cereal crop in the developing world and is the staple food considered a semi-aquatic annual grass plant. About 20 species of the genus *Oryza* are recognized, but nearly all cultivated rice is O. sativa L. A small amount of *Oryza glaberrima*, a perennial species, is grown in Africa. So-called "wild rice" (*Zizania aquatica*), grow in the Great Lakes region of the United States, is more closely related to oats than to rice. (Lupien,J.R.,1993)

Rice from which only husk has been removed, but layers of bran and most of the germ retained, is known as brown rice. Rice from which husk,germ and bran layers have almost completely been removed by power machinery is known as milled rice. Rice milled to a high degree is known as white rice. Rice from which husk, germ and bran layers have been partly removed without the use of power machinery is known as hand-pounded rice. Rice milled to a high degree and then coated with some foreign substances such as glucose or talcum is called polished rice. Paddy specially processed by steaming or soaking in water, then heated and dried, is called parboiled paddy. It can then be milled to various degree or home pounded. It is called parboiled milled or parboiled home pounded. (Bakhru,H.K.,1990)

2.1.1 Food Value

Starch constitutes the bulk of the rice grain. The protein content of the rice is lower than that of wheat, but is of superior quality and utilized better by the body than the wheat protein.

Table 1

Proximate Composition of Raw Milled Rice*

Food value		Minerals and Vitamins	
Moisture	13.7%	Calcium	10mg
Protein	6.8%	Phosphorus	160mg
Fat	0.5%	Iron	3.1mg

Minerals	0.6%	Small amount of
Fibre	0.2%	Vitamin B Complex
Carbohydrates	78.2%	
	100 %	

*Value per 100 gms edible portion Calorific Value - 345kcal
(Cited: Bakhru,H.K.,1990. Food that heal. The natural way to go ..ealth)

2.1.1.1 Nutritive Value of Rice Proteins

The protein content of rice varies from 6 - 9 percent depending on the variety. The chief protein is glutelin. Small quantities of albumin, globulins and prolamins are also present. The amino acid content of rice protein is given in table 2. The proteins are limiting in lysine, and threonine and to a lesser extent in methionine. The protein efficiency ration of rice proteins ranges from 1.8 - 2.2 and is increased to about 2.4 - 2.6, when supplemented with a mixture of lysine and threonine and to 2.8 - 3.0 when supplemented with lysine, threonine and methionine.

Table 2
Amino Acid Content of Rice Proteins

Amino acid	Amount
Lysine (g/16gN)	3.8
Threonine (g/16gN)	3.7
Tryptophan (g/16gN)	1.1
Methionine (g/16gN)	1.7
Cystine (g/16gN)	1.8
Total s - amino acids (g/16gN)	3.8
Chemical score(%)	60

(Cited: Kent, N.L., 1982. Technology of cereals)

2.1.2 Utilization of Rice

Milled rice is consumed largely in the boiled state. In the United States some rice is sold in a precooked or partly precooked condition. It also is used in the manufacture of breakfast foods such as puffed rice, flaked rice, and rice crispies. Broken rice is used for human food as well as for the making of alcoholic beverages.

2.1.2.1 Rice Flour

White rice flour is made from second head rice using pearlier, granulator, pulverizer and finishing machine. It may be made from parboiled or non parboiled rice, and it may be enriched (with iron, thiamin and niacin) on unenriched.

Brown rice flour is made from brown rice which has not been milled or polished.

Rice flour is used in refrigerated biscuit manufacture to prevent sticking; in baby foods, as a thickener; in waffled and pancake mixes, as a water absorbent.

Rice bread has been made from rice flour, using a gum, hydroxy propylmethylcellulose, as a substitute for gluten. The low contents of sodium, protein, fat and fibre and the high content of rice bread as an alternative to wheat bread for persons suffering from inflamed kidneys, hypertension and coeliac disease. (Kent, N.L.,1982)

2.2 COCONUT

The coconut (*Cocos nucifera*) is known as a "wonder food". It is a near perfect diet, as it contains almost all the essential nutrients needed by the human body. It is also considered a sacred fruit and holds a very high place in all religious ceremonies. (Bakhru,H.K.,1990)

Coconut reached East Africa, and possibly Panama before 1492. Thereafter, it gradually spread to all the topical areas of the world. It is now widely cultivated in India, Sri-Lanka, Indonesia, Philippines, the East Indies, the West Indies, and the islands of the Indian and Pacific Oceans. Coconut tree grows abundantly along the entire coast of the sea and it thrives well in loose sandy soil. Its age varies from 80 to 200 years.

(Bakhru,H.K.,1990)

2.2.1 Food Value

The coconut is highly nourishing, strengthening and fattening food article. The coconut has a high oil content which is easily digestible. It is more easily utilized by the body than all other fats. The protein content of coconut is of high quality, containing all the amino-acids. It is also rich in potassium, sodium, magnesium and sulphur. The energy value of the dried coconut is very high. (Bakhru,H.K.,1990)

The chemical composition of coconut and its products is given in table 3.

Table 3

Chemical composition of Coconut and its products.

	Coconut kernel (tender)	coconut kernel from mature nuts	Desiccated coconut	Copra	coconut milk (from mature nuts)
Moisture(%)	90.8	46.3	3.5	4.8	89.8
Protein(%)	0.9	4.1	6.3	7.1	0.8
Fat(%)	1.4	37.3	57.4	64.4	7.2
Crude Fibre(%)	_	3.4	7.5	5.9	_
Carbohydrate(%)	6.3	7.9	24	16.1	2
Calorific Value(kcal/100g)	41	48	618	672	76

(Cited: Swaminathan,M.,1988. Hand book of food science and experimental foods)

2.2.2 Desiccated coconut

Desiccated coconut was first manufactured from imported nuts in England and the USA in the year 1880s. This is uneconomic compared with production in coconut-growing countries, and by 1890 manufacture was under way in Sri-Lanka.

2.2.2.1 Manufacture

Desiccated coconut is made from the white part of kernel, the brown testa or parings having been removed. The white meat is disintegrated or shredded and dried at 60^0 - 75^0C to a moisture content of less than 2.5 per cent.

In Sri-Lanka husked coconuts are delivered to the factory, usually after three or four weeks of being stacked in the field before husking. The shells are chipped off by male workers

("hatcheters") using a special type of small hatchet. Damaged kernels or germinated nuts are rejected and used to make low-grade copra.

Formerly the kernels were well washed in running water and fed to the disintegrators. Owing to the occurrence of salmonella infection in samples of desiccated coconut supplied to Europe and to Australia, it is now the practice, after washing the kernels, to sterilize them by immersing in boiling water. Kernels are put into wire baskets which can be held in boiling water for the necessary time.

After sterilization the kernels are fed to the disintegrators, which reduce them to a wet meal. Alternatively, cutters are used which produce "fancy cuts" such as thread and chip.

The more usual indirect fixed driers operate at a temperature inside the drier of about 75° - 80° C and drying takes 45 to 50 minutes. With direct oil firing at an inlet temperature of 120° C and of saturated air at outlet of 80° C, drying time is reduced to 25 minutes. The dried product is allowed to cool on galvanized tables, and is sifted into grades, usually with a double glassine liner.

(Child,R.,1974)

2.2.2.2 Quality Standards

Quality standards laid down by the Sri-Lanka government's Bureau of Standards, are enforced by the Coconut Development Authority. The standards currently prevailing are:

Colour	:- Natural white
Taste & Smell	:- Sweet and pleasant and free from cheesy, smoky,, soapy, sour or other undesirable flavours.
Moisture	:- Not exceeding 3 percent for coarse, medium, fine and superfine grades and 3.5 per cent for the special grades.
Oil Content	:- Not less than 68 per cent.
Acidity	:- Note in excess of 0.3 per cent of free fatty acids calculated as lauric acid.
Foreign Matter	:- Free from all foreign matter including shell, coconut fibre, metal particles and textile fibres.
Bacterial Contamination	:- No bacterial contamination at all of the salmonella group in 50g samples.
Parings	:- Brown specks due to paring in medium and coarse grades not to exceed 10 particles per 100g taken at random. (C.D.A)

2.2.2.3 Uses

Desiccated coconut is used in a variety of major consumer project areas. In the confectionery industry it goes into chocolate bars, nut based chocolate products and candy. In the food processing trade it is used extensively in packaged and canned [illegible] products. [illegible] frozen food industry provides an outlet for it, in the ice cream trade and the frozen food trade. In the bakery industry it is a widely used ingredient in cakes and biscuits, toppings and desserts. The restaurant and hotel service trade is another major consumer. And finally it is a well known and widely used item in home baking. (C.D.A)

2.2.3 Coconut Oil

Coconut oil consists of different types of fatty acids, the average composition of which is as table 4.

Table 4

The Average Fatty Acid composition of Coconut Oil.

Saturated		
C6	Caproic	0.5%
C8	Caprylic	8.0%
C10	Capric	7.0%
C12	Lauric	48.0%
C14	Myristic	17.0%
C16	Palmitic	9.0%
C18	Stearic	2.0%

Unsaturated		
C16 : 1	Palmitoleic	0.2%
C18 : 1	Oleic	6.0%
C18 : 2	Linoleic	2.3%

(Cited: U.S.C.C.R.I, Health effects of all natural coconut oils)

Table 5

Linoleic acid content of rice(milled) and coconut.

Values per 100g edible potion	Rice (milled)	Coconut
Calories	360	600
Total fat (g)	0.6	62.7
Saturated fat (g)	0.3	57.4
Poly unsaturated fatty acid (g)	0.2	0.9
Linoleic acid (g)	≤ 0.2	≤ 0.9
Linoleic acid % of total fat	33	≤ 1.5
Linoleic acid % calories provide	≤ 0.5	≤ 0.1

(Cited: Rajalakshmi,R.,1981. Applied nutrition)

2.3 LIPIDS

Lipids are important constituents in foods and in food preparation.

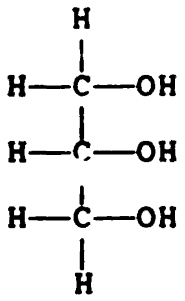
Fats are important in many types of recipes. They are used as the medium of heat transfer when foods are fried. Some fats contribute flavour to foods.

2.3.1 Chemistry of Fats

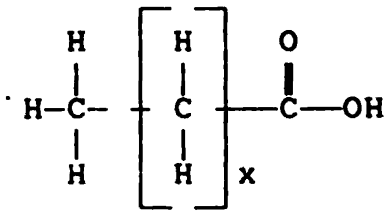
Glycerol:-

Fats are composed of two substances, glycerol and fatty acids. Glycerol is a three - carbon alcohol containing three hydroxyl (-OH) groups. Fatty acids are organic acids that

contain a chain of carbon atoms linked to a terminal organic acid radical or carboxyl group (-COOH). A variety of fatty acids occurs in the various common fats and oils.

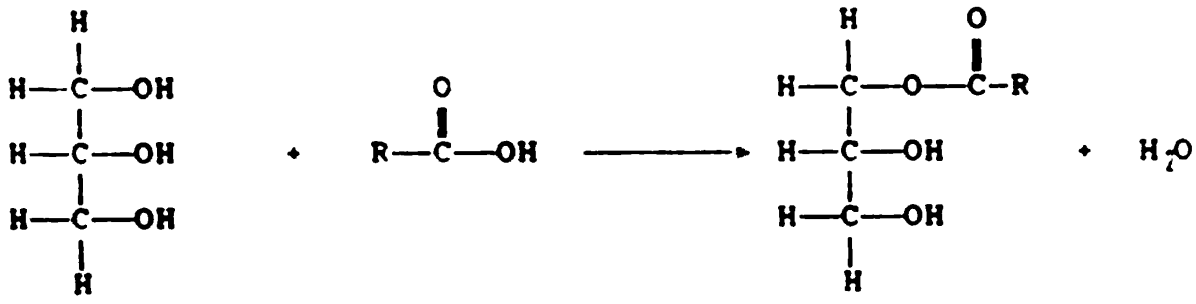


Glycerol

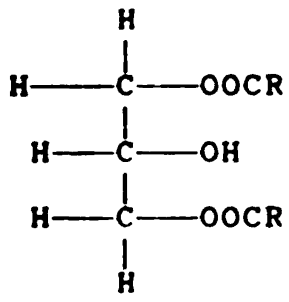


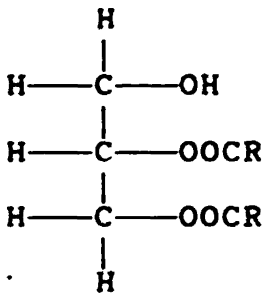
Fatty Acid

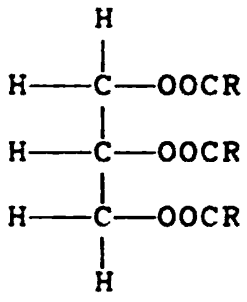
When fatty acids esterify with the hydroxyl (-OH) group of glycerol, a fat is formed. The compound formed when one hydroxyl group is esterified with a fatty acid is termed a monoglyceride; two hydroxyl groups combined with two fatty acids are a diglyceride; and all three hydroxyl groups reacting with three fatty acids form a triglycerides. In the equation (which illustrates the formation of a monoglycerde and a molecule of water), the R represents the carbon chain of the fatty acid. Actually, esterification can occur on any of the three carbons of glycerol, and therefore other permutations occur in addition to the one shown.



Glycerol Fatty acid Monoglyceride







Monoglyceride Diglyceride Triglyceride

Fatty acids

The chemical composition of the fatty acids is responsible for the variable characteristics of fats. Fatty acids naturally occurring in foods usually contain an even number of carbon atoms ranging in chain length from four to twenty two carbon atoms, and they either may be saturated with hydrogen or contain a varying number of double bonds capable of adding hydrogen, in which case they are known as unsaturated fatty acids. These double bonds are located in positions that are characteristic for each particular unsaturated fatty acids. When a fatty acid has more than one double bond, it is said to be polyunsaturated.

The backbone carbon chain of the fatty acid is a linear configuration except where a double bond occurs. At a double bond, the chain may continue in its same configuration (trans) or the next portion of the chain may proceed at a different angle, as illustrated. When the chain proceeds in an altered direction, the configuration is classified as cis. In nature, the most commonly occurring unsaturated fatty acid is oleic (18 carbons) and the most common saturated fatty acid is palmitic (16 carbons). (Mcwilliams,M.,1979)

2.4 RANCIDITY

Fats may spoil because they have become rancid. Rancidity is the presence of an undesirable odour or flavour caused by the oxidation or hydrolysis of fats. Inhydrogenated fats a preliminary change called reversion[a type of flavour and odour degradation usually associated with vegetable, fish, and other highly unsaturated oils. This flavour degradation is thought to

be brought about by oxidation of linolenic - type acids (Furia,T.E.,1972)] causes highly unsaturated fats to develop a slightly fishy flavour before true rancidity occurs. Actual rancidity is classified as oxidative or hydrolytic.

(McWilliams,M.E.,1974)

2.4.1 Oxidative Rancidity

There are three types of rancidity due to oxidation, i.e., autoxidation, photoxidation and enzymic-oxidation.

2.4.1.1 Autoxidation

Atmospheric oxidation (autoxidation) is the chief factor in quality deterioration of fats and fatty portions of foods. Fats and fat like substances undergo oxidative deterioration which results in off - flavours and off - odours. In extreme cases toxic by-products have resulted from oxidative reactions. Generally fat oxidation does not progress to a point where toxic by-products are a factor. That portion of fat which is reactive at any given time is quite small. It is estimated that not more than about 10% of the unsaturated molecules may undergo breakdown due to atmospheric oxidation. The resulting products of fat oxidation, however are organoleptically potent and adversely affect the marketability of the food.

(Furia,T.E.,1972)

2.4.1.1.1 Mechanism of Autoxidation

Unsaturated and saturated fatty acids and their esters can be oxidized by the usual chemical oxidizing agents, such as nitric acid, chromic acid, ozime, potassium permanganate, hydrogen peroxide, etc. Such reactions are important industrially and form the basis of certain useful analytical methods, but are not of prime importance to the food technologist. Autoxidation (atmospheric oxidation) under the relatively mild processing and storage conditions of the food industry is of utmost importance because of the resultant malodour and malflavour producing aldehydes and ketones.

Autoxidation of unsaturated lipid substances may be divided into two general areas: (1) the oxidation of the highly unsaturated fats, particularly the polyunsaturated fats,

resulting in polymeric end products, and (2) the moderately unsaturated fats which result in rancidity, reversion, and other types of off-flavours and off-odours.

The path of lipid autoxidation, as well as the resulting end products, depends to a large extent upon the conditions of the oxidation; i.e., temperature, catalysts fatty acids-types, the distribution and geometry of the double bonds, and the amount of oxygen available. The flavour and odour of the more highly saturated animal fats and hydrogenated oils whose unsaturated acids consist largely of mono-unsaturated acids are not significantly altered during the early phases of oxidation. The onset of rancidity in such fats is both sudden and definite.

The oxidation of fatty substances is autocatalytic and has the characteristics of a "chain reaction", which may be broken into three stages or links: (Furia,T.E.,1972)

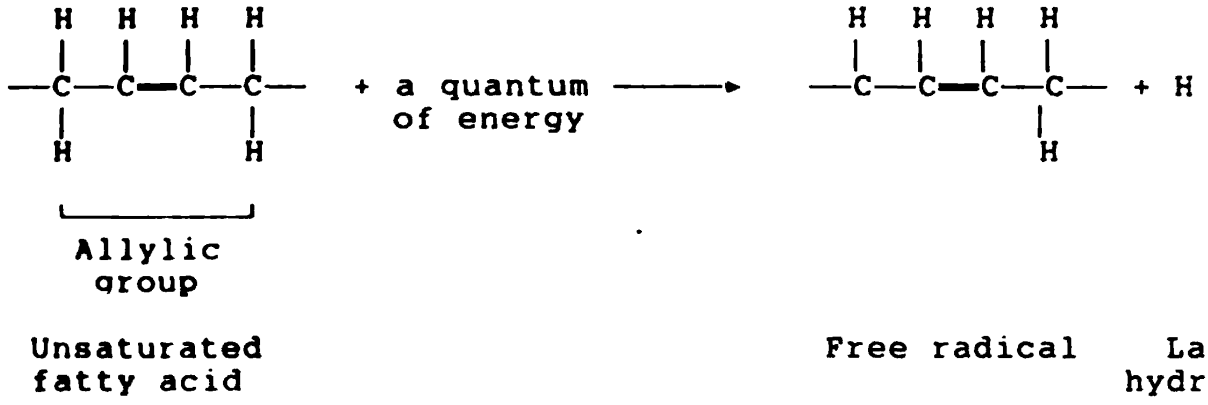
1. Initiation :- Production of R. or RO_2 radicals
2. Propagation:- $R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot}$
3. Termination:- $RO_2^{\cdot} + RH \longrightarrow RO_2H + R^{\cdot}$

   Interaction of radicals to produce non-initiating and non-propagating products. (RH represents alkene substrate, H represents allylic hydrogen).
   (Gunstone, F.D., & Norris,F.A., 1983)

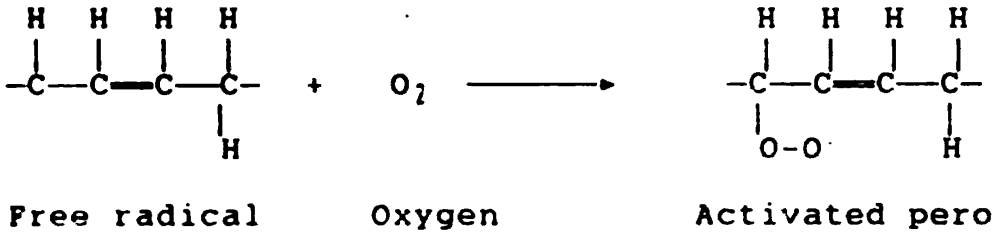
The nature of the initiation reaction is still uncertain, though it is known that hydroperoxides once formed furnish additional initiating radicals. The reaction of alkenes with singlet oxygen to produce hydroperoxides may play a key role in the initiation of autoxidation. The propagation sequence involves the production of a radical R from the alkene RH and its subsequent reaction with oxygen. The radical results from the alkene by reaction at the allylic position and is resonance-stabilized. This affects the structure of the final product. The termination reactions have not been extensively studied.
(Gunstone,F.D., & Norris,F.A.,1983)

eg:- According to the theory held currently, a hydrogen on a carbon adjacent to one carrying a double bond is displaced by a

quantum of energy to give a free radical.

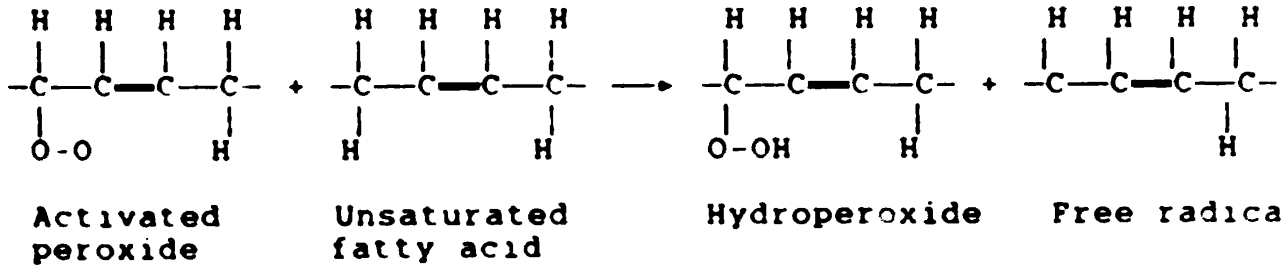


Both heat (for Autoxidation) and light(for photoxidation) are common sources of the energy which gives rise to free radicals. Molecular oxygen can unite with the carbon which carries the free radical to form an activated peroxide as follows:



The energy from this activated peroxide can displace a hydrogen from another unsaturated fatty acid and thus activate it. The latter becomes a free radical.

The displaced hydrogen unites with the activated peroxide to form a hydroperoxide. This part of the reaction is:



In this way the energy which catalyzes the oxidation of fatty acid is not squandered but is passed on to another fatty acid where it repeats the process. A free radical is particularly troublesome because it is the beginning of a self-perpetuating reaction whereby the oxidation of many unsaturated

fatty acid radicals is catalyzed. A hydroperoxide is very unstable, decomposing into compounds with shorter carbon chains. These include fatty acids, aldehydes, and ketones, which are volatile and which contribute to the unpleasant odour of rancid fat. (Charley,H.,1970)

Autoxidation is facilitated by pro-oxidants and inhibited by antioxidants. Pro-oxidants, such as metals(iron, copper, nickel, or cobalt) or other radical initiators, operate by promoting the initiation step in the chain reaction or they may inhibit the activity of antioxidants. (Gunstone,F.D.,1983)

Facts which must be considered when solving stability problems are that, in most cases,

1. The primary products from autoxidation reactions are odourless and tasteless;
2. At higher temperatures and/or oxidation levels, secondary direct addition of oxygen may take place at double bonds of oleic and linoleic acids leading to the formation of various non-volatile oxygen containing compounds;
3. These secondary products are generally odourless but not tasteless;
4. Flavour and odour of the ketonic and aldehydic group are extremely intense.

In recent years, gas chromatography has been used to identify and isolate these various off-flavour producing substances. Evens has shown that oxidative off-flavours consist of a number of odoriferous aldehydes, ketones, and other short-chain volatile compounds, (Furia,T.E.,1972)

2.4.1.2 Photo-oxidation

Photo-oxidation involves reaction of an alkene with oxygen in the presence of light and a suitable sensitiser. Sensitisers such as riboflavin activate the alkene and produce the same products as autoxidation, but other sensitisers such as erythrosine or methylene blue convert oxygen to its more reactive singlet state. This reacts with alkenes in a non-radical concerted process (the ene reaction): oxygen becomes attached to

accompanied by double bond migration.

Photo-oxidation is much quicker than autoxidation and the difference in reactivity between oleate, linoleate, and linolenate (1:1.3:2.3) close to the number of double bonds in this esters. The hydroperoxides produced in this way differ from those resulting from autoxidation.

It has been suggested that autoxidation of natural oils may be initiated by photo-oxidation due to pigments remaining in the oil even after processing. (Gunstone,F.D.,1983)

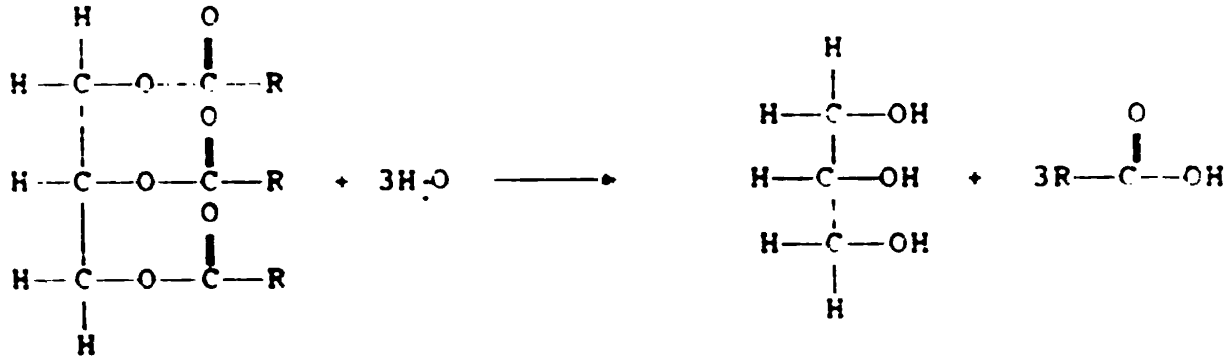
2.4.1.3 Enzymic oxidation

Lipoxygenase, widely distributed throughout the plant kingdom but also existing in animals promotes reaction between oxygen and some unsaturated acids. The natural substrate appears to be linoleic acid, but other acids are also oxidized.

Lypoxygenase contains one atom of iron in its molecule and exists in three states described as the colourless(native) enzyme, the yellow enzyme, and the purple enzyme. It promotes both aerobic and anaerobic reactions. (Gunstone,F.D.,1983)

2.4.2 Hydrolytic Rancidity

In hydrolytic rancidity, the triglyceride reacts with water and for each molecule of water involved one molecule of fatty acid is released. When a molecule of fat reacts with three molecules of water, glycerol and three fatty acids are formed, as shown bellow.



A triglyceride Water Glycerol Fatty acid

(Charley,H.,19[illegible])

Such reversible reactions are catalyzed by lipases(enzymes that act on fats) and certain other enzymes, acids, and high temperatures.

Enzymatic reactions are slowed by cold temperatures, but the exact temperatures needed to adequately slow the reaction rates of particular enzymes vary with different species. In liver, hydrolytic rancidity develops rapidly at 37°C,but at -10°C the enzymes needed for the reaction are less effective and the changes proceed very slowly. In contrast, poor flavour can be caused in fish by hydrolytic changes even in storage at -14°C, but storage at -22°C greatly retards these reactions.

The presence of water naturally encourages hydrolytic rancidity, but this type of rancidity can occur even at the moisture level of dehydrated foods. When hydrolysis produces free fatty acids with a chain length of twelve or fewer carbons, a rancid flavour is apparent.

(McWilliams,M.E.,1974)(Faria,T.E.,1972)

2.5 FOOD ADDITIVES

Modern food industry demands the use of food additives to maintain the quality and storage life of foods. Early days foods were preserved with incidention additives that resulted from cooking. Food was also preserved extensively in ancient times by heating, drying, salting, pickling, fermenting and smoking(Hope,J.E.,1973). Substances like common salt, vinegar, fruit acids, sugar/sugar syrup, honey have been used in various preparations. Primarily they act as preservatives.

The use of synthetic food additives became very common in the recent past.

Food additives may be defined as the substance which are deliberately added to food to achieve certain desirable technological quality. i.e.

1 To facilitate the processing.

2. To extend the storage life.

A familiar example of an additive to extend the storage life is antioxidants, which are added to retard the onset of rancidity and extend the shelf life Some packaging materials are impregnated

with antioxidants to aid in extending shelf life.

3. To improve appearance of the final food.
4. To achieve desirable organoleptic properties.

It is generally recognized that all chemicals (including food) are toxic to animals and man if large doses are administered. Therefore a limit in daily intake of these additives should be maintained by studying the safety of these additives with long Acceptable Daily Intake (ADI) is estimated and are related to the body weight.

The additives are permitted in foods when there is enough data available with respect to the toxicity level etc. Therefore, it is very important that one should never use a non-permitted additives in food.

In Sri-Lanka the legal document to control the use of Food Additives is called "Food Act No 26 of 1982". Under the Food Act various regulations are formed. The main classes of food additives are:

1. Preservatives
2. Colouring matters
3. Sweetening agents
4. Emulsifiers, stabilizers
5. Anticaking agents
6. Sequesternts
7. Flavours
8. Acids, buffers and bases
9. Firming & crisping
10. Enzymes
11. Enzymes
12. Flour & bread additives

2.5.1 Preservative additives

In Sri-Lanka the use of food preservatives is controlled by the Food (preservatives) Regulations 1991, formed under the Food Act, No 26 of 1982.

Preservatives can be defined as a substance inhibiting, retarding or arresting the process of fermentation, acidification or other deterioration/decomposition of food and it does include the following classes of preservatives.

1 Any permitted antioxidant
2 Any permitted artificial sweetener
3 Any permitted bleaching agent
4 Any permitted colouring matter

5. Any permitted emulsifier
6. Any permitted improving agents
7. Any permitted stabilizer
8. Vinegar
9. Soluble carbohydrate sweetening
10. Common salt
11. Herbs, spices etc.
12. Smo.. .., agents

2.6 ANTIOXIDANTS

2.6.1 Flavour Stability and Antioxidants

Off-flavours and of odours in oils and fats are caused by the reaction of oxygen with the unsaturated fatty acids.

In general, oxidation may be prevented or hindered by:

- Avoiding contact with oxygen
- Reducing unsaturation in the product
- Avoiding conditions favoring oxidation, namely:
  - exposure to light
  - elevated temperatures
  - presence of pro-oxidants

(Gunston,F.D.,1983)

2.6.2 Role in Fat

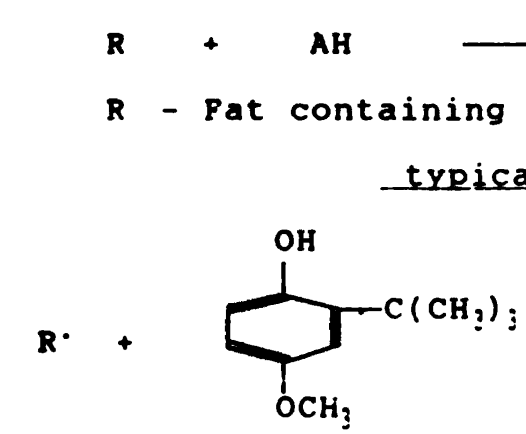
Antioxidants are either naturally occurring substances or additives that help to minimize the development of oxidative rancidity in fats. Some antioxidants retard oxidation by donating hydrogen to an unsaturated fatty acid and thus prevent the formation of the compounds capable of adding oxygen. Others prevent metals from catalyzing the oxidation reaction.

(McWilliams,M.E.,1974)

2.6.3 Mechanism of Antioxidants

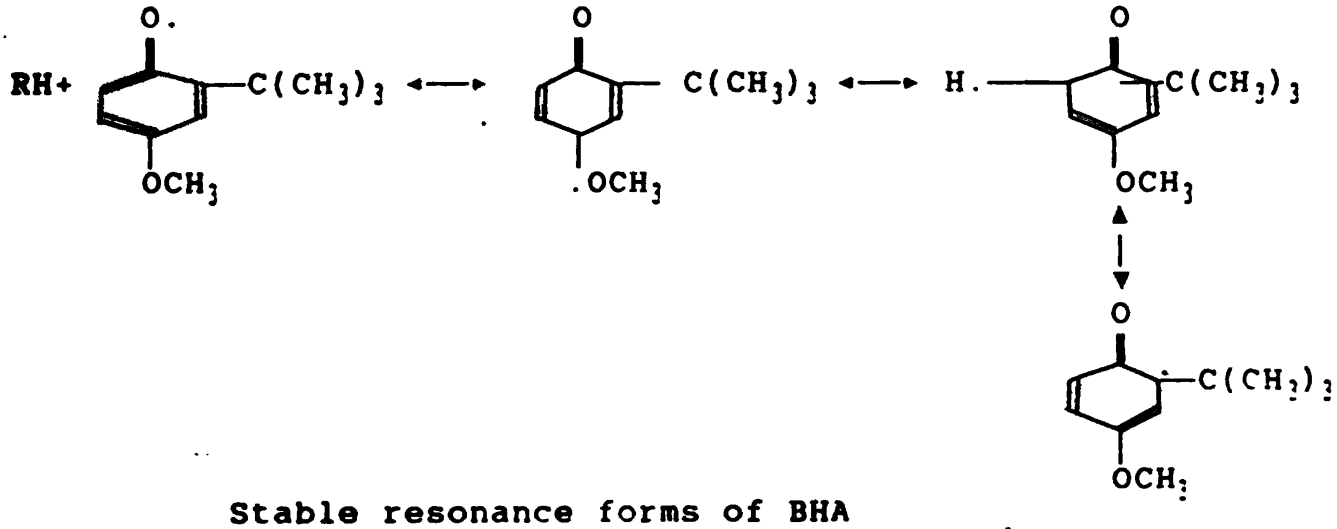
Antioxidants are usually thought to function as free radical acceptors, thus terminating the oxidation at the initiation step as shown in follow.

$$RH \xrightarrow{-H} R \xrightarrow{O_2} ROO \xrightarrow{RH} ROOH + R$$



typical antioxidant reaction

Butylated
Hydroxyanisole



Stable resonance forms of BHA

In the absence of an antioxidant, a hydrogen atom is lost from the allylic carbon in the fatty acid group with the formation of a fatty free radical (R·). The latter is readily susceptible to attack by atmospheric oxygen, resulting in the formation of peroxides and hydro peroxides. When, for example, a phenolic - type antioxidant is present, it functions as a free radical acceptor forming a stable compound that will not propagate further oxidation of the glyceride. Here the antioxidant is a "primary" one. There is also a second class, so-called "synergists", which promote or synergies the action of other antioxidants, but having little effect if present alone. Examples are citric, phosphoric, thiodiproponic, ascorbic, and

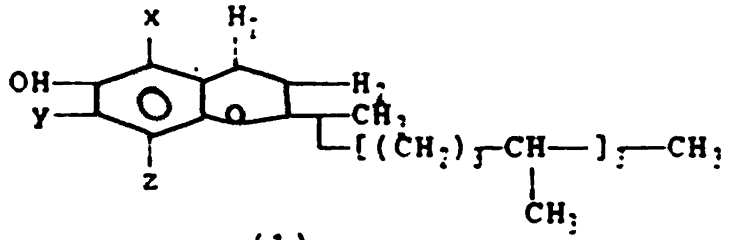
tartaric acids. These function by tying up ("chelating") pro-oxidant metals in the oil and to some extent by inhibiting peroxide decomposition and by regenerating or sparing of primary antioxidants. [(Gunstone,F.D.,1983) & (Furia,T.E.,1972)

2.6.4 Primary Antioxidants

2.6.4.1 Tocopherol

Natural fat and oils are much more resistant to oxidation than pure triglycerides because of the presence of antioxidants, so - called "inhibitors", in the former. Moreover, vegetable oils resist oxidative rancidity better than animal fats because of their higher content of these natural antioxidants, now identified mostly as tocopherols. The antioxidant action of tocopherols was first demonstrated by Olcott and Emerson, and later tocopherols were identified as the active substances in the "inhibitols" previously isolated from a variety of vegetable oils.

The four principal tocopherols are designated alpha, beta, gama, and delta (α, β, τ, δ). These Tocopherols(1) differ in the number and location of methyl groups in the aromatic ring. In general, antioxidant activity diminishes as one goes from δ to τ to β to α tocopherol.

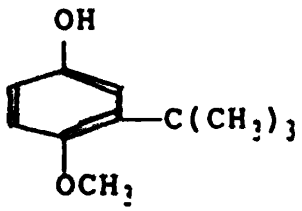


(1)

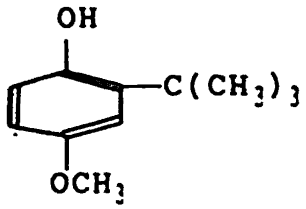
Tocopherol	x	y	z
α	CH3	CH3	CH3
β	CH3	H	CH3
τ	H	CH3	CH3
δ	H	H	CH3

2.6.4.2 Butylated Hydroxy Anisole (BHA)

Butylated hydroxy anisole is a mixture of 2a (~ 15%) and 2b (~ 85%). It was approved in the United States in 1948 for food use and has since found widespread use in other countries. Although not very effective in vegetable oils. BHA antioxidant action has a carry - through effect in baked and fried foods containing fats and oils. It may be used in combination with other pr[illegible] antioxidants [illegible]t possesses a [illegible]iceable phen[illegible] odour, especially when heated to high temperatures.



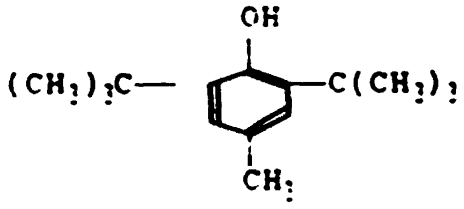
(2a)



(2b)

2.6.4.3 Butylated Hydroxy Toluen

BHT (3), previously used extensively in non-food applications, was cleared in the United State in 1953 for use in food oils. It has relatively low antioxidant effectiveness in vegetable oils, but, like BHA, is often used with other primary antioxidants because of its carry - over effectiveness in baked or fried products. Pending tests in progress in connecting with a review of all "GRAS" (Generally Regarede As Safe) substance, the use of BHT is temporarily restricted in the United States to current levels in foods for which it is now approved.



(3)

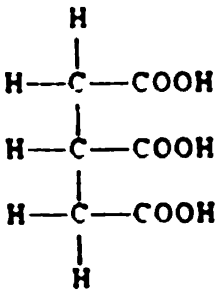
2.6.5 Synergistic Antioxidants

Although the exact nature of the synergism is not well understood, much of the effect may be due to inactivation of prooxidant metals present in the oil as a result of chelation

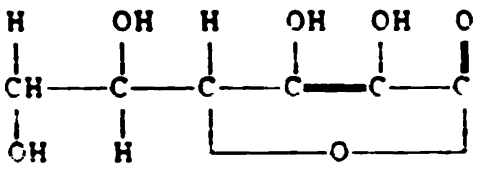
with metals. Copper and iron are most important on a practical basis. It is often assumed that these metals are present as soaps formed by the action of free fatty acids in the oil during processing from seed to crude oil; however, this could not be demonstrated in several plant tests. It appears more likely that metals are bound organically in some form other than soap as a prooxidant component of the seed. This must be broken down and the me . inactivated b. .elation with s : synergists as .ic acid, some heat is generally required.

When prooxidant metals inactivated or removed, primary antioxidants have an easier task. On the other hand, in the presence of prooxidant metals, at least in vegetable oils like soy bean, primary antioxidants are little value. It is, therefore, recommended that citric acid always be added in the cooling stage of deodorisation. Since citric acid decomposes at 150°C (302°F), well bellow deodorisation temperatures, this practice appear logical, although, for some reason, a few processors have had good results adding citric before deodorisation.

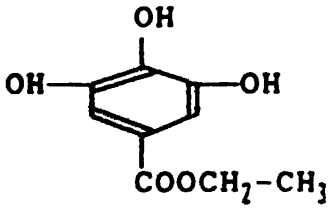
Although citric acid is the most commonly used synergist (or metal chelant), other acids, like ascorbic and tartaric, have been used. The phospholipid, lecithin, is also effective, but may introduce colour or flavour problems. Phosphoric acid is very effective, but the level of usage is critical. Excessive amounts can cause the development of melon-like and cucumber-like off flavours with resultant lower flavour scorch in aged oils, despite im|rove oxidative stability. (Gunstone,F.D.,1983)



Citric acid



Ascorbic acid



Propyl gallate

2 5.6 Selection of antioxidants

The selection of antioxidants for food fats is limited in the United States to those compounds which have been approved under applicable regulations. Among other qualifications, these antioxidants must have adequate potency, contribute no off-flavour or odours to the products in which they are used, and be economically attractive when compared to other methods for inhibiting fat oxidation, such as vacuum packaging, low temperature storage and others. Ethoxyquin is approved only for use in animal feeds, dehydrated forage corps, and some speciality uses. It has a very low order of potency in rendered animal and vegetable fats compared to other food-approved antioxidants.

The antioxidant formulations most commonly used in edible products contain various combinations of BHA, BHT and/or propyl gallate together with citric acid in a suitable solvent. The higher gallates, such as dodecyl gallate, while approved for food use in several foreign countries, are most widely used. Several S - containing compounds, such as thiodipropionic acid and dilaurylthiodipropionate, are approved for use in edible fats, but their relative ineffectiveness has discouraged their use in food. Also S - containing compounds often produce some odour and flavour problems in most food fats. They are widely used, however, in combination with phenolic-type antioxidants in the stabilization of some food packaging films, particularly the polyolefins.

The tocopherols, gum guaiac, and similar natural antioxidants usually lack potency in most products compared to combinations of BHA, BHT and propyl gallate.

One of the major problems associated with using antioxidants under commercial conditions has been the failure to achieve complete dispersion. When large quantities of rendered fats and

oils are being stabilized, extreme care should be exercised to completely blend the antioxidant into the fat so solution occurs. When adding antioxidants to food products containing relatively small quantities of lipids, the problem is intensified.

The following application methods concerning the commercial usage of antioxidants much necessarily be general fine equipment availability, physical nature of products, and other factors vary widely. All unsaturated fats should be stabilized as soon as possible after being processed, particularly when subjected to heat. The oxidative reaction is autocatalytic, so that even a short delay without antioxidant protection can under extreme conditions, be injurious to organoleptic quality. Even the best of antioxidant systems employed in food products will not inhibit rancidity in a product which was reached the end of its induction period. i.e., the formation of sufficient quantities of stable hydroperoxides. If possible, antioxidants should be added to a fat prior to heat processing. When heating a fat to increase the solubility of antioxidants, minimum conditions should be used since excessive heat also catalyzes breakdown reactions. Infection systems, where portions of an antioxidant have proved quite practical in large installations. Smaller operations usually depend on batch process with subsequent agitation.

Chelators, included in most antioxidant formulations, must be approved from a toxicological stand point, cause no odour, flavour or colour problems, and be effective at the concentrations used. Citric acid is the most widely used chelator in the food industry. It is used alone and in combination with the phenolic - type antioxidants. This natural acid (which is also used as a flavour modifier is approved on a "no limit" basis by the U. S. Food and Drug Administration. Since citric acid is only slightly soluble in food fat, propylene glycol is often used as a co-solvent to assist in dissolving the acid in fats. Phosphoric acid is used on a limited basis as a metal chelator, particularly in some vegetable oils and some fish fats. It also has food additive uses in addition to its metal chelator properties.

Various monoglrceride and lecithin - citric acid reaction

products and esters, eg:- isopropyl citrate, have been approved as food additives and are easily incorporated into the fat. Generally, these esters are not as effective chelators as citric acid. (Furia,T.E.,1972)

2.6.7 Commercial Antioxidants

Commercial use of antioxidants to stabilize fatty food on a major scale began in 1947 in the U.S. Farller attempts using natural antioxidants had not been too successful. The first satisfactory antioxidant combination was a mixture of BHA, propyl gallate, and citric acid to stabilize lard for shortening purposes. This combination of antioxidants is currently in wide use. Increasing shelf life with antioxidants, however, has spread to practically all major fats and food products which are subject to oxidative deterioration. (Furia,T.E.,1972)

2.6.8 U. S. Government Regulations

In general, approved antioxidant compounds may be added to products subject to regulation under the Food, Drug and Cosmetic Act at no more than 0.02 per cent based on the fat content of the food.

Products covered under the Meat Inspection Act and Poultry Inspection Act can generally be treated with approved types of antioxidants at concentrations up to 0.01 percent of an individual antioxidant and a combined total of not more than 0.02 per cent of all approved antioxidants based on the weight of the fat. Regulations and tolerances applying to the use of antioxidants are quite complex and are being modified continually. The allowable concentrations of some antioxidants are given in table 6.

Table 6
Allowable concentrations of some antioxidants

Antioxidant	Limitation
1.BHA (Butylated hydroxy anisol)	0.02% of fat or oil content including essential (volatile) oil content of the food.
2.BHT (Butylated .droxy toluen'	
3.Propyl gallate	
4.Dilauryl thiodipropionate	
5.Thiodipropionic acid	
6.Gum guaiac	0.1% in edible fats and oil
7.Tocopherols	No listed limits

(Cited: Gunston, F.D., Norris,F.A.,1983. Lipida in foods, chemistry, Biochemistry and technology)

2.7 PACKAGING

The main aims of packaging are to keep foods in good condition until they are consumed (to give them their expected shelf life) and to encourage customers to buy the food. For many the success of a small business depends on the type and cost of available packaging. Correct packaging extends the shelf life of food which allows it to be distributed to more distant markers. Good packaging also attracts customers to buy a product in preference to competitor's brands and can develop an image of quality or value for money, so that people buy the same products again.

In many parts of the world, foods are wrapped in simple materials such as reused newsprint, leaves or papers. These are normally only used for foods that are eaten soon after purchase (eg:- snack foods and bakery goods) and need little protection by packaging. Food with a longer expected shelf life have different needs and may require more sophisticated packaging to protect them against air, light, moisture, crushing, insects, or micro-organisms. For this food packaging should perform the following functions:

* It should provide a barrier against dirt and other contaminants thereby keeping the food clean.
* It should prevent losses. (eg:- packages should be securely closed to prevent leakage.)
* It should protect food against physical and chemical damage (eg:- bruising, crushing or the harmful effects of air and light).
* It should be convenient for handling and storage during distribution, sale and in the home.
* It should help the customer to identify the food and give instructions on how to use it correctly.

(Fellows,P.,1993)

2.7.1 Appropriate packaging for Coconut Products

Coconut food products must be divided into liquids, semi - liquids and solids to consider them from the packaging standpoint. Without exception, however, they are vulnerable to the deleterious effects of moisture and oxygen, moisture leading to growth of yeasts and moulds, and oxygen to rancidity. Any new technologies put forward have therefore to keep these primary problem areas in mind. At the same time, new technologies and materials can in some cases deliver the required package performance with lower weights, volumes and costs.

(Robson.N.C.,1992)

2.7.1.1 Polypropylene

Polypropylene is a clear glossy film with a high strength, and is puncture resistant. It has low permeability to moisture, gases, and odours, which is not affected by changes in humidity. It stretches, although less than polythene. It has good resistance to oil, and therefore can be used successfully for packaging oily products. (Fellows,P.,1992)

2.8 SENSORY EVALUATION

The production of finished foods, either by the farmer or the food processor, implies that the consumer will accept these products and pay the requisite price, i.e., that the product has

a certain quality. It is easier to recognize quality than to define it. Quality is obviously some sort of mental summation of the physical and chemical properties of the food. Many sensory factors are involved but the relation of each to palatability is not unknown. So chemical and physical tests, although can be correlated with quality, must be supplemented with sensory tests. In order to determine differences between food a variety of sensory testing procedures have been developed. These are used to select sensitive panels, to insure uniform quality, or to defect the difference in food quality between processes, raw materials, or storage conditions.

(Amerome,A.M.,1973)

Definition :- Sensory evaluation is a scientific discipline used to evoke, measure and interpret reactions to the characteristics of foods and materials as they are perceived by the senses of smell, taste, touch, and sound. (Stone,H.,1985)

2.8.1 Practical Requirements of sensory tests

The practical requirements for sensory tests are;

1. <u>Panel members and panel leader</u>:-

For difference taste, at least 10 panel members are required. A flavour profile panel consists of 4 - 6 panel members. They should be given adequate training in the methods of analysis.

2. <u>Test room</u>:-

A room with individual booths should be provided for flavour profile analysis. A round table has been introduced for the panel. The panel members do the analysis first individually in recording the results. Only after all the panel members have completed the analysis, an open discussion of the results takes place. The test rooms should be air conditioned to keep temperature and humidity constant. About 70° F (20°C), 60% relative humidity and light intensity 150 Lx are recommended. The room should be free from odour and noise.

3. <u>Sample preparation and presentation</u>:-

Method of preparing samples and the most appropriate means

of presenting them have to be determined before the start of experiment. The temperature of the test products should be standardized and kept constant.

4. Techniques of smelling and tasting:-

For odour tests of food products, a special technique is frequently used to perceive the aroma more clearly; smelling is done with a short, rapid sequence of short "sniffs".

Tasting of coffee and tea is done by slurping one teaspoonful of the liquid. By this slurping technique the same effect is produced as by swallowing. Wine and milk tasters roll the liquid on their tongue, so that it reaches all parts of the tongue where taste buds are located.

5. Testing time and fatigue:-

It is important to select the correct time for conducting sensory tests. Panel members should feel fresh. The morning time between 10 and 11 a.m. is considered by many as the most suitable for conducting sensory tests.

(Swaminathan,M.,1988)

2.8.2 The Main Types of Tests Available in Sensory Evaluation

Foods may be evaluated by human sensory organs either to learn whether they are likely to be acceptable to the general public or to learn whether foods differ in certain respects. The former is called "preference testing" and the later "difference testing". Panels of judges appropriate for performing the two functions differ. The distinctions between the two types of testing are reflected both in the number of judges that constitute a panel and in the qualifications of its members as individuals.

2.8.2.1 Preference Testing

A fairly large number of individuals, representative of the public or a large segment of it, is essential for preference testing if results are to be valid. As they develop a new product, commercial enterprises use such food - testing panels to get an indication of the way the public will react to it before they attempt to put it on the market. (Charley,H.,1970)

2.8.2.2 Difference Testing

The other type of food-testing panel is used as an instrument to assess differences in odour, taste, texture, and other aspects of food quality.

For this testing we use paired, duo-trio or triangular tests. In the paired test two samples are presented and the difference should be specific. Appropriate statistical analyses will then reveal how much confidence we can have in the results.

In the duo-trio and triangular tests the difference need not be specific. In the duo-trio tests a standard is presented and then as two unknowns the standard and another sample. The chance of choosing the correct sample is again fifty-fifty and as before the test must be repeated several times and statistical measures of significance applied.

In the triangular test three samples are presented, two of which are the same. In this case the taster has only one-third chance of selecting the correct odd sample by chance. However, as in the other procedures repetition is needed to permit statistical analyses of the significance of the results.

In many cases it is necessary to test more than two samples. Here ranking and scoring procedures are often used. Ranking is simple but one must be certain that the samples are all being ranked on the same quality (taste, colour, flavour, etc.). Furthermore, ranking does not tell us how much difference there is between samples. The results, however, can be statistically evaluated and the significance differences between samples determined. In other words, scoring can be used to rank samples. The question of whether the value assigned to each component of the total score is correct is not easy to establish and the additiveness of the separate scores is questionable .

Because of these problems hedonic scoring has recently been used. In this score care the samples are rated as to the degree to which they liked or disliked. A seven step hedonic score care might have the following steps: like very much, like moderately, and dislike very much. The results can be transformed to scores and analyzed statistically.

(Watts,B.M.,1983)

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Apparatus

Apparatus used for the preparation of instant rice pittu mixtures

* Electronic balance
  (FX-3200, A & D Company Ltd.,Japan)
* Electric Dryer
  (Mala, Sole-Exporters,Indiana International)
* Bowls (glass)
  (Capacity - 3 l)
* Trays (Aluminium)
  (Size - L*W*D, 400*300*50 mm)
* Measuring cylinder (glass)
  (Capacity- 100 ml,Subdivision- 1 ml)
* Electric sealer
  (TEW - Impulse sealer, Type - TISH-300, Max. Seal length-300mm, Heat time - 0.2 ~ 1.3 (second), Dimensions - L*W*D, 18*4.5*8.5 cm)
* Oriented polypropylene(OPP) bags

  Width - 15cm
  Length - 15cm
  Gage - 300
  Thickness - 0.075mm

Apparatus used for the preparation of pittu by pre-prepared instant rice pittu mixtures

* Bowls (glass, capacity - 2 l)
* Measuring cylinder (glass)
* Steamer

* Gas cocker
(Goldstar, model - TG-252 SP, Dimension - (H*W*D),163*590*427 mm)

Apparatus used for the determinations of moisture contents

* Watch glasses
(Diameter - 80 mm & 60 mm)
* Desiccators (pyrex glass)
* Electronic balance
* Electric oven
(Memmert Universal oven, Model - UM 300)

3.1.2 Raw materials

Raw materials used for the preparation of instant rice pittu mixtures

* Rice flour
Moisture content - 13%
Fines percent by mass min. by 300 µm - 97.5% (passing through)
* Desiccated coconut powder
Moisture content - 3%
Colour - White
Grade - Medium
* Salt(fine crystals)
* Water (Hardness less than 3000 ppm.)

Raw materials used for the preparation of rice pittu for sensory evaluations

* Prepared instant rice pittu mixtures
* Water

Antioxidants used as preservatives

* Citric acid
* Ascorbic acid (vitamin C)
* Alpha(α) tocopherol (vitamin E)
* Butylated hudroxy anisol (BHA)
* Garlic powder

3.2 METHODS

3.2.1 Preparation of samples

Two forms of antioxidant incorporation methods were used in order to evaluate the effect of antioxidants, on prevention of autoxidation process in pittu mixes. These methods were conditionally called; **Wet incorporation** and **Dry incorporation.**

In the preparation of the pittu mixtures, rice flour, desiccated coconut and salt were kept constant at a ratio of 75:25:2 respectively. The rest and most important ingredient, antioxidants were incorporated with the samples in accordance with the stipulated dose levels prescribed in the food act.

Five types of antioxidants were used for the both incorporation methods. (Table 7)

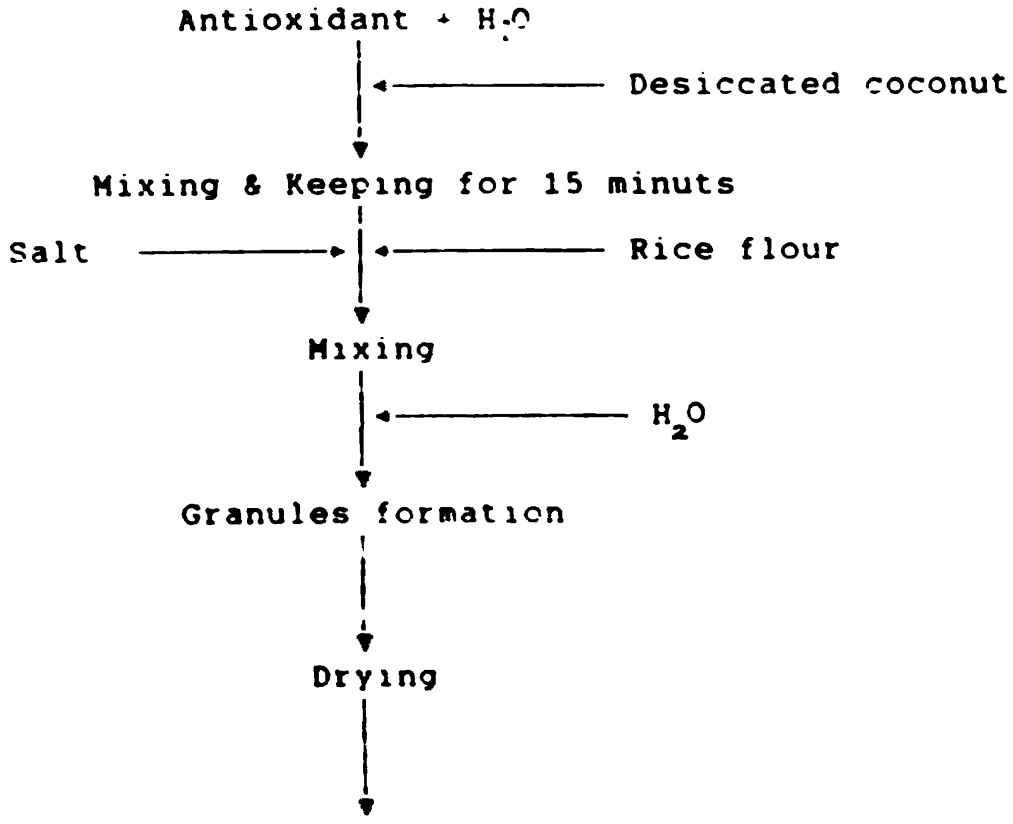
Table 7

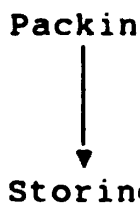
Type of antioxidants and there percentages

Type of antioxidant	Percentages
1. Combination of Citric acid & Vitamin C	. 0.2 % 0.2 %
2. Vitamin C (ascorbic acid)	0.2 %
3. Vitamin E (α tocopherol)	0.2 %
4. Butylated hydroxy anisol (BHA)	0.02 %
5. Garlic powder	1 %

3.2.1.1 Method of wet incorporation

Flow chart





Procedure for the preparation of a sample:-

An antioxidant (eg:- BHA) was weighed in to a bowl and dissolved in 20ml of cold water (hardness should be less than 3000ppm). 25g of desiccated coconut powder was added in to it and mixed well. After that it was kept for 15 minutes. Then 75g of rice flour, 2g of salt were added in to it and mixed well. 25ml of water was added into the mixture in small quantities and mixed by hand to form granules. Prepared granules were spreaded on a tray as a thin layer (thickness - about 0.5 cm) and kept it in the dryer until its moisture content reduces less than 5% (at 80^0 -90^0 c for 1 1/2 hrs.). After cooling the above dried sample, it was packed in oriented polypropylene (opp) bags and completely sealed.

As this procedure 12 samples were prepared for one type of antioxidant and totally 60 samples were prepared for 5 types of antioxidants (amount according to type has been mentioned at table 7).

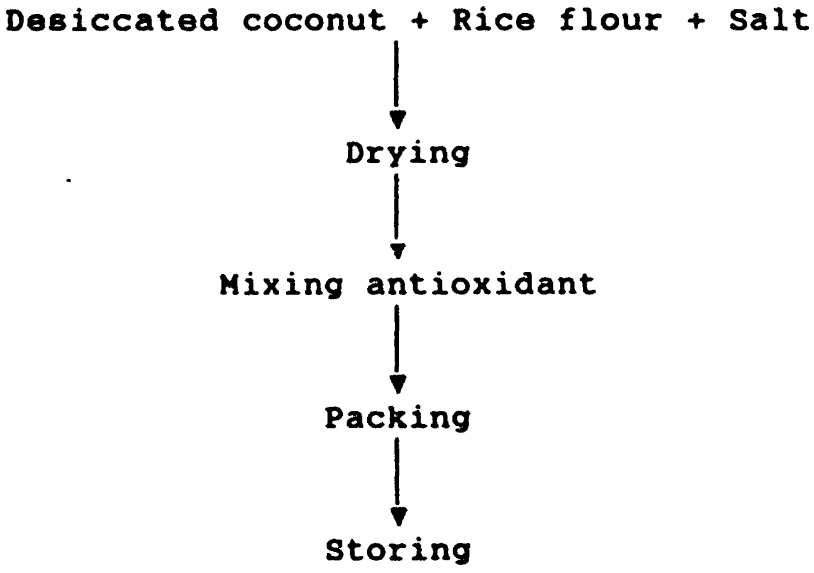
12 control samples were prepared as above procedure without applying antioxidants.

Those all samples (72 samples) were packed and labelled separately.

* In the case of vitamin C, it should be incorporated with the mixture after drying in order to minimize the distraction of vitamin due to high temperature.
* Vitamin E should be directly mixed with desiccated coconut powder, because it's a fat soluble vitamin.

3.2.1.2 Method of dry incorporation

Flow chart



Procedure for the preparation of a sample:-

2g of salt, 25g of desiccated coconut powder and 75g of rice flour were measured out into a bowl and mixed well. Prepaired mixture was spreaded on a tray as a thin layer (thickness - about 0.5cm) and kept it in the dryer (at 80^{0}c -90^{0}c for 30 - 45 minutes) until its' moisture content was reduced less than 5%. After that antioxidant was added to the dried sample by mixing it with a small quantity of the sample, and mixed well. After cooling the dried sample, it was packed in an oriented polypropylene (opp) bag and completely sealed.

As this procedure 12 samples were prepared for one type of antioxidant and altogether 60 samples were prepared for 5 types of antioxidants (types and amounts have been mentioned at table: 7)

12 control samples were also prepared as done in the above procedure without applying antioxidants.

Those all samples (72 samples) were packed and labelled separately.

3.2.2 Determination of the moisture contents

Moisture contents of all dried samples were determined by oven dry method.

Procedure:-

A previously weighed watch glass was taken (W_0g) and a sample of dried pittu sample was placed in it and weighed it again (W_1g). Weight of the sample (5g) was obtained by subtracting weight of the empty watch glass. The watch glass with the sample was placed in an oven that was set at 105°c for 3 hrs. before the operation. Then the dried sample was taken out from the oven and kept in a desiccator and weighed. This process of drying was repeated by cooling and weighing at 30 minute intervals until the difference between any two consecutive weighings does not exceed one milligram (W_2g).

Weight of the watch glas = W_0g

Weight of the sample + Watch glass → = W_1g

Weight of the sample + Watch glass, after drying until get a constant weight → = W_2g

Weight of the initial sample = $(W_1 - W_0)$g

Weight of the moisture = $(W_1 - W_2)$g

Moisture content as a percentage of the initial weight = $\frac{(W_1 - W_2)}{(W_1 - W_0)} * 100\%$

3.2.3 Preparation of instant rice pittu mixtures for sensory evaluation

** In order to prepare dry incorporated samples for the sensory evaluation, it was mixed with water to obtain granules. In this procedure the sample was mixed well by hand to form granules while adding water in small quantities.

** Wet incorporated samples were mixed with water and then steamed before the evaluation.

3.2.4 Sensory evaluation for steamed pittu

Sensory properties of both forms of antioxidant incorporated samples (wet form and dry form) were evaluated separately after forming of a 10 member sensory panel. They were asked to rank and score the 5 samples of each form in between 0 - 10 numerical limits based on their own feelings as against control sample of the each form on 3 attributes of taste, smell and appearance.

The score cards/ballot papers used for scoring as in format A for wet incorperate samples and as format B for dry incorparate samples. The sample codes also mention in table 8 according to antioxidants and its applied form.

This sensory evaluation procedure was carried out monthly with 3 replications for each and every treatment for 3 months. At the same time evaluation was done with 3 replications just after the preparation of samples.

** Format A

Ballot paper used for the sensory evaluation of wet incorperation samples

Name:- ..... Date:-						
Good (7-10) Satisfactory (4-6) poor (0-3)						
Attributes	WCO	WCA	WAS	WTO	WGA	WBU
Taste						
Smell						
Appearance						

** Format B

Ballot paper used for the sensory evaluation of dry incorperation samples

Name:- ..... Date:-						
Good (7-10) Satisfactory (4-6) Poor(0-3)						
Attributes	DCO	DCA	DAS	DTO	DGA	DBU
Taste						
Smell						
Appearance						

** Sample codes have been named as follows.

Table 8

Sample codes according to applied form and type of antioxidants.

Applied form	Antioxidant	Sample code
Wet	Citric acid + Vitamin C	WCA
	Vitamin C (ascorbic acid)	WAS
	Vitamin E (α tocopherol)	WTO
	Garlic powder	WGA
	BHA	WBU
	- (control)	WCO
Dry	Citric acid + Vitamin C	DCA
	Vitamin C (ascorbic acid)	DAS
	Vitamin E (α tocopherol)	DTO
	Garlic powder	DGA
	BHA	DBU
	- (control)	DCO

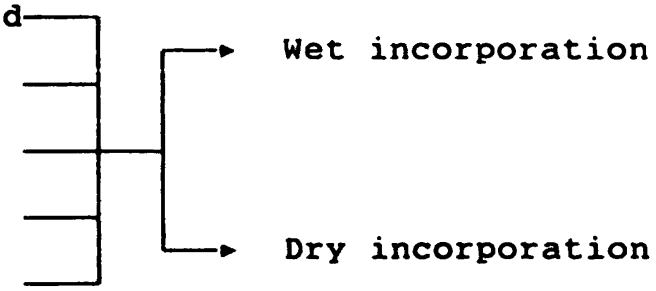
CHAPTER 4

RESULTS AND DISCUSSION

The aim of this study is evaluating the effectiveness of various antioxidants in order to prevent the rancidity due to autoxidation process in desiccated coconut mixed rice pittu. To accomplish this, 5 types of antioxidants were incorporated into the pittu mixture in two different ways; wet incorporation and dry incorporation forms, as mentioned in detail at chapter 3.

The types of antioxidants and their incorporation forms can be sketched as follows.

1. Citric acid + Ascorbic acid
2. Ascorbic acid
3. α Tocopherol
4. Garlic powder
5. BHA



The effect of the each antioxidant on both forms were evaluated statistically according to sensory evaluation tests, for the period of 3 months with monthly intervals. Taste, smell, and appearance were the sensory properties which was evaluated at these tests.

The results of each test for both incorporation forms are summarized in table 9 and 10. Statistically analysis of the above results are given in appendix I and II.

4.1 RESULTS

Table 9

Results of the Wet Incorporation Method

Time	Wet Form		
	Taste	Smell	Appearance
Just after preparation	Not significant difference among any type	Significant difference between WGA and all other types	Not significant difference among any type
After one month	-Do-	-Do-	-Do-
After two months	Significant difference between WCO and all other types	Not significant difference among any type	-Do-
After three months	Significant difference between WBU & WCO WBU & WAS WBU & WCA --- WTO & WCO WTO & WAS WTO & WCA --- WGA & WCO WGO & WAS WGO & WCA	Significant difference between WGA & WCO WGA & WAS WGA & WCA --- WBU & WCO WBU & WAS WBU & WCA --- WTO & WCO WTO & WAS WTO & WCA	-Do-

(For further details refer appendix I)

Table 10

Result of the Dry Incorporation Method

Time	Dry Form		
	Taste	smell	Appearance
Just after preparation	Not significant difference among any sample	Significant different difference between DGA and all others	Not significant difference among any sample
After one month	-Do-	Not significant different between any sample	-Do-
After two months	Significant difference between DBU & DCO DBU & DAS DBU & DCA --- DTO & DCO DTO & DAS DTO & DCA --- DGA & DCO DGA & DAS DGA & DCA	-Do-	-Do-
After three months	Significant difference between DBU & DCO DBU & DAS DBU & DCA DBU & DGA DBU & DTO --- DTO & DCO DTO & DAS DTO & DCA --- DGA & DCO DGA & DAS DGA & DCA	Significant difference between DBU & DAS DBU & DCO DBU & DCA DBU & DTO --- DGA & DAS DGA & DCO DGA & DCA DGA & DTO --- DTO & DAS DTO & DCO DTO & DCA	-Do-

(For further details refer appendix II)

4.1.1 TASTE

Taste factor is one of major organoleptic properties in a food product that gives clear indication of quality and acceptability of the food through the sensory organs. Hence this sensory factor could be used as yardstick in order to measure the degree of palatability.

Just After Preparation:-

There was not a significant difference between any sample of both incorporation methods. All of them were in acceptable condition in terms of taste.

After One Month of Preparation:-

Statistical evaluation revealed that the quality of the products was same as just after preparation of both methods. Taste of control samples was also acceptable without rancid taste.

After Two Months of Preparation:-

Statistical there was a significant difference between control and rest of the treatments in wet form. The taste of control sample was somewhat deteriorate, specially in the direction of taste, which was being rancid.

But at dry form, only BHA,tocopherol and garlic powder incorporated samples were in an acceptable level in contrast to the control sample. Control sample was in extremely rancid condition and citric acid + ascorbic acid, ascorbic acid incorporated samples gave slightly rancid taste comparing to the control.

After Three Months of Preparation:-

At wet form, it has been revealed that only BHA, a tocopherol and garlic powder incorporated samples were in acceptable level whereas citric acid + ascorbic acid, ascorbic acid, incorporated samples and control sample failed to maintain a good taste due to their rancidity.

At dry form BHA incorporated sample was significantly different from the rest. That is only BHA incorporated sample

was of acceptable level without rancidity. α tocopherol and garlic powder incorporated samples were significantly different with citric acid + ascorbic acid and ascorbic acid incorporated samples while lost there antioxidative capability after three months. However α tocopherol and garlic powder incorporated samples were rancid slightly.

4.1.2 SMELL

Smell is also one of the most important organoleptic properties in foods which cites whether food is in good odour prior to intake.

Just After Preparation:-

As far as the smell is concerned in both forms, there was no significant difference between control and other treatments except for garlic powder incorporated sample as it had a peculiar smell of garlic.

After One Month of Preparation:-

At the wet form, the intensity of the garlic smell was less than as it was in an fresh preparation. Except that the smell of other samples was acceptable.

At the dry form there were not any difference in any sample, including in garlic powder incorporated sample. Smell of the all samples was in acceptable level.

After Two Months of Preparation:-

At wet form, all samples were in an acceptable level. Although the control sample had a slightly rancid taste, the rancid smell was not spreaded through the product.

At dry form only the control sample gave rancid smell, being the rest were in acceptable level.

After Three Months of Preparation:-

At wet form citric acid + ascorbic acid and ascorbic acid incorporated samples gave rancid smell as control. But BHA, garlic powder, and tocopherol incorporated samples maintained an acceptable degree of smell.

At dry form, BHA and garlic powder incorporated samples were significantly different from others. That is BHA and garlic powder incorporated samples were in acceptable level, while garlic powder incorporated sample was slightly rancid in taste. As far as the tocopherol incorporated sample is concerned, it was significantly different from citric acid + ascorbic acid, ascorbic acid and control, because its (tocopherol incorporated sample) rancid smell was not too much higher comparing to others.

4.1.3 APPEARANCE

Appearance is also one of the important organoleptic properties of a food, which causes for its palatability.

When considering the appearance of both forms, there were no any difference in any sample just after preparation and also upto three months.

4.2 DISCUSSION

The term rancidity can be expressed as undesirable odour or flavour due to hydrolysis and/or oxidation of fat/oil. Therefore fat containing food products like the instant pittu mixture, rancidity is a major factor in the degradation of its quality, i.e., oil of the desiccated coconut can cause this problem. Rice flour also contain negligible amount of oil compare to desiccated coconut.

Among the types of rancidity, hydrolytic rancidity and oxidative rancidity are important processes which is connected with the quality degradation of instant rice pittu mixtures.

Hydrolytic rancidity is the reaction where by water molecules lead splitting of fatty acids from the glycerol portion of the fat molecule. Such reactions are catalyzed by lipase (enzymes that act on fats) and certain other enzymes. The presence of water naturally encourages this rancidity. Water activity also helps to the growth of micro organisms and produce enzymes which leads this reaction.

Therefore by reducing the moisture content and maintain at a lower level (about less than 5%), hydrolytic rancidity can be prevented easily. Moisture content can be reduced by drying and

the lowered moisture level can be maintained by using proper moisture proof packing materials. This can be fulfilled by using oriented poly propylene bags, because they are low permeabile to moisture, (as well as gas and odours).

In this experiment the mixtures were allowed to stay in the drier at 80^0 - 90^0C for 1 1/2 hours in order to reduce the moisture level less than 5% according to the long term factory processing experience has revealed this moisture level is adequately low to prevent both micro-biological (spoilage due to micro organisms) and biochemical (rancidity) activities. The products (after drying) were allowed to cool prior to packing. The purpose of this was to prevent the damage of packing material and to get the mixture free of extra moisture.

By considering these factors, hydrolytic rancidity can be prevented easily by using physical conditions as described above.

When considering oxidative rancidity, it can be divided into three forms, namely autoxidation, photo oxidation and enzymic oxidation.

There is a little possibility in occurring of photo oxidation on this mixture, because all the treatments were kept in house.

Enzymic oxidation also can be neglected, because lipolytic enzymes are not in the ingredients, which was used for the preparation of pittu mixtures, if present they can be inactivated due to heat, while drying at the temperature of 80^0 -90^0 C.

Autoxidation process is a radical chain reaction involving initiation, propagation and termination steps (describe in chapter 2), and the resultant end products (fatty acid, aldehyde, ketone) cause for the rancid flavour. Specially the moderately unsaturated fats under go for this reaction. Palmitoleic(C16:1), oleic(C18:1), and linoleic(C18:2) are the unsaturated fatty acids which contain in coconut oil, and they can be considered that may under go for autoxidation. Pro-oxidants such as metals(iron, copper, nickel, cobalt) or other radical initiators facilitated initiation step in the chain reaction of autoxidation.

So, when considering the autoxidation process, prevention is a very difficult task under natural conditions, because it is

an auto catalytic reaction and oxygen is always present in and around the food. This problem can be overcome easily by vacuum and nitrogen packing. But using antioxidants is economically active when comparing to above methods. So to prevent the autoxidation process antioxidants can be used under the local regulation.

When considering this research which was done to evaluate the effectiveness of the antioxidants, it proves the theory that there is an effect by using antioxidants. Because the control samples of both forms were unable to keep more than one month without rancid.

When expressing in details, in the absence of an antioxidant, a hydrogen atom was lost from the allylic carbon of the fatty acid group with the formation of a fatty free radical. The latter is readily susceptible to attack by atmospheric oxygen, resulting is the formation of peroxides and hydro peroxides. They are very unstable, decomposing into compounds with shorter carbon chains. These include fatty acids, aldehydes and ketons, which are volatile and which contribute to the unpleasant odour and taste. The reason for occurring the rancidity in control sample after one month may be due to the fact that peroxide formation during storage is slow at first due to an induction period. This period can vary from a few weeks to several months according to the particular oil or fat, the temperature, etc.

According to the result of the wet form incorporation method, it is possible to consider BHA, α tocopherol and garlic powder (allicin is the compound contain in garlic powder which has antioxidant characteristics) are reactive than citric acid + ascorbic acid and ascorbic acid (these antioxidants incorporated samples were unable to keep more than two months) BHA and α tocopherol are phenolic type antioxidants, functions as a free radical acceptor forming a stable compound that will not propagate further oxidation of the glyceride. (This type of antioxidants are called as "primary" antioxidants). Citric acid and ascorbic acid are called as "synergists",they promote the action of other antioxidants, but have little effect if present

alone.

So, we can consider citric acid + ascorbic acid and ascorbic acid not reactive further or were completely oxidize between 2 - 3 months.

Taste, smell and appearance are the sensory characteristics which were considered at this sensory evaluation tests. Among them if one attribute of a sample is not in a favourable condition, that is, when considering the taste, it can be rancid slightly, but the smell can be in good condition. Because the rancidity of the sample is not too much higher to spread a rancid smell, that can identify as organoleptic property.

When comparing the result of the both forms of antioxidant incorporation methods, it is possible to identify a considerable differences between them. The only reason which can cause for this difference is, at the dry form, antioxidants were directly incorporated to the mixture of rice flour, desiccated coconut and salt. By this method also it may not be able to achieve complete dispersion.

But at the wet form incorporation method, antioxidants were added directly into the desiccated coconut, after dissolving in water, and also kept it for 15 minutes. These conditions cause for a well absorption and dispersion of the antioxidant into desiccated coconut.

CHAPTER 5
CONCLUSION

* BHA kept an antioxidant effect for more than three months in both incorporation forms (wet incorporation form and dry incorporation form).

* α tocopherol (vitamin E) was effective as an antioxidant for more than three months in wet incorporation form and two months in dry incorporation form.

* In the wet incorporation form, garlic powder was able to suppress the autoxidation process for more than three months, and in the dry incorporation form only for two months.

* The combination of citric acid and vitamin C (ascorbic acid) had an antioxidant effect for two and one month respectively for wet and dry incorporation forms.

* Ascorbic acid alone kept its antioxidative capacity only for two and one month for wet and dry incorporation forms respectively.

Among the two types of antioxidant incorporation forms (wet incorporation and dry incorporation forms) BHA was the most effective one, being active for more than three months in both forms. But when comparing the effectiveness of other types of antioxidant in both forms, wet incorporation form was superior to the dry form. Therefore, the wet incorporation form is the most effective antioxidant incorporation method.

CHAPTER 6

RECOMMENDATIONS AND SUGGESTIONS

6.1 RECOMMENDATIONS

* In wet incorporation form BHA, α tocopherol(vitamin E), and garlic powder were the effective antioxidants. Out of them BHA, and vitamin E were artificially made. But vitamin E is well known to the people and is taken with other vitamins as vitamin preparations in form of vitamins, capsules, etc. Since artificial additives like BHA are not preferred by consumers, consumer acceptance for BHA mixed instant rice pittu mixtures may be not significant.

  Garlic powder is a natural antioxidant compound. But it gives a peculiar smell and taste when mixing with pittu mixture. But not all consumers may like this taste and smell. So it can be recommended to include garlic powder into one type of formula for consumers who prefer this taste and vitamin E can be recommended to mix with the other type for the rest of consumers.

6.2 SUGGESTIONS

* Citric acid and ascorbic acid (vitamin C) are synergistic antioxidants. They are not effective long when using alone. It can be assumed to have a better effect by using combinations with primary antioxidants, eg:- α tocopherol (vitamin E) can be used with ascorbic acid (vitamin C).

* In this study the ratio of the ingredients (rice flour 75 : desiccated coconut 25 : salt 2) was constant. This ratio was taken basing on formula previously established by the factory, in order to produce a pittu mixture which has good

characteristics. But good effect can be obtained by changing the ratio between desiccated coconut and rice flour. While keeping antioxidants at the same level constantly. But the type of the antioxidant could be changed.

* It can be suggested to increase shelf life of the product by using another packing materials that have a nigher moisture proof ability.

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APPENDIX I

STATISTICAL ANALYSIS OF THE NUMERIC DATA OF SENSORY TESTS FOR WET INCORPORATION METHOD.

Duration :- Just after preparation

Attribute:- Taste

Testers.	WCO			WCA			WAS			WTO			WGA			WBU		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	7	8	8	7	8	8	7	8	8	7	8	8	6	7	7	7	8	8
2	8	8	8	8	8	8	8	8	8	8	8	8	7	8	7	8	8	8
3	7	7	8	7	7	8	7	7	8	7	7	8	7	6	7	7	8	8
4	10	9	9	9	9	9	9	9	9	10	9	9	8	8	8	10	9	9
5	8	8	9	8	8	9	8	8	9	8	8	9	9	9	9	9	8	9
6	7	7	7	8	7	7	8	7	7	8	7	7	9	7	7	8	7	7
7	7	8	7	6	8	7	6	8	7	7	8	7	7	9	7	8	8	7
8	8	8	8	7	8	8	7	8	8	7	8	8	6	7	7	7	8	8
9	9	8	9	9	8	9	9	8	9	9	8	9	9	8	9	9	8	9
10	9	9	8	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9

Mean values of the above table

Testers	WCO	WCA	WAS	WTO	WGA	WBU	Total
1	7.67	7.67	7.67	7.67	6.67	7.67	45.02
2	8.00	8.00	8.00	8.00	7.33	8.00	47.33
3	7.33	7.33	7.33	7.33	6.67	7.67	43.66
4	9.33	9.00	9.00	9.33	8.00	8.33	52.99
5	8.33	8.33	8.33	8.33	9.00	8.67	50.99
6	7.00	7.33	7.33	7.33	7.67	7.33	43.99
7	7.33	7.00	7.00	7.33	7.67	7.67	44.00
8	8.00	7.67	7.67	7.67	6.67	7.67	45.35
9	8.67	8.67	8.67	8.67	8.67	8.67	52.02
10	8.67	9.00	9.00	9.00	8.67	9.00	53.34
total	80.33	80.00	80.00	80.66	77.02	80.68	478.69 (G.T)

Correction factor (CF) = $(GT)^2$/Total No. of observations
= $478.69^2/60$
= 3819.07

Sum of squares between types (SS_{types}) = $(80.33^2/10 + 80^2/10 + 80^2/10 + 80.66^2/10 + 77.02^2/10 + 80.68^2/10) - (CF)$
= $(38200.29/10) - (3819.07)$
= 3820.03 - 3819.07
= 0.96

Sum of squares between testers ($SS_{testers}$) = $(45.02^2/6 + 47.33^2/6 + 43.66^2/6 + 52.99^2/6 + 50.99^2/6 + 43.99^2/6 + 44^2/6 + 45.35^2/6 + 52.02^2/6 + 53.34^2/6) - (CF)$
= $(23060.02/6) - (3819.07)$
= 3843.34 - 3819.07
= 24.27

Sum of squares error (SS_{error}) = $(SS_{total}) - [(SS_{type}) + (SS_{testers})]$
= 29.75 - [0.96 + 24.27]
= 4.52

NOVA Table

Source	SS	DF (Degree of Freedom)	MS (Mean Square)	F	F^{5}_{df},5%
Between types	0.96	(6-1)	(0.96/5) 0.19	(C.19/0.1)1.90	2.45
Between testers	24.27	(10-1) 9	24.27/9 2.69		
Error	4.52	[59-(9+5)] 45	4.52/45 0.10		
Total	31.44	(60-1) 59			

alculated value of F for types = 1.90
ritical table value of F at 5% level = 2.45

he calculated value of F is smaller than the critical value if F at 5% level table. Therefore, there is mo significant ifference in taste, among the 6 types of samples.

Attribute :- Smell

Testers	WCO			WCA			WAS			WTO			WGA			WBU		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	9	9	9	9	9	9	9	8	9	9	9	8	8	7	8	9	9	9
2	8	9	9	7	8	8	7	8	8	8	9	9	7	7	7	8	9	9
3	7	7	7	7	8	8	7	8	8	7	8	8	6	6	5	7	7	8
4	[illegible]	7	7	6	7	[illegible]	6	7	7	6	[illegible]	7	4	4	[illegible]	6	7	7
5	9	8	8	9	8	7	9	8	8	9	9	8	8	7	6	9	9	8
6	6	6	7	6	6	8	6	6	7	6	6	7	8	8	8	6	7	6
7	5	6	6	5	6	6	5	6	7	5	6	6	5	6	6	5	6	6
8	6	5	5	5	6	6	5	6	5	5	6	6	7	7	7	5	6	6
9	8	7	7	8	7	7	8	7	7	8	8	7	6	6	5	8	7	7
10	8	8	7	8	7	8	8	7	8	8	8	9	7	5	7	8	8	9

Mean values of the above table

Testers	WCO	WCA	WAS	WTO	WGA	WBU	Total
1	9.00	9.00	8.67	8.67	7.67	9.00	52.01
2	8.67	7.67	7.67	8.67	7.00	8.67	48.35
3	7.00	7.67	7.67	7.67	5.67	7.33	43.01
4	6.67	6.67	6.67	6.67	4.33	6.67	37.68
5	8.33	8.00	8.33	8.67	7.00	8.67	49.00
6	6.33	6.67	6.33	6.33	8.00	6.33	39.99
7	5.67	5.67	6.00	5.67	5.67	5.67	34.35
8	5.33	5.67	5.33	5.67	7.00	5.67	34.67
9	7.33	7.33	7.33	7.67	5.67	7.33	42.66
10	7.67	7.67	7.67	8.33	6.33	8.33	46.00
Total	72.00	72.02	71.67	74.02	64.34	73.67	427.72 (G.T.)
Mean	7.20	7.20	7.17	7.40	6.40	7.37	

* CF = 427.72²/60 = <u>3049.07</u>

* $(SS)_{Total}$ = 3128 - 3049.07 = <u>78.93</u>

* $(SS)_{Tre}$ = (30553.33/10) - 3049.07 = <u>6.26</u>

* $(SS)_{testers}$ = (18630.41/6) - 3049.07 = <u>55.99</u>

* $(SS)_{error}$ = $(SS)_{total}$ - [$(SS)_{type}$ + $(SS)_{testers}$]
= 78.93 - [6.26 + 55.99]
= <u>16.68</u>

ANOVA Table

Source	SS	DF	MS	F	$F^{5}_{45,5\%}$
Between type	6.26	5	1.25	3.38	2.45
Between testers	55.99	9	6.22		
Error	16.68	45	0.37		
Total	78.93	59			

Calculated value of F for types = 3.38
Critical table value of F at 5% level = 2.45

The calculated value of F is greater than critical value of F at the 5% level table. Therefore there is a significant difference of smell, among the 6 types of samples, since there is an overall significant effect of types, the individual means should be ordinarily examined to find this out. Once test that allows investigation of all possible pairs of means in a sequential manner is the Newman-Keuls test.

The main steps in this test are as follows:-

1. Enter the ANOVA table and take the error mean square, and obtain the standard error of the mean for each treatment.

Standard error (Sy^{-}) = $\sqrt{}$ error mean square/No. of testers
= $\sqrt{}$ $(MS)_{error}/(n)_{testers}$
= $\sqrt{}$ 0.37/10
= <u>0.19</u>

2. Arrange the K (6) means in order from low to high

WGA	WAS	WCO	WCA	WBY	WTO
6.40	7.17	7.20	7.20	7.37	7.40

3. Enter the studentized range table of 5% significant range, for the corresponding error DF (45) and p= 2,3,4,5,6(K) and list the k-1 ranges.

P :	2	3	4	5	6
Range:	2.87	3.44	3.79	4.04	4.23

4. Multiply these ranges by standard error (0.19) to form a group or K-1 least significant ranges(LSR).

P :	2	3	4	5	6
LSR :	2.87*0.19,	3.44*0.19,	3.79*0.19,	4.04*0.19,	4.23*0.19
	0.55	0.65	0.72	0.77	0.80

5. Due to no. of means exceed more than 5 we should use DMR(Dunkan Multiple range) values instead of LSR values.

DMR values = (LSR value) * significant studentized factor(R) for DF_{error}(45) and no. of testers (10).

DMR values:-

0.55*1.17,	0.65*1.17,	0.72*1.17,	0.77*1.17,	0.80*1.17
0.75	0.76	0.84	0.90	0.94

6. Test the observed ranges between means beginning with largest versus smallest, which is compared with the DMR value for p = K(6), then test largest versus second smallest with the DMR value for p = K-1 ; and so on. This should be continued for largest versus smallest.

WTO - WGA = 7.40- 6.40 = 1.00 > 0.94 significant difference (s.d.)
WTO - WAS = 7.40 - 7.17 = 0.23 < 0.90 - not s.d.
WTO - WCO = 7.40 - 7.20 = 0.20 < 0.84 - not s.d.
WTO - WCA = 7.40 - 7.20 = 0.20 < 0.76 - not s.d.
WTO - WBU = 7.40 - 7.37 = 0.33 < 0.75 - not s.d.

WBU - WGA = 7.37 - 6.40 = 0.97 > 0.90 - s.d.
WBU - WAS = 7.37 - 7.17 = 0.20 < 0.84 - not s.d.
WBU - WCO = 7.37 - 7.20 = 0.17 < 0.76 - not s.d.
WBU - WCA = 7.37 - 7.20 = 0.17 < 0.75 - not s.d.

WCA - WGA = 7.20 - 6.40 = 0.80 < 0.84 - not s.d.
WCA - WAS = 7.20 - 7.17 = 0.03 < 0.76 - not s.d.
WCA - WCO = 7.20 - 7.20 = 0.00 < 0.75 - not s.d.

WCO - WGA = 7.20 - 6.40 = 0.80 > 0.76 - s.d.
WCO - WAS = 7.20 - 7.17 = 0.33 < 0.75 - not s.d.

WAS - WGA = 7.17 - 6.40 = 0.77 > 0.75 - s.d.

Attribute :- Appearance

ANOVA Table

Source	SS	DF	MS	F	$F^{5}_{2,5\%}$
Between type	0.23	5	0.054	1.25	2.45
Between testers	39.85	9	.43		
Error	1.93	45	0.04		
Total	42.01	59			

Calculated value of F for types = 1.25
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore there is no significant difference in appearance among the 6 types of samples.

Duration :- After one month of preparation

Attribute :- Taste

ANOVA Table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	5.57	5	1.11	2.06	2.45
Between testers	7.57	9	0.84		
Error	24.11	45	0.54		
Total	37.24	59			

Calculated value of F for types = 2.06
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at the 5% level table. Therefore there is no significant difference in taste, among the 6 types of samples.

Attribute :- Smell

ANOVA Table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between type		5	1.31	6.89	2.45
Between testers	6.5281	9	9.10		
Error	.50	45	0.19		
	8.43				
Total	96.45	59			

Calculated value of F for types = 6.89
Critical table value of F at 5% level = 2.45

The calculated value of F is greater than critical value of F at the 5% level table. Therefore there is a significant difference in smell among the 6 types of samples.

Standard error($S\bar{Y}$) = $\sqrt{(MS)_{error}/(n)_{testers}}$
= $\sqrt{0.19/10}$
= 0.14

Means are in order from low to high for treatments.

WGA	WCO	WAS	WCA	WTO	WBU
6.43	7.20	7.20	7.30	7.37	7.40

Studentize table values(STV). [$(DF)_{error}$=45 & p = 2,3,4,5,6]

2.87	3.44	3.79	4.04	4.23

LSR values = STV * $S\bar{Y}$

2.87*0.14,	3.44*0.14,	3.79*0.14,	4.04*0.14,	4.23*0.14
0.4	0.48	0.53	0.57	0.59

DMR values = LSR * R(1.17)

0.40*1.17,	0.48*1.17,	0.53*1.17,	0.57*1.17,	0.59*1.17
0.47	0.56	0.62	0.67	0.69

WBU - WGA = 7.40 - 6.43 = 0.97 > 0.69 - significant difference
WBU - WCO = 7.40 - 7.20 = 0.20 < 0.67 - not s.d.
WBU - WAS = 7.40 - 7.20 = 0.20 < 0.62 - not s.d.
WBU - WCA = 7.40 - 7.30 = 0.10 < 0.56 - not s.d.
WBU - WTO = 7.40 - 7.37 = 0.07 < 0.47 - not s.d.

WTO - WGA = 7.37 - 6.43 = 0.94 > 0.67 - s.d.
WTO - WCO = 7.37 - 7.20 = 0.17 < 0.62 - not s.d.
WTO - WAS = 7.37 - 7.20 = 0.17 < 0.56 - not s.d.
WTO - WCA = 7.37 - 7.30 = 0.07 < 0.47 - not s.d.

WCA - WGA = 7.30 - 6.43 = 0.87 > 0.62 - s.d
WCA - WCO = 7.30 - 7.20 = 0.10 < 0.56 - not s.d.
WCA - WAS = 7.30 - 7.20 = 0.10 < 0.47 - not s.d.

WAS - WGA = 7.20 - 6.43 = 0.77 > 0.56 - s.d.
WAS - WCO = 7.20 - 7.20 = 0.00 < 0.47 - not s.d.

WCO - WGA = 7.20 - 6.43 = 0.77 > 0.47 - s.d.

ttribute :- Appearance

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between type	5.72	5	1.14	1.61	2.45
Between testers	17.99	9	1.99		
Error	31.88	45	0.71		
Total	55.59	59			

alculated value of F for types = 1.61
ritical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value f F at the 5% level table. Therefore there is no significant ifference in appearance, among the 6 types of samples.

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ration :- After two months of preparation

ttribute :- Taste

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between type	189.96	5	37.99	118.72	2.45
Between testers	16.68	9	1.85		
Error	14.42	45	0.32		
Total	221.06	59			

alculated value of F (118.72) for types is greater than the itical value of F (2.45) at the 5% level table. Therefore ere is a significant different in taste among 6 types of pples.

1. Standard error (SY^-) = $\sqrt{}$ 0.32/10 = 0.18

2. Means :- WCO WAS WCA WGA WTO WBU
   2.90 7.47 7.53 7.53 7.87 7.87

3. Studentize table values(STV) for DF_{error}= 45 and p = 2,3,4,5,6
   2.87 3.44 3.79 4.04 4.23

4. LSR values = (STV * SY^-)
   2.87*0.18, 3.44*0.18, 3.79*0.18, 4.04*0.18, 4.23*0.18
   0.52 0.62 0.68 0.73 0.76

5. DMR values = (LSR * R)
   0.52*1.17, 0.62*1.17, 0.68*1.17, 0.73*1.17, 0.76*1.17
   0.61 0.73 0.80 0.85 0.89

6. WBU - WCO = 7.87 - 2.90 = 4.97 > 0.89 - significant difference
   WBU - WAS = 7.87 - 7.47 = 0.40 < 0.85 - not s.d.
   WBU - WCA = 7.87 - 7.53 = 0.34 < 0.80 - not s.d.
   WBU - WGA = 7.87 - 7.53 = 0.34 < 0.73 - not s.d.
   WBU - WTO = 7.87 - 7.87 = 0.00 < 0.61 - not s.d.

   WTO - WCO = 7.87 - 2.90 = 4.97 > 0.85 - s.d.
   WTO - WAS = 7.87 - 7.47 = 0.40 < 0.80 - not s.d.
   WTO - WCA = 7.87 - 7.53 = 0.34 < 0.73 - not s.d.
   WTO - WGA = 7.87 - 7.53 = 0.34 < 0.61 - not s.d.

   WGA - WCO = 7.53 - 2.90 = 4.63 > 0.80 - s.d.
   WGA - WAS = 7.53 - 7.47 = 0.06 < 0.61 - not s.d.
   WGA - WCA = 7.47 - 2.90 = 4.57 < 0.61 - not s.d.

   WCA - WCO = 7.53 - 2.90 = 4.63 > 0.73 - s.d.
   WCA - WAS = 7.53 - 7.47 = 0.06 < 0.61 - not s.d.

   WAS - WCO = 7.47 - 2.90 = 4.57 > 0.61 - s.d.

Attribute :- Smell

ANOVA table

Source	SS	DF	MS	F	$F^5_{45,5\%}$
Between type	7.47	5	1.55	0.29	2.45
Between testers	65.49	9	7.28		
Error	241.51	45	5.37		
Total	310.74	59			

Calculated value of F for types = 0.29
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at the 5% level table. Therefore there is mo significant difference in smell, among the 6 types of samples.

Attribute :- Appearance

ANOVA table

Source	SS	DF	MS	F	$F^{5}_{:,5\%}$
Between types	1.28	5	0.26	1.63	2.45
Between testers	38.03	9	4.23		
Error	7.21	45	0.16	.	
Total	46.52	59			

Calculated value of F for types = 1.63
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at the 5% level table. Therefore there is no significant difference in appearance, among the 6 types of samples.

Duration :- After three months of preparation

Attribute :- Taste

ANOVA table

Source	SS	DF	MS	F	$F^{5}_{:,5\%}$
Between types	651.68	5	130.34	352.27	2.45
Between testers	6.51	9	0.39		
Error	16.43	45	0.37		
Total	674.62	59			

Calculated value of F (352.27) for types is greater than the critical value of F (2.45) at the 5% level table. Therefore there is a significant different in taste among 6 types of samples.

1. Standard error (SY) = $\sqrt{0.37/10}$ = 0.19

2. Means:- WCO WAS WCA WGA WTO WBU
1.00 1.17 1.53 7.60 7.80 8.03

3. Studentize table values (STV) for DF_{error} = 45 and p = 2,3,4,5,6.
2.87 3.44 3.79 4.04 4.23

4. LSR values = (STV * SY)
2.87*0.19, 3.44*0.19, 3.79*0.19, 4.04*0.19, 4.23*0.19
0.55 0.65 0.72 0.77 0.8

5. DMR values = (LSR * R)
0.55*1.17, 0.65*1.17, 0.72*1.17, 0.77*1.17, 0.8*1.17
0.64 0.76 0.84 0.9 0.94

5. WBU - WCO = 8.30 - 1.00 = 7.03 > 0.94 - significant difference
   WBU - WAS = 8.03 - 1.17 = 6.86 > 0.90 - s.d.
   WBU - WCA = 8.03 - 1.53 = 6.50 > 0.84 - s.d.
   WBU - WGA = 8.03 - 7.60 = 0.43 < 0.76 - not s.d.
   WBU - WTO = 8.03 - 7.80 = 0.23 < 0.64 - not s.d.

   WTO - WCO = 7.80 - 1.00 = 6.80 > 0.90 - s.d.
   WTO - WAS = 7.80 - 1.17 = 6.63 > 0.84 - s.d.
   WTO - WCA = 7.80 - 1.53 = 6.27 > 0.76 - s.d.
   WTO - WGA = 7.80 - 7.60 = 0.20 < 0.64 - not s.d.

   WGA - WCO = 7.60 - 1.00 = 6.60 > 0.84 - s.d.
   WGA - WAS = 7.60 - 1.17 = 6.43 > 0.76 - s.d.
   WGA - WCA = 7.60 - 1.53 = 6.07 > 0.64 - s.d.

   WCA - WCO = 1.53 - 1.00 = 0.53 < 0.76 - not s.d.
   WCA - WAS = 1.53 - 1.17 = 0.36 < 0.64 - not s.d.

   WAS - WCO = 1.17 - 1.00 = 0.17 < 0.55 - not s.d.

Attribute :- Smell

ANOVA table

Source	SS	DF	MS	F	$F^{5}_{45,5\%}$
Between type	223.83	5	44.77	12.37	2.45
Between testers	51.99	9	5.78		
Error	162.82	45	3.62		
Total	438.64	59			

Calculated value of F (12.37) for types is greater than the critical value of F (2.45) at the 5% level table. Therefore there is a significant difference in smell among 6 types of samples.

1. Standard error (SY) = $\sqrt{362/10}$ = 0.60

2. Means :-

WCO	WAS	WCA	WGA	WTO	WBU
2.64	3.13	3.17	6.57	6.73	7.14

3. Studentize table values (STV) for DF_{error}=45 and p = 2,3,4,5,6

2.87	3.44	3.79	4.04	4.23

4. LSR values = (STV * SY)

2.87*0.60,	3.44*0.60,	3.79*0.60,	4.04*0.60,	4.23*0.60
1.72	2.06	2.27	2.42	2.54

5. DMR values =(LSR * F)

1.72*1.17,	2.06*1.17,	2.27*1.17,	2.42*1.17	2.54*1.17
2.01	2.41	2.66	2.83	2.97

6. WBU - WCO = 7.14 - 2.64 = 4.50 > 2.97 - significant difference
WBU - WAS = 7.14 - 3.13 = 4.01 > 2.83 - s.d.
WBU - WCA = 7.14 - 3.17 = 3.97 > 2.66 - s.d.
WBU - WGA = 7.14 - 6.57 = 0.57 < 2.41 - not s.d.
WBU - WTO = 7.14 - 6.73 = 0.41 < 2.01 - not s.d.

WTO - WCO = 6.73 - 2.64 = 4.09 > 2.83 - s.d.
WTO - WAS = 6.73 - 3.13 = 3.60 > 2.66 - s.d.
WTO - WCA = 6.73 - 3.17 = 3.56 > 2.41 - s.d.
WTO - WGA = 6.73 - 6.57 = 0.16 < 2.01 - not s.d.

WGA - WCO = 6.57 - 2.64 = 3.90 > 2.36 - s.d.
WGA - WAS = 6.57 - 3.13 = 3.44 > 2.41 - s.d.
WGA - WCA = 6.57 - 3.17 = 3.4 > 2.01 - s.d.

WCA - WCO = 3.17 - 2.64 = 0.53 < 2.41 - not s.d.
WCA - WAS = 3.17 - 3.13 = 0.04 < 2.41 - not s.d.

WAS - WCO = 3.13 - 2.64 = 0.49 < 2.01 - not s.d.

Attribute :- Appearance

ANOVA table

Source	SS	DF	MS	F	$F_{5,45}$,5%
Between type	0.35	5	0.07	1.40	2.45
Between testers	45.84	9	5.09		
Error	2.35	45	0.05		
Total	48.54	59			

Calculated value of F for types = 1.40
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at the 5% level table. Therefore there is no significant difference in appearance, among the 6 types of samples.

APPENDIX II
STATISTICAL ANALYSIS OF THE NUMERIC DATA OF SENSORY TESTS FOR DRY INCORPORATION METHOD

Duration :- Just after preparation
Attribute :- taste

ANOVA table

Source .	SS	DF	MS	F	F^{5}_{45},5%
Between types	0.22	5	0.04	0.22	2.45
Between testers	26.67	9	2.96		
Error	7.96	45	0.18		
Total	34.84	59			

Calculated value of F for types = 0.22
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore, there is no significant difference of taste among 6 types of samples.

Attribute :- Smell

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	13.88	5	2.77	7.29	2.45
Between testers	47.79	9	5.62		
Error	17.24	45	0.38		
Total	78.86	59			

Calculated value of F (7.29) for types is greater than the critical value of F (2.45) at the 5% level table. Therefore there is a significant different in smell among 6 types of samples.

1. Standard error (SY) = $\sqrt{(MS)_{error}/(n)testers}$
$= \sqrt{0.38/10}$
$= \underline{0.20}$

2. Means :-

DGA	DCO	DAS	DTO	DCA	DBU
5.83	7.30	7.07	7.10	7.17	7.20

3. Studentize (STV) table values for DF_{error}=45 and p = 2,3,4,5,6

2.87	3.44	3.79	4.04	4.23

4. LSR values = (STV * SY)

2.87*0.20,	3.44*0.20,	3.79*0.20,	4.04*0.20,	4.23*0.20
0.57	0.69	0.76	0.81	0.85

5. DMR values = (LSR * R)

0.57*1.17,	0.69*1.17,	0.76*1.17,	0.81*1.17,	0.85*1.17
0.67	0.81	0.89	0.95	1.00

6. DBU - DGA = 7.20 - 5.83 = 1.37 > 1.00 - significant difference
DBU - DCO = 7.20 - 7.03 = 0.17 < 0.95 - not s.d
DBU - DAS = 7.20 - 7.07 = 0.13 < 0.89 - not s.d
DBU - DTO = 7.20 - 7.10 = 0.10 < 0.81 - not s.d
DBU - DCA = 7.20 - 7.17 = 0.07 < 0.67 - not s.d

DCA - DGA = 7.17 - 5.83 = 1.34 > 0.95 - s.d
DCA - DCO = 7.17 - 7.03 = 0.14 < 0.89 - not s.d
DCA - DAS = 7.17 - 7.07 = 0.04 < 0.81 - not s.d
DCA - DTO = 7.17 - 7.10 = 0.07 < 0.67 - not s.d

DTO - DGA = 7.1 - 5.83 = 1.27 > 0.89 - s.d
DTO - DCO = 7.1 - 7.03 = 0.07 < 0.81 - not s.d
DTO - DAS = 7.1 - 7.07 = 0.03 < 0.67 - not s.d

DAS - DGA = 7.07 - 5.83 = 1.24 > 0.81 - s.d
DAS - DCO = 7.07 - 7.03 = 0.04 < 0.67 - not s.d

DCO - DGA = 7.03 - 5.83 = 1.20 > 0.67 - s.d.

Attribute :- Appearance

ANOVA table

Source	SS	DF	MS	F	$F^{5}_{:,5\%}$
Between types	0.48	5	0.10	1.60	2.45
Between testers	72.60	9	8.07		
Error	2.69	45	0.06		
Total	75.77	59			

Calculated value of F for types = 1.60
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore there is no significant difference in appearance among the 6 types of samples.

Duration :- After one month of preparation

Attribute :- Taste

ANOVA table

Source	SS	DF	MS	F	$F^{5}_{:,5\%}$
Between type	3.15	5	0.70	1.02	2.45
Between testers	16.16	9	1.80		
Error	30.91	45	0.69		
Total	50.58	59			

Calculated value of F for types = 1.02
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at the 5% level table. Therefore, there is no significant difference in taste, among the 6 types of samples.

Attribute :- Smell

VA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	0.62	5	0.12	0.60	2.45
Between testers	58.58	9	6.50		
Error	8.84	45	0.2		
Total	68.04	59			

Calculated value of F for types = 0.60
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore, there is no significant difference in smell, among the 6 types of samples.

Attribute :- Appearance

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	4.66	5	0.93	1.79	2.45
Between testers	11.02	9	1.22		
Error	23.19	45	0.52		
Total	38.87	59			

Calculated value of F for types = 1.79
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore, there is no significant difference in appearance, among the 6 types of samples.

Duration :- Two months after preparation

Attribute :- Taste

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	184.74	5	36.95	94.74	2.45
Between testers	10.87	9	1.21		
Error	17.57	45	0.39		
Total	213.18	59			

Calculated value of F (94.74) for types is greater than the critical value of F (2.45) at the 5% level table. Therefore there is a significant difference in taste, among 6 types of samples.

1. Standard error (SY^{-}) = $\sqrt{0.39/10}$
   = 0.20

2. Means :-

DCO	DAS	DCA	DGA	DTO	DBU
3.93	4.04	4.07	7.33	7.43	7.77

3. Studentize table values (STV) for DF_{error} = 45 and p = 2,3,4,5,6
   2.87, 3.44, 3.79, 4.04, 4.23

4. LSR values = STV * SY^{-}

2.87*0.20,	3.44*0.20,	3.79*0.20,	4.04*0.20,	4.23*0.20
0.57	0.68	0.75	0.80	0.84

5. DMR values = LSR * R

0.57*1.17,	0.68*1.17,	0.75*1.17,	0.80*1.17,	0.84*1.17
0.67	0.80	0.88	0.94	0.98

DBU - DCO = 7.77 - 3.93 = 3.84 > 0.98 - significant difference
DBU - DAS = 7.77 - 4.04 = 3.73 > 0.94 - s.d.
DBU - DCA = 7.77 - 4.07 = 3.7 > 0.88 - s.d.
DBU - DGA = 7.77 - 7.33 = 0.44 < 0.80 - not s.d.
DBU - DTO = 7.77 - 7.43 = 0.34 < 0.67 - not s.d.

DTO - DCO = 7.43 - 3.93 = 3.50 > 0.94 - s.d.
DTO - DAS = 7.43 - 4.04 = 3.39 > 0.88 - s.d.
DTO - DCA = 4.43 - 4.07 = 3.36 > 0.80 - s.d.
DTO - DGA = 7.43 - 7.33 = 0.10 < 0.67 - not s.d.

DGA - DCO = 7.33 - 3.93 = 3.40 > 0.88 - s.d.
DGA - DAS = 7.33 - 4.04 = 3.29 > 0.80 - s.d.
DGA - DCA = 7.33 - 4.07 = 3.26 > 0.67 - s.d.

DCA - DCO = 4.07 - 3.93 = 0.14 < 0.80 - not s.d.
DCA - DAS = 4.07 - 4.04 = 0.03 < 0.67 - not s.d.

DAS - DCO = 4.04 - 3.93 = 0.11 < 0.67 - not s.d.

Attribute : Smell

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	0.62	5	0.12	0.95	2.45
Between testers	45.28	9	5.03		
Error	5.83	45	0.13		
Total	51.73	59			

Calculated value of F for types = 0.97
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore there is no significant difference in smell, among the 6 types of samples.

Attribute :- Appearance

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	1.02	5	0.20	1.33	2.45
Between testers	53.11	9	5.90		
Error	6.52	45	0.15		
Total	60.65	59			

Calculated value of F for types = 1.33
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore there is no significant difference in appearance, among the 6 types of samples.

Duration :- After three months of preparation

Attribute :- Taste

ANOVA table

Source	SS	DF	MS	F	F^{5}_{45},5%
Between types	408.32	5	81.66	240.18	2.45
Between testers	7.14	9	0.79		
Error	15.25	45	0.34		
Total	430.71	59			

Calculated value of F (240.18) for types is greater than the critical value of F (2.45) at the 5% level table. Therefore there is a significant difference in taste, among 6 types of samples.

1. Standard error (SY) = $\sqrt{0.34/10}$
= 0.18

2. Means :-

DCO	DAS	DCA	DGA	DTO	DBU
0.83	0.90	1.07	4.00	4.07	8.10

3. Studentize table values (STV) for DF_{error} = 45 and p = 2,3,4,5,6
2.87, 3.44, 3.79, 4.04, 4.23

4. [illegible] values = STV [illegible]
2.87*0.18, 3.44*0.18, 3.79*0.18, 4.04*0.18, 4.23*0.18
0.52 0.62 0.68 0.73 0.76

5. DMR values = LSR * R
0.52*1.17, 0.62*1.17, 0.78*1.17, 0.73*1.17, 0.76*1.17
0.61 0.73 0.80 0.85 0.89

6. DBU - DCO = 8.10 - 0.83 = 7.27 > 0.89 - significant difference
DBU - DAS = 8.10 - 0.90 = 7.20 > 0.85 - s.d.
DBU - DCA = 8.10 - 1.07 = 7.03 > 0.80 - s.d.
DBU - DGA = 8.10 - 4.00 = 4.10 > 0.73 - s.d.
DBU - DTO = 8.10 - 4.07 = 4.03 > 0.61 - s.d.

DTO - DCO = 4.07 - 0.83 = 3.24 > 0.85 - s.d.
DTO - DAS = 4.07 - 0.90 = 3.17 > 0.80 - s.d.
DTO - DCA = 4.07 - 1.07 = 3.00 > 0.73 - s.d.
DTO - DGA = 4.07 - 4.00 = 0.07 < 0.61 - not s.d.

DGA - DCO = 4.00 - 0.83 = 3.17 > 0.80 - s.d.
DGA - DAS = 4.00 - 0.90 = 3.10 > 0.73 - s.d.
DGA - DCA = 4.00 - 1.07 = 2.93 > 0.61 - s.d.

DCA - DCO = 1.07 - 0.83 = 0.24 < 0.73 - not s.d.
DCA - DAS = 1.07 - 0.90 = 0.17 < 0.61 - not s.d.

DAS - DCO = 0.90 - 0.83 = 0.07 < 0.61 - not s.d.

Attribute :- Smell

ANOVA table

Source	SS	DF	MS	F	F .5%
Between type	149.44	5	29.89	90.58	2.45
Between testers	108.15	9	12.02		
Error	15.04	45	0.33		
Total	272.63	59			

Calculated value of F (90.58) for types is greater than the critical value of F (2.45) at the 5% level table. Therefore there is a significant difference in smell among 6 types of samples.

1. Standard error (SY) = $\sqrt{0.33/10}$
= 0.18

2. Means :-

DAS	DCO	DCA	DTO	DGA	DBU
2.60	2.63	2.90	5.20	6.00	6.23

3. Studentize table values (STV) for DF_{error} = 45 and p = 2,3,4,5,6
2.87, 3.44, 3.79, 4.04, 4.23

4. LSR values = STV * SY
2.87*0.18, 3.44*0.18, 3.79*0.18, 4.04*0.18, 4.23*0.18
0.52 0.62 0.68 0.73 0.76

5. DMR values = LSR * R
0.52*1.17, 0.62*1.17, 0.78*1.17, 0.73*1.17, 0.76*1.17
0.61 0.73 0.80 0.85 0.89

6. DBU - DAS = 6.23 - 2.60 = 3.63 > 0.89 - significant difference
DBU - DCO = 6.23 - 2.63 = 3.60 > 0.85 - s.d.
DBU - DCA = 6.23 - 2.90 = 3.33 > 0.80 - s.d.
DBU - DTO = 6.23 - 5.20 = 1.03 > 0.73 - s.d.
DBU - DGA = 6.23 - 6.00 = 0.23 < 0.61 - not s.d.

DGA - DAS = 6.00 - 2.60 = 3.40 > 0.85 - s.d.
GDA - DCO = 6.00 - 2.63 = 3.37 > 0.80 - s.d.
DGA - DCA = 6.00 - 2.90 = 3.10 > 0.73 - s.d.
DGA - DTO = 6.00 - 5.20 = 0.80 > 0.61 - s.d.

DTO - DAS = 5.20 - 2.60 = 2.60 > 0.80 - s.d.
DTO - DCO = 5.20 - 2.63 = 2.57 > 0.73 - s.d.
DTO - DCA = 5.20 - 2.90 = 2.30 > 0.61 - s.d.

DCA - DAS = 2.90 - 2.60 = 0.30 < 0.73 - not s.d.
DCA - DCO = 2.90 - 2.63 = 0.27 < 0.61 - not s.d.

DCO - DAS = 2.63 - 2.60 = 0.03 < 0.61 - not s.d.

Attribute :- Appearance

ANOVA table

Source	SS	DF	MS	F	$F^{5}_{\infty},5\%$
Between types	0.55	5	0.01	0.17	2.45
Between testers	72.85	9	8.09		
Error	2.76	45	0.06		
Total	75.66	59			

Calculated value of F for types = 0.17
Critical table value of F at 5% level = 2.45

The calculated value of F is smaller than the critical value of F at 5% level table. Therefore there is no significant difference in appearance, among the 6 types of samples.

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