Development and Analysis of Lipid Based Edible Coatings for Walnut Kernels

by

Abhinav Jain (114004)
Alan Pious (114012)
Alisha Kar (114013)
Deepanshu Verma (114043)
Shweta Gupta (114148)

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Dr. Ashutosh Upadhyay
Department of Food Science and Technology

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DECLARATION

We hereby declare that the thesis on the topic "*Development and analysis of lipid based edible coating for walnut kernels*" submitted here under the guidance of Dr Ashutosh Upadhyay, Dean Associate and HOD food Science and Technology, NIFTEM for the partial fulfillment of the award for the degree of Bachelor of Technology in Food Technology and Management is original except for source material explicitly acknowledged.

It is also declared that, this work has not been previously included in a thesis/dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

We also acknowledge that we are aware of University policy and regulation on honesty in academic work, and of disciplinary guidelines and procedures applicable to breaches of such policy and regulations.

ABHINAV JAIN ALISHA KAR ALAN PIOUS DEEPANSHU VERMA

SHWETA GUPTA

CERTIFICATE

This is certifying that the group consisting of

1. Abhinav Jain (114004)
2. Alan Pious (114012)
3. Alisha Kar (114013)
4. Deepanshu Verma (114043)
5. Shweta Gupta (114148)

Have successfully completed their thesis on "*Development and analysis of lipid based edible coating for walnut kernels*" under the supervision of their guide Dr Ashutosh Upadhyay.

As per their declaration, the thesis is an authentic work on the issue carried out at National Institute of Food Technology Entrepreneurship and Management

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ABSTRACT

Nuts are high value products particularly rich in fatty acids. The major portion of fatty acid in nuts is unsaturated containing primarily mono-unsaturated and poly-unsaturated fatty acids rendering multifarious health benefits associated with their consumption. However in conjunction to the enhanced health, these fatty acids pose an added instability to the nuts. This can be directly linked to the fact that an increase in the content of double bonds (due to unsaturation) increases the susceptibility to rancidity. Walnuts specifically have very high lipid content (65%) out of which over 70% is PUFA. This can be attributed to an increased instability of walnut kernels upon storage over longer duration due to very high number of double bonds which readily break down to form alkyl free radicals upon exposure to extrinsic parameters such as light, temperature, metals etc and form hydroperoxides. The hydroperoxides further decompose to form secondary oxidation products like saturated and unsaturated carbonyl compounds. Walnuts also have certain lipid peroxidation bio-catalysts which triggers the rate of auto-oxidation reaction.

The work was directed at curbing this problem by development of a lipid based edible coating for walnuts. Edible coatings in general are of 3 major type viz., polysaccharide based, protein based and lipid based and they could be conglomerated to form composite coat according to the desired functionality. A lipid layer owing to its enhanced hydrophobicity renders the coating water vapour impermeable. Impregnating the carrier lipid (palm stearin/palm superolein) with bee wax, acetylated monoglyceride and anti-oxidant resulted in the formation of lipid based coating which could substantially reduce the rate of oxidative rancidity. Bee wax is considered as GRAS which has exceedingly strong barrier properties towards gases as well as water vapour and acetylated monoglyceride has film forming abilities along with barrier properties against oxygen and water vapour. Butylated Hydroxy Toluene is used as the anti-oxidant which has free radical scavenging activity and thereby prevents the onset of oxidation and lengthens the lag phase for oxidation. Two types of edible coatings were made exploiting the diverse chemical properties of carrier- 1) Unsaturated carrier (palm superolein) 2) Saturated carrier (palm stearin). Palm superolein provided for a better appearance of the

coat in terms of gloss and transparency. However palm stearin rendered better functionality in terms of reducing the rate of oxidative rancidity in walnut kernels. The above hypothesis was analysed by carrying out various assays for rancidity profiling by measuring primary oxidation products (Peroxide value) and secondary oxidation products (TBARS, p-Anisidine value) and thereby determining the overall quality of walnut kernels by measuring the TOTOX value (Total oxidation value). The results so obtained substantiated the hypothesis, as the coatings developed worked effectively and increased the shelf stability of walnut kernels by reducing the rate of auto-oxidation. This was suggested by the amount of oxidation products so formed in each of the categories over an accelerated shelf life study for 29 days.

CHAPTER 1 – INTRODUCTION

Botanically, nuts are dry, "one-seeded indehiscent fruits" that does not split open at the seam when it reaches maturity, however the word nut is commonly used for any fruit/seed consisting of a kernel/seed (edible) inside a hard, tough inedible shell (Jaynes,1973).

1.1 General Overview – Nuts

Nut trees often grow in areas that are not-suitable for other crops and some of them give high yields.Nuts have been a valued product to man from the earliest times and remain a gourmet food today. Being referred as nature's perfect little packages, nuts are nutritionally rich, have proven health benefits and are delicious. Nuts can be classified as tree-nuts i.e. almonds, walnuts, hazelnuts, pecans, pistachio and cashews and ground-nuts i.e. peanuts. Nuts offer for a given weight twice the amount of protein than any other food product. On the nutrition basis nuts can be divided into three broad groups, the high fat or oil group, the high protein group and the high starch group. In high fat group, the majority of the nuts contain 50 percent or more of fat, examples being cashew-nut, walnut, pecan etc. Most of the common everyday dessert nuts are rich in oil. Groundnut is an example of a nut with high protein content which contains 25 per cent protein, other nuts like almonds and pistachios are also rich in protein (around 20 percent). Protein rich nuts also have high percentage of fat; the total richness in both protein and fats of this group of nuts explains why some nuts like groundnuts are very popular. Only a few nuts are rich in carbohydrates, for example, chestnut contains 50 per cent carbohydrates which are relatively poor in fats and proteins.

Nuts calorific values are mainly derived from fats, ranging from approximately 568-674 kcal/100g. Nuts are have low levels of saturated fatty acids and arehighly rich in unsaturated fatty acids (i.e. mono and polyunsaturated fatty acids). Among nuts, walnuts are rich in omega-3 fatty acid and alpha- linolenic acid (F. Shahidi et al., 2013). Some common high value nuts that are very popular in customers have been described here:

Product	Percent Fat	Fatty Acids as % of Total Fat	
		Mono-Unsaturated	Polyunsaturated
Almond	52–55	65–77	17–21
Brazil nut	67	35	36
Cashew	46	59	17
Hazelnut (filbert)	63	79	10
Macadamia	77	79	2
Peanut	36–54	48–61	18–32
Pecan	68	54–62	25–34
Pine nut	62	38	42
Pistachio	49	68	15
Sesame seed	54	38	44
Sunflower seed	50	19	66
Walnut, English	62–67	23	63–90

Table 1.1: *Lipid profiling of various nuts (ref: Trezza and Krochta in Application of edible Protein coatings to Nuts and Nut-containing)*

1.1.1 Walnuts (*Juglans regia*):

Walnuts are one of the oldest tree-nuts cultivated which belongs to the family of juglandaceae and genus juglans. Walnut is the seed of a drupe or drupaceous nut. China is the leading world producer, followed by the USA, Iran, Turkey, Ukraine, Romania, France and India (Marcela, 2010).

There are many varieties of walnut grown in different parts of the world like English walnut (juglansregia L) which is cultivated in USA, France, Italy, Poland, Turkey, Chile, China, Germany, Bulgaria, Northern India and Australia. The European walnut is the best known variety and it is found in India. It grows in the hilly areas of Punjab, Himachal Pradesh and Uttarakhand. Some walnuts are also exported from India. Apart from the nut, walnut wood contributes to commercial importance. Black walnut (juglansnigra) which is a native of North America , west to Michigan and most of the Midwestern states. White walnut or butternut (juglanscinerea) which is also from North American tree, and found in the eastern region but it is less important from a commercial point of view (R B N Prasad, 2003).

Walnut is a nutrient rich nut with high protein and essential fatty acids. It is rich in unsaturated fatty acids, Poly unsaturated fatty acid (PUFA). Walnut oil when extracted from nut is of greenish, yellow or almost colourless with a pleasant odour

and a nutty flavour. Oil content in walnut kernels contains approximately 60 % (can be vary from 52 to 70 %) (Cemile, 2012).Minor components of walnut oil include tocopherols, phospholipids, sphingolipids, sterols, hydrocarbons and volatile compounds (Marcela 2010). They are a good source of phosphorous, potassium and magnesium as well as proteins and vitamin E.

Component	Unit	Per 100 g kernels	Proportion RDI	RDI (component)/ RDI (calories)*
Water	g	4.07		
Energy	kJ	2738.00	0.33	1.00
Protein	g	15.23	0.27	0.82
Total lipid (fat)	g	65.21	2.17	6.58
Ash	g	1.78		
Carbohydrate	g	13.71	0.11	0.33
Fibre, total dietary	g	6.70	0.18	0.55
Sugars, total	g	2.61		
Sucrose	g	2.43		
Starch	g	0.06		
Minerals				
Calcium	mg	98.00	0.10	0.30
Iron	mg	2.91	0.36	1.09
Magnesium	mg	158.00	0.40	1.20
Phosphorus	mg	346.00	0.49	1.48
Potassium	mg	441.00	0.09	0.27
Sodium	mg	2.00	0.00	0.00
Zinc	mg	3.09	0.28	0.85
Copper	mg	1.59	1.67	5.06
Manganese	mg	3.41	1.48	4.48
Selenium	µg	4.90	0.09	0.27

Table 1.2: *Composition, recommended daily intake (RDI) and ratio of RDI (component)*

1.1.2 Pecans (*Carya illinoinensis*)

Pecans are native to North American trees but now it has been progressed into a global agricultural crop. Pecan fruit is an oval to oblong nut, 2.6-6 cm long and 1.5-3 cm broad, dark brown which splits off at maturity to release the thin-shelled nut. It has a buttery 3nisid. Pecans belong to the same family as the walnut and have the same distinctive texture and brain-like shape, but a slightly sweeter taste. Pecans are a good source of dietary fiber, manganese, magnesium, phosphorous, zinc and thiamin. Pecans are also a good source of protein, iron and B vitamins (N Shakuntala 2008).

1.1.3 Almonds (*Prunus amygdalus*)

Almonds are the product of trees which grows in tropical and subtropical region. In India, it is found in Kashmir and Himachal Pradesh. Almonds are a drupe fruit which has thick, leathery and grey-green coat which called as hull, inside it, has hard and woody shell which is called endocarp which has seed inside it, called nut. There are two cultivated varieties of almonds P. amygdalusvaramara and P. amygdalisvardulcis. The former one is bitter almond (having oil yield is 40 %) and the later variety is sweet almond (having oil yield is 50 %). Almond kernel is often eaten fresh it is obtained after bleaching, roasting, frying and salting. (N Shakuntala 2008).

1.1.4 Cashewnut (*Anacardium occidentale*)

The cashewnut tree is originally native to northeastern Brazil. In India, it is cultivated mostly on the West coast. India is the second largest consumer in the world. The tree bears fruits consisting of a pear-shaped swollen basal portion or pedicle which is succulent or juicy, and bright orange or yellow in colour. It is edible and known as cashew apple.

The nut contains an acrid juice which is a powerful vesicant and burns the skin, the nut is slightly curved from the centre while the kernels covered by a thin, reddish brown skin or testa. Cashew is nutrient rich and concentrated food.. Its oil consists mostly of glycerides of oleic (73.8 percent) and linoleic acids(7.7 per cent. The fruit is edible and used in making beverages. Since the raw juice is rich in sugar (11.5 per cent), it can be used for the production of alcohol. A fermented liquor “kaju” is made from the cashew apples. Distilled kaju is “feni” and is quite a popular beverage in the cashew growing of our country. (N Shakuntala 2008)

1.1.5 Nuts and dried fruits consumption pattern of Indian consumer

The nut and dried fruit industry in India is currently fixed at INR 15,000 crores (~ USD 2 billion) and is projected to grow to INR 30,000 crores (~ USD 4

billion) by 2020, conferring to the Chairman of Royal Dried Fruits Range, a city-based dried fruits retailer (Business Standard, October 2016).

While the intake of nuts and dried fruits may be much more extensive today, the medieval values of heritage and exceptionality continue to rest with this first-class category. Other than spices, they are the only other category that reveals the ideal exoticism of antique India. Even until a few decades ago, the use of nuts and dried fruits was controlled to special occasions, where their primary uses would be as a food "enricher" – adding richness to the taste as well as nourishing value. Dried fruits and nuts have been a part of festive food items such as spicy Indian gravies "enriched" with cashew paste or pulav (Indian rice) comprising roasted almonds, cashews and raisins or kheer, and payasam (different kinds of Indian dessert) embellished with various dried fruits and nuts.(INC, march 2017)

The newly-charted health discourse in India: These activities can be better valued against the backdrop of the rapidly shifting socio-economic-cultural landscape of India, with enhanced prospects, optimism and ambition. We see new speeches emerging in the consumer lifestyle and phrasing, one of which is the increase in health cognizance. In this conversation, both energy and endurance are becoming key, not just to stay active in the short-term but to also build strength for endurance to stay ahead in the long run. Lifestyle changes, limited control over diet, the degraded quality of basic foods due to the rampant use of pesticides and chemicals and increasing pollution have triggered concern.

To this effect, consumers are now cleverly adding nuts and dried fruits as a new inclusion in their eating regime. The availability of the snack in multiple formats such as salty snacks and dried fruit mixtures (where the nuts and fruits are sometimes broken and mixed) aid variety and make it more palatable for snacking occasions. Cereals such as corn flakes, oats and muesli, themselves making a relatively new breakfast format, have now introduced new flavours such as honey coated almonds, dried fruits and other nuts. (INC, March 2017)

1.1.6 Health Benefits of Nuts

Number of studies have justified that a diet rich in plant foods and with a high ratio of polyunsaturated to saturated fats can substantially reduce the risk of cardiovascular disease and stroke (Bernert and Browner, 1995; Haumann, 1998).

Walnuts contain exceptionally high levels of PUFAs, that have been studied to modify human serum lipid profiles and reduced cholesterol in men to recommended levels (Sabaté et al., 1993). Hu et al. 1998 showed that consumption of nuts as a part of healthy die can reduce the risk of coronary heart disease. Other nuts being relatively high in PUFAs, can likewise contribute to healthy diets.

1.2 Spoilage in Nuts

Nuts generally have high lipid content and are quite rich in essential fatty acids (especially linoleic and linolenic acids). They contain significant amounts of mono- and polyunsaturated fatty acids (associated health benefits) which makes them vulnerable to oxidative stresses (oxidative rancidity) contributing towards quality deterioration and finally spoilage, if stored badly or for too long. Factors leading to these changes can be varied. Light, moisture, oxygen, high temperature, lipid biocatalysts and certain other factors contribute towards these lipid changes thus the loss of quality and potential safety of nuts. Other than lipid oxidation, sogginess due to moisture uptake, flavour loss and microbial spoilage can also affect the overall quality of nuts.

1.2.1 Rancidity

Lipids are heterogeneous compounds with a high affinity towards non-polar solvents such as hydro-carbons, and include oils, fats and phospholipids. Lipid structures are formed by esters of long chain fatty acids, steroids and terpenes which function as depot storage of energy metabolites in adipose tissues. Various organoleptic character notes such as flavour, aroma, colour, texture and mouth-feel pertaining to the quality parameters of specific food products is affected by lipid composition. Lipids acts as a reservoir of nutrition by providing metabolic energy, fat soluble vitamins (e.g., A, D, E, K) and essential fatty acids (e.g., linoleic acid, linolenic acid, arachidonic acid) (Allen J. St. Angelo 1996) .

Oxidation and hydrolysis of oils and fats owing to a decrease in their shelf life leads to rancidity (G.Talbot). Lipid oxidation also known as oxidative rancidity is the reaction of fats and oils with molecular oxygen resulting in off flavour development.

Lipolysis or hydrolytic rancidity is caused due to high moisture content and enzymes (such as lipases) which liberate free fatty acids from the triglycerides to form diglycerides and monoglycerides. Rancidity is marked by the characteristic off-flavour and odour of the oil, resulting in deterioration of food (Hamilton 1994).

1.2.1.1 Oxidative Rancidity

Oxidative rancidity is a three step process in which the fat initially absorbs oxygen from the air and forms peroxide in the fat, which further breaks down to form secondary products like aldehydes and ketones (Rossell and Pritchard). The catalytic systems responsible for lipid oxidation are light, temperature, enzymes, metals, metalloproteins and micro-organisms (Allen J. St. Angelo 1996). The mechanism of oxidative rancidity could be via- (1) Auto-oxidation (2) Photo-oxidation (3) Enzymatic oxidation (F. Shahidi).

Auto-oxidation

Auto-oxidation in lipids occurs in three stages viz., initiation, propagation and termination (F. Shahidi). It is a self 7nisidine reaction with certain pro-oxidants like heavy metal ions that accelerate it and anti-oxidants that slow it down (G.Talbot). It occurs in two phases, the first being the induction phase where in the oxidation rate is slow and steady until it has reached a specific point after which the rate of oxidation increases markedly resulting in the second phase (Sophe Williamson 1998).

The initiation stage is marked by an alkyl free radical breaking off from the triglyceride moiety due to removal of a hydrogen atom. In case of unsaturated fatty acids hydrogen atom breaks from the methylene group adjacent to the C-C double bond. (G.Talbot).

In the propagation stage, the alkyl free radical formed reacts with a molecule of Oxygen to form hydroperoxyl free radical, which being highly reactive attacks another triglyceride molecule extracting an hydrogen to form relatively stable hydro-peroxide however forming further another triglyceride free radical. And the reaction further propagates in the presence of oxygen to form more and more hydro-peroxides.

Termination takes place when two free radicals combine with each other. Thus, the end of auto-oxidation is marked by break down of hydro-peroxides into aldehydes

due to the hydro-peroxides being unstable resulting in their break down into alkoxy free radicals by loss of hydroxyl free radicals (G.Talbot).

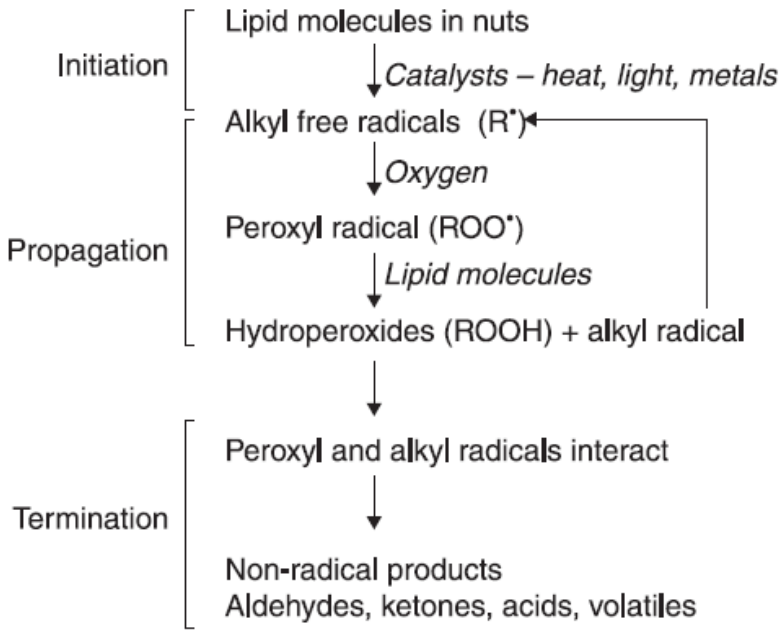

Figure 1.1*: Mechanism of auto-oxidation (ref: F. Shahidi et al. 2013)*

Photo-oxidation

Various aliphatic and aromatic oxidised compounds such as ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxies, sulfoxides, sulfones, phenols, anhydrides, quinones and alcohols are formed as a result of continuing free radical chain reaction of fatty acid molecule (Lee, 2003). Photo-oxidation follows 2 mechanisms- in presence of light and in presence of photosensitizer (G.Talbot).

In the presence of visible and short wave UV light fatty acids are activated to form free radicals which react with triplet oxygen to form singlet oxygen. In presence of sensitizer, two types of photo-oxidation has been studied viz., Type 1 and Type 2.

In Type 1 photo-oxidation, the sensitiser which is inherently present in the fatty acid is activated in the presence of light to form a radical which can react with triplet oxygen for example, riboflavin (B. Matthaus). This occurs via formation of excited photosensitiser, which leads further formation of an intermediate due to a

reaction between excited triplet state of sensitiser and an acceptor substrate. Subsequently the intermediate further react with triplet oxygen to produce the products of oxidation.

In Type 2 photo-oxidation, there is formation of reactive singlet oxygen due to the light activated sensitiser reacting with triplet oxygen, which further reacts with fatty acid molecule (B. Matthaus). Singlet oxygen is highly reactive with double bonds in unsaturated fatty acids resulting in the formation of different positional hydroperoxides and a shift of the double bonds. As compared to Type 1 photo-oxidation reaction, it involves an additional step of conversion into singlet oxygen which directly affects the double bonds in fatty acid chains (G.Talbot).

Enzyme Catalysed oxidation

Enzymatic oxidation involves the enzyme lipoxygenase (oxido-reductase), which leads to the formation of oxidation products like hydroperoxides which might further get converted to hydroperoxides. Lipoxygenases are necessarily present in all living cells in the cell membrane. They catalyse the reaction between oxygen and cic, cis unsaturated fatty acids to form hydroperoxides. Lipoxygenases mostly utilize free fatty acids as their substrate however triacyglycerides with low specificity are also used up as substrate (B. Matthaus).

1.2.1.2 Hydrolytic Rancidity

Hydrolytic rancidity is the result of the interaction of moisture and enzymes with the oil (Allen and Hamilton, 1994). The glycerides in the oil hydrolyse when they react with water to form free fatty acids, diglycerides, monoglycerides and glycerol. These degradation products cause deteriorative changes in the flavour profile of the oil causing emanation of unpleasant odour. The rate of hydrolysis is however dependent upon fatty acid content of the oil, amount of dissolved water in the oil and the storage conditions to which the oil is exposed to (Gunstone and Padley, 1997).

It occurs via two different mechanisms. In the first one, keto acids are produced by a reaction between triglycerides and water in the presence of heat. The keto acids being highly unstable lose CO_2 to form methyl ketones and hydroxyl fatty acids which in turn are the precursors for the formation of –γ lactone and –δ lactones. Depending on the chain length and substrate the methyl ketones have objectionable or acceptable off flavours (Hamilton, 1994).

In the second pathway, hydrolytic rancidity occurs due to presence of water and an activated lipase. The resulting hydrolytic breakdown of triglyceride molecule releases one, two or three molecules of fatty acids. As the fatty acid molecules are released the triglyceride breaks down initially into a diglyceride, then into monoglyceride and finally into glycerol. Depending on the chain length of the free fatty acids produced they have different flavour thresholds above which they are apparent as off flavours (G. Talbot).

1.2.1.3 Products of fat oxidation

Decomposition of glycerol backbone in triacyglycerols (TAG) or phospholipids into volatile compounds with low molecular weights produces off aromas as associated with rancidity. With an increase in fat unsaturation, the likelihood of lipid oxidation increases. The factors directly affecting fat oxidation during the initiation stage are enzymes, metals, metalloproteins, light, high processing temperature, and irradiation which is further accelerated by the reactions that produce free radicals and reactive O_2 species.

Lag Phase

During formation of hydroperoxides by the propagation stage of autoxidation which undergoes formation of free radicals, there comes a stage when the accumulation of lipid oxidation products slow down. This is directly related to the rate of free radicals synthesis governing hydroperoxide formation, which is known as the lag phase. Maximization of lag phase slows down rancidity (Leann M Barden-2014).

Primary Oxidation Products

Due to lipid molecules being exposed to heat and light, there is break down of alkyl free radicals from triacyglycerol (TAG) which subsequently take up Oxygen

from the atmosphere to form Peroxyl free radicals to further form Hydroperoxide which is the primary product obtained during the propagation stage of auto-oxidation of lipids. Peroxide value performed by iodometric titration using starch as indicator and sodium thiosulphate as titrant gives a measure of hydroperoxides content in the lipid fraction.

Secondary Oxidation Products

Peroxides and alky radicals react further to undergo the termination stage of auto-oxidation wherein the peroxide decomposes to form secondary oxidation products like aldehydes, ketones, acids and volatiles (F.Shahidi and J.A John).Secondary oxidation products are most likely formed as a result of heating, radiation or presence of other radical initiating agents. The secondary products are due to either peroxide scission alone or simultaneous peroxide and chain scission (Hoffman, 1989). P-Anisidine value estimates the amount of –α and –β unsaturated aldehydes (mainly 2-alkenals and 2,4-dienals) formed as a result of secondary oxidation by forming a chromogen which is measured spectrophotometrically (S.SuzanneNielson- Fourth Edition). Thiobarbituric acid assay is a method for measuring malondialdehyde (a product of secondary oxidation) by formation of a pink chromogen at high temperature and low pH quantified spectrophotometrically (Marc Pignitter and Veronica Somoza-2012).

1.2.1.4 Implication to Nuts

The onset of lipid oxidation and degree of deterioration majorly depends upon nut type, storage conditions, and composition of the nut or food in which nuts have been used. The fatty-acid profile of nuts majorly influences the induction period and oxidation rate of nut lipids. The relationship is established as; higher the content of poly-unsaturated fatty acids in the product (increase in the number of double bonds – increases the susceptibility to oxidation), higher will be the oxidation rates. This is the reason for nuts like walnuts, pecans and peanuts becoming rancid relatively faster at ambient conditions with reference to other nuts like chestnuts and cashews. However being a dried product, certain raw nuts and many roasted nuts have relatively short shelf-lives, ranging from a few weeks to a few months.

When specifically stating the problem for walnuts, development of unacceptable off-flavours resulting from oxidation of lipids, is a major problem.The

main constituent of walnuts is the oil (around 65 % w/w), predominantly constituted by unsaturated fatty acids (PUFA – around 72% and MUFA – around 14% of total lipids) and hence, is highly prone to oxidative reactions. Due to these oxidative changes walnuts can lose their characteristic flavours over prolonged storage.Since, there is a high percentage of linolenic acid and linoleic acids in walnuts, they contributes most towards the objectionable flavours,a process known as flavour reversion in walnuts, due tothe abundantly formed oxidation products likehexanal, octenals,and heptenals (linoleic oxidation products) and propanal and heptadienals (linolenic oxidation products) (Belitz and Grosch, 1987).

1.2.2 Flavour Loss/Fade

Flavour is the most important criteria in terms of customer acceptability for any food. Nuts have associated characteristic flavours which attribute towards their high value not just in terms of their cost but its overall sensory profile that further results in the increase in their desirability in customer markets.

Nuts tend to lose their characteristic flavours over prolonged storage in ambient conditions.'Flavour fade' is a term that is typically used for describing flavour loss in peanuts during storage (Dimick, 1994). The flavour associated with roasted peanuts is attributedto a large number of volatile compounds i.e., certain ketones, alcohols, pyrazines, sulphur containing compounds (such as methanethiol, carbon 12 nisidine 12 ,and dimethyl sulphide) and some hydrocarbons (Walradt et al.,1971; Crippen et al., 1992). Flavour fade in peanuts is primarily due to the maskingof pyrazines and other flavour compounds by low-molecular weight aldehydesproduced during lipid oxidation (Warner et al., 1996). Sensory scores defining the flour of peanuts directly correlates with the changes in pyrazine content (Braddock et al.,1995). Thus this is conclusive that flavour loss and changes can be controlled via reduction in oxidative rancidity (Dimick, 1994; Warner et al., 1996).

Other nuts also show similar loss in flavours associated with oxidative rancidity. Roasted hazelnut flavour which is attributed to low levels of2-methylpropanal, 2-methyl- and 3-methyl butanol, and perhaps dimethyl sulphide (Sheldon et al., 1972) is lost via similar factors to those found for peanuts. This

hasbeen quantitatively confirmed by Keme et al., (1983a, b). Flavour characteristics for nuts like walnuts and pecans also get affected in the similar fashion. Flavour fadecan limit the shelf life of nuts and nut-containing products. Flavour of nuts generally “fades away” as lipids oxidise, the nuts not only lose the desirablenut flavour, but they also gain the undesirable rancid flavour.

1.2.3 Mass transfer – Moisture and Oil Migration

Moisture migration and moisture uptake is a major mode of quality deterioration for nuts.Generally, a small range of optimal moisture content exists for nuts. Increase in moisture content above these optimum values can make nuts susceptible to microbial spoilage and also can affect the textural properties. For example, peanuts are best stored at an optimum moisture content of about 7%(Woodroof, 1966). Above this value, they are highly prone tomould growth.Moisture uptake can also result in stale and soggy nuts. Cosler (1958 a, b) andAlikonis and Cosler (1961) demonstrated the effect of moisture in determining the quality of a variety of nuts, apart from rancidity. Many studies have also associated the increasesin moisture content o increased rates of oxidative rancidity. Hall, 1991 concluded that the oxidation rates were greatest when the water activity for the samples was around 0.60 to0.75. When imbedded in other foods like confections and ice creams, moisture migration from the surrounding components to the nut phase often leads to loss in crunch and increased flavour fade.

Oil migration from nuts to other components in a food is also one of the primary concernsin the confectionery industry. This transfer of oil can severely affect the shelf-life of products such as chocolate-coated walnuts and Brazil nuts.In these kinds of products it leads to the softening of the chocolate, hardening ofthe nut centre, and formation of ‘surface bloom’ (layer of fat – migrated to the surface and recrystallized – which in turn effects sensory properties and consumer perception of the product)(Minifie, 1980; Talbot, 1996; Couzens and Wille, 1997).

1.3 Preservation strategies

Nuts can be preserved from deterioration and the quality of nuts can be maintained for significantly longer durations by altering the storage conditions, addition of anti-oxidants, and by packaging to reduce their interaction with the external environment. With careful selection of these three factors, the shelf-life of nuts, or in case any food product can be enhanced substantially.

Traditional approaches to preservation of nuts include low temperature storage i.e. cold storage to lower the lipid degradation rates; addition of synthetic antioxidants like BHT or BHA to prevent the food substance from oxidative stresses; and finally packaging that includes metal containers or plastic packaging to reduce the kernel interactions with outer environments.

1.3.1 Cold Storage:

Reduced temperature storage like refrigeration or freezingof nuts is one of the simplest and most reliable methods to preserve them from deterioration.For example nuts likewalnuts and pecans can be effectively stored fortwo years or more without any significant difference in quality when frozen. When nut oils are frozen, it is assumed that the low freezing temperatures halts the oxidative rancidity development and preserves the quality – thus having a great implication in research. The same has been confirmed through various studies and thus is commercially practiced and recommended for long-term storage purposes (Woodroof, 1966; Ahmed and Storey, 1975; NPSA, 1997). Refrigeration is thus a common recommended method for storage of nuts like walnuts, peanuts and pecans. But the method has its own disadvantages like unavailability of low temperature storages in the supply chain and high cost related to the maintenance of cold temperatures. Ecological factors like high energy input for refrigeration purposes and release of ozone-deteriorating gases in the environment is also a rising concern.

1.3.2 Additives: Anti-oxidants:

As nuts are generally stored and displayed at ambient temperatures on shop floor, the preservation can be a challenge.Also when nuts are used in other food products, the optimum storage temperatures of those food products can be different

from those of nuts and hence, the qualityand shelf life of nuts can be severely compromised. Thus, addition of certain additives to prevent nuts from oxidative stresses becomes necessary. *Antioxidants* are a type of edibleadditives that acts to delay the onset of rancidity in lipids by preventing foods from oxidative changes. They act as free radical (singlet oxygen) quenching and scavenging agents, which are responsible for initiating the oxidationof unsaturated fatty acids.They do not completely prevent lipid oxidation, but considerably lengthens the shelf-life period.

Anti-oxidants approved for usage in foods are broadly classified into two general categories – synthetic/chemical anti-oxidants and natural anti-oxidants.

Syntheticantioxidants are petroleum products and are generally comprising of certain phenolic compounds like butylated hydroxyanisole(BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone(TBHQ). Other compounds include gallate esters (such as propyl- and octyl-gallate). Many studies have concluded that these anti-oxidants alone or in combinations when added to walnuts, chocolate-coated peanut butter/nut confections, and othernut-containing products, effectively enhanced the shelf-lives (Hoover and Nathan, 1980, 1981).

Natural antioxidants on the other hand are extracted from plants and include vitamins (vitamin C& E), carotenoids (15nisidi and lycopene), and a wide variety of other naturally occurring compounds.Compounds like vitamins can also be synthesised commercially. Extracts from plants (e.g. Basil, Moringa) and spices (e.g. pepper, turmeric) can have shown high antioxidant properties (Chipault et al., 1952).

1.3.3 Packaging of Nuts:

Packaging plays a very critical role reducing the rate of quality loss in foods. Packaging is also essential component of distribution and storage. Selection of packaging can greatly influence the quality and shelf life ofnuts. Different packaging materials are used to contain nuts i.e. glass jars (air tight) metal containers and plastic containers.Metal and glass packaging act as a total barrier to external environment and thus enhancing the shelf-life to great extents. But being of high cost are generally not used, or if used than only in premium products. High-oxygen-barrier plasticfilms, such as polyvinylidene chloride (PVDC), ethylene vinyl alcohol(EVOH) and metalized filmscan also be used for packaging of nuts. These packaging solutions

provide less protection and preservation than glass or metal but are very cheap in and thus are used extensively.

Other effective strategies include modified atmosphere packaging, i.e. vacuum packaging andnitrogen flushing. High-barrier materials are to be used while adopting these techniques as to limit the amount of oxygen diffusion into, or nitrogen diffusion out of, the package during storage. Light exposure is also to be minimised to protect nuts from phot-induced oxidation.

1.3.4 Modern Approach (Shifting towards more eco-friendly options)

Growing environmental awareness in terms of non-biodegradable wastes generated from packaging materials and high energy inputs, there is a shift towards more nature-friendly options for preventing and preserving foods. Development of biodegradable packaging solutions that are compatible with the environment is need of the hour. Preservation techniques like waxing of fresh fruits and dry fruits/nuts have been used from ages to prevent them from moisture loss.

Edible films and coatings thus can be a very effective and eco-friendly approach towards enhancing the nut's shelf-life, while providing various other important functions in reference with textural and sensory attributes. From last two decades,edible films and coatings has been a topic of great interest in scientific community due to their wide applications and functionality in enhancing and maintaining the overall quality of nuts. The concept of edible packaging and coating has been discussed in brief in the next section.

1.4 Edible films and coatings – a general overview

Edible coatings and films have been used for centuries to prevent food from moisture and preserve them for longer storage times. In most cases the terms film and coating have been used interchangeably to indicate the surface of a food which is covered by a relatively thin layer of material that is edible in nature. However, a film is occasionally differentiated from a coating as a stand-alone wrapping material, whereas a coating is applied and formed directly on the food surface itself. Starting from the limited commercial applications of edible coatings like in case of wax coats

on fruits for better gloss and surface characteristics, the use of edible coatings in commercial applications is on the rise. Today, edible films use has expanded rapidly for retaining quality of a wide variety of foods, with total annual revenue exceeding $100 million.

According to Attila E. et al. edible coatings and films can be defined as “Any type of materials used for enrobing (various foods to extend shelf-life of the product that may be eaten together with food with or without further removal is considered an edible film or coating.” The coating material prevents moisture losses, while allowing a selective exchange of gases through it. Various other functions are also associated with edible coating like surface sterility, appearance enhancement, adhesion and carrier of additives etc. The thick ness of edible coats is generally less than 0.3 mm (Attila E. et al.).

1.4.1 Preservation aspect – Insight

In the past few decades, the field of edible films and coatings and their application have attracted significant attraction within the scientific community. Edible films and coatings have been successfully used in the shelf-life extension of many different products: meat and meat derivatives (Oussalah et al. 2004), fish and derivatives (Gómez-Estaca et al. 2007), fruits (Pérez-Gago et al. 2006), and vegetables (Ponce et al. 2008) etc.

Edible coatings can extend the shelf-life period and improve the standard of food by the management of mass transfer, wetness and oil diffusion, gas porosity (O_2& CO_2), and flavour and aroma losses and by maintaining and enhancing the mechanical and physical characteristics, colour and appearance of foods. On the other hand edible film as a solid sheet may be applied between food elements or on the surface of the food system so as to inhibit migration of wetness, oxygen, CO2, aromas and lipids. Edible films with adequate mechanical properties might conjointly function as edible packaging for designated foods (Monique Lacroix, Khanh Dang Vu, 2014). Edible coatings even have the potential for maintaining the standard of food when the packaging is opened by protective against wetness amendment, element uptake, and aroma loss.

1.4.2 Functions of edible coatings

The main role of edible coatings is to preserve the top quality of a nutrient. Edible coatings are applied in active packaging, thanks to their useful properties and since they eliminate or inhibit the expansion of microorganisms in food merchandise. Active antimicrobial packages are designed to operate once in direct contact with food as they incorporate antimicrobial additives. Several natural substances such as enzymes, bacteriocins and essential oils are studied for their useful properties as active ingredients for edible package.

Consumers require fresh and minimally processed foods that are exempt from chemically synthesized substances, and they look for those food enriched with natural substances that bring health benefits and maintain nutritional and sensory characteristics (Falguera, Pagan, &Ibarz, 2011). Therefore, in recent times the efforts of researchers have been focused on searching for new naturally occurring substances that act as possible alternative sources of antioxidants and antimicrobials (Ponce et al., 2008). Rojas-Grau, Tapia, Rodrıguez, Carmona, and MartinBelloso (2007) proved the ability of edible coatings based on sodium alginate and gellan gum to transport N-acetylcysteine and glutathione as antibrowning agents, besides the positive effect of the addition of vegetable oils in these edible coatings to increase resistance to water vapor transport in minimally processed fruits of Fuji apple. Moreover, it was also stated that the coatings can keep the vegetable oil enriched with essential fatty acids (u3 and u6) encapsulated

Edible films and coatings also act as vehicles for edible additives and active ingredients that could be added to the formulations. Coatings are often used to encapsulate selective additives which may have product enhancing or functionality such as an antioxidant action. Other possibilities include the encapsulation of antimicrobial agents, aroma compounds, and pigments, ions that stop browning reactions or nutritional substances such as vitamins (Debeaufort et al. 1998). The selection of a film-forming agent, and additives to be carried, depends mainly on the specific characteristics and requirements of the food product to be coated.

The most important general prerequisites to be fulfilled by edible films and coatings can be described as (Debeaufort et al. 1998):

- High barrier and mechanical properties with stable physical integrity
- Enough biochemical, physicochemical, and microbial stability
- Free of toxics and safe for health
- Good sensory qualities
- Simple and cheap technology
- Non-polluting
- Low cost of raw materials and process

1.4.3 Structural matrix: carbohydrates, proteins and lipids

Edible coatings and films area unit typically classified according to their structural material. In this method, films and coatings are supported proteins, lipids, polysaccharides or composite. Let's say, a composite film could accommodates lipids and hydrocolloids combined to make a bilayer or a cluster (Krochta, Baldwin, &Nisperos-Carriedo, 1994) In some recent studies the assembly of edible and perishable films by combining numerous polysaccharides, proteins and lipids is taken into account with the aim of taking advantage of the properties of every compound and therefore the natural action between them. The mechanical and barrier properties of these films not solely rely on the compounds employed in the chemical compound matrix, however additionally on their compatibility. (Altenhofen, Krause, & Guenter, 2009).

The improvement of edible films composition is in one amongst the most necessary steps of the analysis during this field, since they must be developed per the properties of the fruits and vegetables to that they need to be applied (Rojas-Grau et al., 2009a). Thus, it's important to characterize and check totally different coating solutions on contemporary and minimally processed food, since every one of them has totally different quality attributes to be maintained and increased throughout the storage time (Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2008a)

Polysaccharides and proteins are great materials for the formation of EC and EF, as they show excellent mechanical and structural properties, but they have poor barrier capacity against moisture transfer. This problem is not found in lipids due to their hydrophobic properties, especially those with high melting points such as

beeswax and carnauba wax (Morillon, Debeaufort, Bond, Capelle, & Volley, 2002; Shellhammer & Krochta, 1997)

1.4.4 Edible films and coatings and their role as active packages

The development of coatings supported polysaccharides has brought a major increase within their applications and in the quantity of product which will be treated, extending the period of time of fruits and vegetables because of the selective permeability of those polymers to O2 and carbon dioxide. These polysaccharide-based coatings may be accustomed modify the interior atmosphere of fruits, delaying senescence (Rojas-Grau et al., 2009a).

The effectiveness of an edible coating to guard fruits and vegetables depends on the management of wettability (Cerqueira, Lima, Teixeira, Moreira, & Vicente, 2009b), on the film's ability to take care of the practicality of some compounds (plasticizers, antimicrobials, antioxidants) among the matrix, because the loss of those molecules affects the thickness of the film (Park, 1999), and also the solubility in water because it is important to avoid the dissolution of the coating (Ozdemir & Floros, 2008).

The main advantage of victimisation these different ways in situ of typical artificial chemical compound primarily based packages is that the reduction of waste arising from artificial packaging with its world ecological concern, whereas having the potential to limit wetness, aroma, and migration of supermolecule between food elements (Miller and Krochta 1997; Nussinovitch 2009).

1.4.5 Pre-requisites for suitable coating materials for nuts

In recent years, a variety of materials have been tested in order to meet the specific requirements of this type of products. In the search for suitable film-forming materials for the proper coating of nuts, the following requisites should be taken into account.

1. Organoleptic Compatibility
2. Antioxidant Activity
3. Good Adhesiveness on the Surface of the Nut
4. Microbial Stability

5. Good Optical Properties

`Edible films or coatings may also be used to carry or support flavours at the product surface. These flavours will then be promptly released upon consumption of the product. In fact, a few products that use this flavouring concept have already been commercialized. For instance, there are roasted peanuts with a curry-flavoured coating that instantaneously dissolves in the mouth providing the perception of the Indian spice.

Processes for coating nuts and peanuts have been described (Krochta et al., 2005;Cleophas, 2006; Rapp et al., 2006; Mie et al., 2008) including pan coating and coating nuts with a film forming coating to delay the development of rancidity. The film forming coating is applied in a solution that contains an amount of surfactant greater than that which reduces the surface energy of the solution to its lowest level.

There are certain desirable characteristics have been defined by various studies and researches in the field of edible coating:

- Contain no harmful, allergic and non-digestible elements
- Give structural stability and stop mechanical injury throughout transportation, handling, and show Have smart adhesion to surface of food to he protected providing uniform coverage
- Management water migration each in and out of protected food to keep up desired wetness content
- Give semi-permeability to keep up internal equilibrium of gases concerned in anaerobic respiration, therefore retarding senescence
- Stop loss or uptake of elements that stabilize aroma, flavour, biological process and organoleptic characteristics necessary for shopper acceptance whereas not adversely sterilisation the style or look
- Give organic chemistry and microbial surface stability whereas protective against contamination gadfly infestation, bug proliferation, and different varieties of decay.
- Maintain or enhance aesthetics and sensory attributes (appearance, taste etc.) of product

- Function carrier for fascinating additives akin to flavour, fragrance, colouring, nutrients, and vitamins.
- Incorporation of antioxidants and antimicrobial agents will be restricted to the surface through use of edible turns, therefore minimizing price and intrusive style.
- Last however not least – be simply factory-made and economically viable.

1.4.6 Prospects of edible coatings in nuts

Edible coatings having low gas and vapour permeability have potential application in packaging and preservation of nuts. Many studies during the last decade have shown various potential edible coat formulations majorly comprising of proteins, carbohydrates and lipids in different proportions in conjunction with varied plasticizers and emulsifiers, which can significantly improve the shelf-life of nuts and maintain the quality during storage. Application of edible coatings can have varied advantages as stated below-

- Reduction in plastic waste production and high energy inputs – Eco-friendly approach of preservation.
- Significantly enhance the shelf life and storage quality of nut.
- Varied structural and textural enhancements to the nut surface in terms of sensory attributes.
- Can individually coat every single nut and thus eliminating the need of consuming entire batch/packet within short duration, once the packet has been opened.
- When coated nuts are put in food-matrix as ingredients, thus water-migration from food to nuts, and thus sogginess in nuts can be efficiently controlled.
- Oil-migration from nuts to food-matrix can also be efficiently controlled thus improving sensory qualities of foods embedded with nuts.

A comprehensive discussion has been done in the next section regarding edible coatings for nuts and previously done research on the same.

CHAPTER 2 – AIMS AND OBJECTIVES

HYPOTHESIS:

Present study aims to formulate and evaluate an edible lipid based coating for walnut kernels to reduce its susceptibility to auto-oxidation thereby enhancing its shelf-life.

Research Objectives

- Development and standardization of edible lipid based coating using bee wax, acetylated monoglyeride, anti-oxidant and carrier lipid (saturated/unsaturated fat)
- Rancidity profiling of the oil extracted from coated and uncoated walnut kernels by Peroxide value, p-Anisidine value, TBARS, TOTOX value after subjecting to accelerated storage conditions over a period of 29 days.
- Analyzing the efficacy of the coating developed and deducing its further applicability.

CHAPTER 3 – LITERATURE REVIEW

Nuts, because of their high lipid content and rich essential fatty acid profile (mono and poly- unsaturated fatty acids), are particularly prone to lipid oxidation and rancidity. In their natural states, nuts have a natural packaging or barrier in form of hard shells or skins that retains flavour and aroma, regulate gaseous transport (oxygen, water etc.) and protect them from deterioration (Miller and Krochta 1997). However, unfortunately modern dietary choices include processed foods that lack these natural barriers. Therefore, in minimally processed nuts, to control the development of off-flavours that makes the product unacceptable to the consumer, the kinetics of lipidoxidation has to be controlled (Lorena Atarés et al., 2016).

Packaging methods like use of inert gases, or vacuum packing, where in oxygen is removed from the packages (low oxygen packaging), have been used widely to prevent nuts from deterioration. However these methods are incapable of preventing lipid deterioration once the nuts are removed from the package (Hye-Jin Kang et al., 2013).In other words nuts are difficult to preserve during long-term storage even when packed under these special packaging conditions (Mexis, Badeka, Riganakos, Karakostas, &Kontominas, 2009).Another concern with these method is the use of synthetic polymers and laminates. These petroleum based synthetic polymers are utilised in low-oxygen packages, owing to their excellent barrier properties, pose serious environmental threats and ecological problems due to their non-biodegradability (Tharanathan, 2003).

An alternative method to low-oxygen packaging can be the use of a thin continuous edible coating around each nut with very low oxygen permeability to reduce the oxygen concentration within the nuts and delay oxidative rancidity, thus overcoming the cons of low-oxygen packaging (Juan I. Mate et al., 1996).

The primary function of an edible coating on nuts is to prevent or delay lipid oxidation and onset of rancidity (Baker et al., 1994). Further, it can also be used as a carrier of food additives including spices, colorants, flavourings, antioxidants and antimicrobials (Takala, Salmieri,Vu, & Lacroix, 2011). Also, coatings can prevent nuts from absorbing moisture and reacting with other ingredients when used in certain food products like cakes (Baker et al., 1994).

Coating application can help prevent fat deterioration by minimising oxygen penetration into the nut meals (Hye-Jin Kang et al., 2013). Also the synergistic effect of powerful antioxidants carried by the coating can contribute towards protection from oxidation (Debeaufort, Quezada-Gallo, & Voilley, 1998).The residual oxygen concentration within the nut depends on the oxygen permeability of the coating and the oxygen consumption rate within the tissue (Juan I. Mate et al., 1996).

3.1 Published Research and work

Within the last 50 years, over 200 research articles have been published on the potential use of edible films and coatings for enhanced properties (appearance) and shelf life extension of nuts. In spite of the extensive research done on the subject, only few applications are commercially viable. However, as food processing technology has advanced, functionality of food ingredients has improved. New opportunities for food coating applications have been discovered by the exploitation of specific food ingredients (Trezza and Krochta, 2002). Use of proteins, hydrocolloids and lipids as coating materials are highly promising due to their wide array of physical properties (e.g., film forming and barrier properties). Many important reviews have also been published (Koelsch, 1994; Krochta et al., 1994; Gennadios etal., 1997; Debeaufort et al., 1998).

3.1.1 Early works

One of the earliest examples of edible coating for nuts dates back to nineteenth century. During those times sucrose was applied as an edible coat on nuts, almonds, and hazelnuts to prevent oxidation and rancidity during storage (Debeaufort et al. 1998). Corn-zein and shellac wax have also been historically used as coating materials for several nuts. These coats are dissolved and applied using an ethanol solvent having low surface energy thus allowing good wettability and coverage of nut surfaces (hydrophobic) (Tharanathan 2003). But, there were certain drawbacks with ethanol using as a solvent i.e. high cost and inadequate availability of quality food grade ethanol, safety and health issues and the environmental concerns associated. Commercial corn-zein coatings helped reducing rancidity (sensory-detectable) of roasted peanuts, pecans, almonds, and walnuts (Cosler, 1958a; Alikonis andCosler,

1961). Cosler 1958b showed that zein-coated peanut bars and chocolate bars containing zein-coated nuts had increased shelf life compared to products with uncoated nuts. These coatings were a commercial blend of zein, oil, BHT, and other antioxidants. Coatings improved certain appearance properties (gloss) and acted as oil and moisture barrier.

In other early work, Hebert and Holloway (1992) patented a formulation and process for pre-coating of nuts with a protein – base coating to improve uniformity and adhesion of a second seasoning layer. Durst (1967) successfully coated fig bars, brownies and other bakery items with sodium caseinate and gelatin coatings to prevent stickiness and oxidative deterioration.

3.1.2 Recent works

Many studies have been published indicating moisture, oxygen and flavour barrier properties of edible films (Miller and Krochta 1997). Studies presented certain suitable film forming materials (whey protein isolate, soy protein isolate and cellulosic derivatives) for nuts having good oxygen and aroma barrier properties (Han and Krochta 2007; Hye-Jin Kang et al., 2013; Maté and Krochta 1998; Moslem Sabaghia et al. 2015; Leeand Krochta 2002 ;Baldwin and Wood 2006; Trezza and Krochta 2002 ; Miller and Krochta 1997), these properties make them suitable for this use. For overview of past researches refer table 3.1.

More recently, composite coatings based on protein and polysaccharide has come into the picture. Whey and soy protein based coats have shown potential for reducing oxidative degradation and extension of shelf life of simple as well as roasted nuts (Hye-Jin Kang et al., 2013; Trezza and Krochta, 2002). Adherence of coatings on nut's surface is a vital factor for shelf life extension. Lin and Krochta (2005) presented their work on this adherence factor for peanuts by immersing them in an aqueous solution of the surfactants and allowing them to air dry. This process modified and enhanced the surface energy that made them compatible with the hydrophilic whey protein based coatings. Use of surfactants (Span 20) in the coating solutions also enhanced the surface coverage of the peanuts depending on the concentration.

Maté et al. (1996) showed that the effectiveness of coatings can be determined on the basis of coat's thickness and the relative humidity. This conclusion was made while investigating the effects of humidity in peanuts on these coatings. The lack of discontinuities of the coatings was also proved to be a critical factor in improving the shelf life of peanuts.

Nut product	Coating	
Peanuts	Whey protein	Sugar esters, soy lecithin, Sorbitan laureate (Span 20) (*s*)
		Glycerol (*p*)
		Lecithin (*s*)
		Methyl paraben (*am*)
		Vitamin E (*ao*)
		Glycerol (*p*)
		Distilled acetylated monoglycerides
		Glycerol (*p*)
Pecans (pecan kernels)	Methyl cellulose (MC), hydroxypropyl cellulose (HPC), carboxymethyl cellulose (CMC)	Propylene glycol (PG), sorbitol (*p*)
		Lecithin (*s*)
		α-tocopherol (vitamin A), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) (*ao*)
Almonds	Hydroxypropyl methylcellulose (HPMC)	Tween 80 (*s*)
		Ascorbic acid, Citric acid, Ginger oil (*ao*)
p plasticizer, *s* surfactant, *am* antimicrobial, *ao* antioxidant		

Table 3.1: *Original research studies—performed over the last two decades on the application ofedible coatings to nuts (Source- Lorena Atarés et al., 2016)*

3.1.2.1 Protein Based

Till date major work in the field of edible coats for nuts and dry-fruits has been dedicated to protein based coating - owing to their excellent film forming and mechanical properties, high wettability and solubility, and excellent gas barrier

properties with medium or poor water vapour barrier properties (due to their hydrophilicity). In addition to this proteins have wide functionality based on the type and the origin source and are cheaply available. Film-forming protein materials can be derived from many sources which are broadly categorised in two – *Animal Sources* that includes collagen, gelatin, fish myofibrillar protein, keratin, egg white protein, casein, and whey protein or *Plant Sources* like corn zein, wheat gluten, soy protein, peanut protein, and cottonseed protein.

Whey protein–based edible coatings were found effective in reducing lipid oxidation duringthe storage of dry roasted peanuts (Maté and Krochta, 1996; Maté et al., 1996), and walnuts (Mate and Krochta, 1997) depending on levels of plasticizer in coating solution, thickness of dry coating, and conditions of storage. Coated peanuts and walnuts developed an excellent resistance to oxidative rancidity and hexanal levels (degradation product of linoleic acid – indicator of rancidity) were found to be much lower in comparison to uncoated samples (Maté and Krochta, 1998). The results were confirmed through other studies (Lee et al. 2002 ; Lee and Krochta 2002) leading to the conclusion that coating can significantly slow down the oxidation of samples, meaning an extension of shelf-life, thus a direct extension of shelf-life.

RAO Qing et al. (2011) showed the effectiveness of composite coatings of soy protein isolate - starch and antioxidant (citric acid, TBHQ) in lowering the oxidative degradation of walnut kernels for a study of 10.7 months.

Swenson et al. (1953) showed that nuts coated with pectinate, pectate, and zein coatings containing antioxidants like BHA, BHT, and citric acid prevented rancidity to great extent and also maintained their texture.

A summary of protein coating research on nuts is presented here (Table 3.2).

Coating	Nut/Product	Result
Zein	Nuts, nut-containing chocolate bars, and nut clusters	Extended shelf life, reduced rancidity and sogginess at 21 and 38°C, and added gloss
Zein	Almonds Almonds in a chocolate confection	Rancidity not reduced
Caseinate, gelatin	Bakery items	Limited oxidative rancidity and prevented sticking
Whey protein with and without added tocopherol	Walnuts and peanuts	Walnuts: enhanced oxidation due to oil leakage Peanuts: hexanal and peroxides in product reduced over 70 days at 38°C
Whey protein/AMG bilayer coatings	Walnuts	Dramatically reduced oxidation in terms of hexanal and peroxide values at 38°C over 70 days
AMG	Chopped almonds	Onset time of sogginess delayed by sixfold
AMG	Pecans	Small decrease in rancidity and shelf life from sensory panel data
Whey protein isolate	Peanuts	Increased gloss, decreased rancidity in sensory studies
Whey protein isolate	Hazelnuts, peanuts	Increased gloss

Table 3.2: *A summary of application research of Protein/Lipid coating on nuts (Source- Trezza and Krochta, 2002)*

Li Qi et al. (2012) reported an edible coat matrix with egg white proteins glycosylated with xylose through Maillard reaction to improve water barrier properties (EWP-Xyl MRPs). The coat was optimised based on contributions of sugar type, pH, heating temperature and xylose concentration to the Maillard reaction. The results showed that there was a significant reduction in acid value of walnuts.

Coatings based on caseinate have also been successful as an excellent barrier to oxygen and moisture transfer in raisins and peanuts, thus extending shelf life of the products to a considerable extent (Chen, 1995, 2002).

Corn-zein is one of the most commercialised protein based coatings which is used as oxygen,lipid, and moisture barriers for nuts, candies, confectionery products, and other foods(Padua and Wang, 2002). A formulation of quick-drying alcohol-based zein (Optazein, Opta FoodIngredients, Inc., Cambridge, Massachusetts) was used as a coating to improve and maintain qualityof nuts in a “raisin nut mix,” and showed that samples coated with zein had higher sensory ratings than wheat gluten–coated samples (Conca, 2002).

Zhou Bailing et al. (2004) presented a zein composite to inhibit oxidative rancidity of the walnut meat in storage. The results showed that coated walnuts had significantly low peroxide value, saponification value and acid value in comparison to

uncoated samples. It was claimed that zein film coating prolonged the shelf-life by 250 days or more at storage temperature of 20℃.

However, protein coatings allow some level of oxygen permeability that can eventually lead to rancid foods. This oxidation and degradation of foods by the permeation of oxygen can be markedly retarded by incorporating antioxidants into protein films and coatings. These antioxidants diffuse and migrate into the food matrix at an appropriate and controlled manner thus scavenge the free radicals created in the food during the first stage of oxidation.

3.1.2.2 Polysaccharide Based

Different polysaccharides have also got significant attention beside proteins in the development of edible coatings. Polysaccharide coatings are generally poor moisture barriers, but they have moderately low oxygen permeability and, at the same time, selective permeability to O2 and CO2 (Lacroix and LeTien, 2005) thus finding varied applications in producing modified atmospheres around the food product. Other than functional properties their wide availability, low cost, and non-toxicity are some of the crucial parameters for their usage as edible coating materials. Polysaccharide film-forming materials include starch and starch derivatives, cellulose derivatives, alginate, carrageenan, chitosan, pectinate, and various gums. Proteins can be combined with polysaccharides to enhance certain mechanical properties.

Polysaccharides such as carboxy methylcellulose (CMC) and chitosan alone or in conjunction with other constituents have also shown promising results in terms of enhancing shelf-life and overall acceptability of nuts (Moslem Sabaghia et al. 2015; Baldwin and Wood 2006).

Baldwin and Wood (2006) investigated the application of carboxy methylcellulose (CMC) on pecan kernels along with plasticizers (propylene glycol and sorbitol) and several antioxidants (tocopherol, BHA, and BHT). This imparted shine to the coated nuts thus enhancing visual characteristics. It was found that hexanal levels were nearly half in coated kernels with reference to uncoated ones, implementing that coated kernels underwent less oxidation and were less rancid. Coated nuts also scored higher ratings for overall flavour by sensory panels, thus

validating the hypothesis of shelf-life extension through these selected coating materials.

Moslem Sabaghia et al. 2015 presented coating solutions made from chitosan incorporating green tea extracts to reduce lipid oxidation and fungal growth while improving the sensory properties of walnut kernels. It was inferred that chiton-green tea extract coatings when studied for 18 week time period effectively prolonged the shelf-life of the kernels at different concentrations.

Atarés et al. (2011) reported the use of hydroxyl propyl methylcellulose (HPMC) formulations in conjunction with different antioxidants such as ascorbic and citric acid and essential oils of ginger in prevention of oxidative rancidity and extension of shelf-life for toasted almonds. The studies were based on oxygen permeability of the films to characterize the mechanisms that modulated the lipid oxidation protection. It was concluded that acid incorporation had a significant improvement in terms of oxygen barrier performance of the films. The addition of ginger oil, though improved hydrophobicity was not able to retard lipid degradation.

Antonella L. Grosso et al. (2017) demonstrated the effect of carboxy methyl cellulose, methyl cellulose and whey protein coatings on sensory stability of walnuts during storage at room temperature for 210 days. The results suggested that edible coatings can help preserving sensory attributes in walnuts, especially in case of methyl cellulose, improving their shelf-life.

Starch and dextrin formulations also are marketed as protective coatings, integrity maintainers, appearance enhancers, and seasoning adhesives for nuts, snacks, and cereals (Krochta, 2002). Also the prospects of potato starch (modified and simple) as a coating material for walnuts were studied and it was concluded that they had the potential for reduced water vapour and gas permeability that is most critical factor in preserving nuts (M.L. Hurtado et al. ,2001). Uses of rice-starch in reduction of oxidative rancidity for walnuts have also been established (Mona Aghazadeh et al. 2017).

3.1.2.3 Lipids and Wax based

Lipids and waxes are less frequently used as stand-alone edible films and coatings as they are not polymers and thus have poor mechanical properties. However

they are extensively used in conjunction with proteins or carbohydrates to enhance coat's hydrophobicity (Water vapour barrier properties). Waxes are the most common type of lipids used in edible coatings, the use is this much extensive that that the word 'wax' is commonly used to refer any coating, whether or not any lipid substance is present in the coat. Edible lipids majorly include beeswax, candelilla wax, carnauba wax, triglycerides (e.g., milk fat fractions), acetylated monoglycerides, fatty acids, fatty alcohols, and sucrose fatty acid esters.

Dariusz Kowalczyk et al. (2017) demonstrate that those walnut kernels which were coated with an emulsion based on candelilla wax - carboxymethyl cellulose (CMC) with or without L-ascorbic acid (AA) showed retarded lipid oxidation with enhanced gloss but with a partial loss of aroma. The wax gave hydrophobic properties to the coat enhancing its water vapour barrier properties.

Edible coatings made from whey protein isolate (WPI), pea starch (PS), and their combinations with carnauba wax (CW) in different proportions were effective in preventing oxidative and hydrolytic rancidity of walnuts and pine nuts stored at25 ◦C throughout the storage (12 d) but were less effective at 50 ◦C (Ghadeer F. Mehyar et al. 2012).

Lee et al. (2002a, 2002b) reported that whey protein coats in conjunction with lipids resulted in favourable sensory properties of coated peanuts and coated chocolate-covered almonds.

Certain commercial wax based coats and products for use in nuts have been developed by Mantrose-Haeuser Co., Inc. The main motive was not to preserve the nuts but to seal the centres of nuts to improve adhesion or to prevent fat migration, and also as polishing substances for better appearance.

Waxes owing to their complex structures and lipophilic behaviours can be of great importance. Wax have high melting points in relation to fats and can be hard solid at temperatures as high as 55° C. Wax structures also offer structural rigidity and high malleability in conjunction with greatly reduced water vapour permeability and significantly reduced oxygen permeability. Waxes are highly resistant to oxidation and are quite stable thus can be potential candidates for outer coat/covering to prevent coated product from degradation.

3.2 Components of the lipid based coat

3.1.1 Beeswax

Product category	Products	Characteristics
Pharmaceuticals	Drugs, pills, capsules, salve and ointments	Consistancy, binding agent, time release mechanism, carrier of drug

Figure 3.1*: Beeswax specifications (ref: Stefan Bogdanov, April 2016)*

Apismellifera and *Apiscerana* produces bee wax, it's a complex product secreted in liquid form by special wax glands in the abdomen of younger worker bees *(*Masson et Cie, Paris (1968)). During secretion, it is almost white and only after contact with honey and pollen it assumes a variably intense yellowish color and turn brown after about four years, because it contains the cocoon *(*Synthesis and secretion of beeswax in honeybees, Apidologie, 22 (1991)). It is insoluble in water and cold alcohol and hinder the action of acids and gastric juices of honey bees; Partially dissolved in boiling alcohol, and completely in chloroform, in carbon disulphide, and in the essence of hot turpentine (Bee Product Science, Switzerland (2009)).

Bee wax is inert i.e. it does not react with human digestive system and flows through body without any change. Substance are released slowly which are encapsulated in wax. These Properties may also produce problem when wax is stored near toxic chemicals and pesticides or after treatment with various drugs inside the hive (USA, L A S (1978) Beeswax). Bee wax is used from processing to packaging and preservation. Bee wax can also be used as a thickener of biodegradable lubrication gases. Bee wax has antimicrobial properties (Kacaniova M at al. (2012)). Application on Coating of drugs and pills, facilitate ingestion, but retards dissolution. It is also used to chew in order to strengthen the gingival and to increase saliva and stomach juices (Potschinkova, P (1992)).

As per "European Union under the name of E901", bee wax is an authorized food preservative and as a glazing agent on confectionery (excluding chocolate), small products of fine bakery wares coated with chocolate, snacks, nuts and coffee beans. Application is also in food supplements and as a carrier for colors. 1290g bee wax consumption per person and day are permitted. It protects the container against the effects of acids from fruit juices or honey (EFSA (2008) Beeswax (E 901)).

Combination of beeswax, honey and olive oil can be successfully used against dermatitis, psoriasis and also against anal fissures and hemorrhoids and against burns. Oral administration of a mixture of 6 beeswax alcohols called D-002 (50 to 100 mg/day) for 6 weeks may ameliorate arthritic symptoms meanwhile improve clinical evolution in patients with osteoarthritis (Puente, et al. (2014)). Main causes are antioxidant and anti-inflammatory effects of this mixture (Molina; V, M R; Carbajal, D (2015) D-002 (Beeswax Alcohols)).

Dominance of commercial beeswax is now contaminated by acaricides. African beeswax, which is free of acaricides is a good candidate for the near future (Schroeder, et al. (2003)).

3.1.2 Acetylated monoglyceride

They are based on distilled monoglycerides with acetylation of the free hydroxyl group in the range of 50%, 70% and 90%. Acetylation results in the depression of melting point of the corresponding monoglyceride to about 25-30°C. They are stable in crystal form and show no polymorphism. The surface activity of ACETEM depends on their degree of acetylation. Complete acetylation of monoglycerides lead to their not being surface active and behaving as plastic fats which as a result forms flexible films with very low permeability to oxygen and water vapour. Partially acetylated ACETEM have very low surface activity depending on their degree of acetylation and they thereby form gel with water (Lipid Technologies and Applications). Work in the past have shown a tremendous reduction (up to 70%) in the water vapour permeability of films containing 70% acetylated ACETEM (Danisco range of products) forming a laminate with whey protein isolate (Martin Anker, 2001). Addition of acetylated monoglyceride in the edible coating for pecan kernels has resulted in retardation of colour changes and peroxidation further also reducing the formation of secondary oxidation products due to oxidative rancidity (S.D. Senter, 1979). Hexanal production in walnuts (secondary volatile product of oxidative rancidity) was reduced due to incorporation of acetylated monoglyceride when exposed under accelerated condition of 37°C for 70 days (Mate and Krotcha, 1997).

3.1.3 Butylated Hydroxy Anisole

BHA is an anti-oxidant, it exists as a white or slightly yellow, waxy solid with a faint characteristic at room temperature (IARC 1986). It is also availed as preservative in food, food packaging, and animal feed (IARC 1986). Edible fats and fat containing foods accommodate BHA for its antioxidant properties and also in foods cooked or fried in animal oils, because of its high thermal stability and its ability to remain active in baked and fried foods (HSDB 2009). BHA is added to butter, lard, meats, cereals, baked goods, sweets, beer, vegetable oils, potato chips, snack foods, nuts and nut products, dehydrated potatoes, and flavoring agents. It is used in sausage, poultry and meat products, dry mixes for beverages and desserts, glazed fruits, chewing gum, active dry yeast, defoaming agents for beet sugar and yeast, and emulsion stabilizers for shortening (IARC 1986). BHA stabilizes the petroleum wax coatings of food packaging (HSDB 2009). It works as effective stabilizer for essential oils, paraffin, and polyethylenes (HSDB 2009).

BHA is readily lost from thermal processes that generate steam because it is Volatile at 150°C to 170°C (302°F to 338°F) (Warner et al. 1986). BHA is generally recognized as safe for use in food when the total of antioxidants is not greater than 0.02% of fat or oil content as per FDA.

3.1.4 Butylated Hydroxy Toluene

BHT as an antioxidant point of view can be considered a matter of public health concern, because of the potential toxicological effects attributed to BHT itself and to metabolites derived from it (Branen 1975; Babich 1982; Malkinson 1983; Kahl 1984; Bomhard and others 1992; Williams and others 1999). The toxic effects of BHT are thought to be caused by its metabolites rather than by the parent compound (Thompson and Trush 1986).

BHT showed the highest volatilization, in comparison with BHA and TBHQ, reaching 28% during cookie baking at 195 ºC for 10 min and 49% after submitting potatoes to deep-frying in lard at 190 to 200 ºC for 1 h (Warner and others 1986b).

Molecule can be endogenously generated by some freshwater phytoplankton submitted to oxidative stress conditions (Babu and Wu 2008). BHT itself is oxidized and the subsequent derived radical is stabilized by electronic delocalization in the

benzene ring. This way, BHT can stop radical oxidation propagation, which retards lipid oxidation and extends food shelf-life (Warner and others 1986a).

The antioxidant activity of this molecule at high temperatures, such as frying conditions, can greatly vary from that developed at moderate or low temperatures, as during storage. Several authors reported rapid loss of BHT at high temperatures (> 140 ºC) and noticed lower antioxidant activity (Zhang and others 2004; Marmesat and others 2010).

3.1.5 Tocopherol

Tocopherols are monophenolic anti-oxidants consisting of eight naturally occurring homologues consisting of –α, -β, -γ, -δ tocopherols characterised by a saturated side chain consisting of three isoprenoid units and their corresponding unsaturated tocotrienols. However their anti-oxidant activity differs according to the Vitamin E concentration ascribed to them. It has been shown in the past that –α tocopherols have highest vitamin E activity (Eitenmiller, 1997). The order of antioxidant activity is –δ > –γ > –β > –α. It is also important to evaluate the oxidation conditions such as temperature, availability of oxygen, chemical nature and physical state of the lipid to better understand the role of tocopherols as antioxidants. Addition of –α tocopherol in combination with ascorbyl palmitate in acetylated monoglyceride coatings of walnuts has shown a positive effect in delaying rancidity (Mate and Krotcha, 1997). Ascorbyl palmitate is a synergist which enhances the action of tocopherol, thereby reducing the dosage and subsequently reducing cost (Schuler, 1990).

3.1.6 Palm Stearin

Palm stearin is the solid fraction obtained by fractionation of oil when crystallized at controlled temperatures (Lim, T.K. 2010). It is therefore a co product of palm oiland is usually traded at a discount to oil and palm stearin creating it a price effective ingredient in many applications. Palm stearin owing to existing in various polymorphic forms has a wider availability in various applications.

It has different composition than palm olein, the liquid fraction of vegetable oil, particularly in terms of its solid fat content, and so has a lot of variable physical characteristics (Gunstone, Frank D. 2011). Like crude palm fruit oil, palm stearin

contains carotenoids, however physically refined palm oils don't, as they're removed or destroyed within the refinement method.(Gunstone, Frank D. 2011).

Palm stearin consists of mostly glyceryl tripalmitate, with rest of the fat content being glyceryl dipalmitatemonooleate. (Tsung Min Kuo, Harold W. Gardner, 2002)

3.1.7 Palm Superolein

Palm oil is semi-solid at temperature (20°C). The liquid portion can be physically separated from the solid portion of vegetable oil by fractionation. fractionation of the liquid portion is termed “palm olein”, that is often bottled and sold as preparation oils. The solid fat portion is termed “palm stearin” and it's ordinarily accustomed formulate trans-free fats resembling margarin, shortening and vegetable clarified butter.

Palm Olein is further fractionated to a more liquid fraction called **“Super Palm Olein“.** Super Palm Olein has an iodine value of 60-63.Super Palm olein is more suited to cooler climates and has cloud points of regarding 2°C-5°C. due to its carboxylic acid composition and smart aerophilic stability super refined palm olein is great to be used as liquid oil and the more appropriate for cooking. With the exception of its prime quality performance, the advantage is it doesn't alter the style or flavour of deep-fried food because it doesn't have any distinct fragrance. Moreover, it leaves the meal fully dry with no dripping of oil.

CHAPTER 4 – MATERIALS AND METHODS

All the chemicals and reagents mentioned in the experimental analysis and assays were procured from authenticated and listed sources. All experimental methods were taken from reliable published sources with or without minor modifications.

4.1 Overall Design of the Experiment

Experimental design used for the presented study is briefly explained in the figure 4.1. One of the most important raw material, i.e. whole walnuts kernels, were procured from a distributer company named Barries& Nuts, Hydrabad, Telangana in February 2018 and were immediately stored at a temperature of -20° C.

Kernels were supplied in vacuum packaged pouches over which sealed paper-metal foil composite packaging was in place. They had been harvested, mechanically shelled and packaged in as described above November 2018. Experiments were carried out during the period April 2018 – May 2018.

All the procedural methods have been described in the following sections.

4.2 Development of Edible Coat

Chemicals and Reagents:

Acetylated mono-glycerides (Danisco), Beeswax (Hi-Tech Natural Products (India) Ltd.), Palm Stearin (Bunge), Palm Super Olein (Kamani Oils), Butylated hydroxy toluene (BHT) (Thermo Fisher Scientific, USA)

Instruments and Apparatus:

Hot water bath, Precision balance (Sortorius).

Principle:

It was aimed at developing a lipid based edible coating to foster the best barrier properties. The foremost objective behind selecting the raw materials for the coating directed at exploring the organic alternative for the ordinarily used Carnauba wax for multifarious coatings of food products. This ensued for the requirement of a

highly functional blend of emulsifier and fat which could work in synchrony with the wax used as described in figure 4.1 and 4.2.

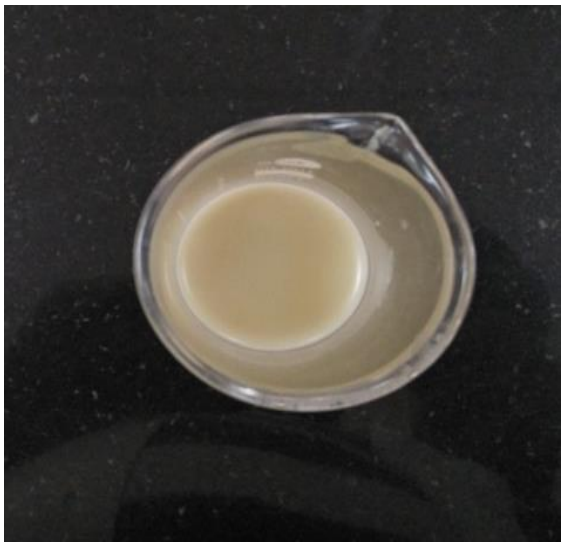

Figure 4.1 and 4.2: *Development of Edible Coat- Trial 1 & Trial 2*

Formulation	Control	Trial 1	Trial 2
Walnut	320g	320g	320g
Bee Wax	-	8.32g (20% coat)	6.24g (15% coat)
Acetylated Monoglyceride	-	8.32g (20% coat)	4.16g (10% coat)
Palm Stearin	-	-	31.2g (75% coat)
Palm Super Olein	-	24.96g (60% coat)	-
Butylated 39nisidin toluene (BHT)	-	0.045g (0.1% coat)	0.045g (0.1% coat)
Total weight of the coat	-	41.645g	41.645g

Table 4.1: *Coat formulation recipes for Trial 1and Trial* 2.

Protocol:

The different ingredients used were weighed in 100ml beakers and separately heated in water bath (refer table 4.1). The bee wax was heated at 90° C for about 25 minutes till a homogenous fluid consistency was obtained. The fat/oil was melted at 60° C for about 20 minutes separately until a homogenous consistency was obtained. To the fat/oil, weighed amount of BHT was added and stirred until get dissolved with no remaining crystals at the bottom. Acetylated mono-glyceride (ACETEM) was then added drop-wise into the fat/oil-BHT solution with continuous stirring. The fat/oil and ACETEM mixture was then mixed part-by-part with consistent stirring into the melted wax till a homogenous solution was obtained at 90° C. The walnut kernels

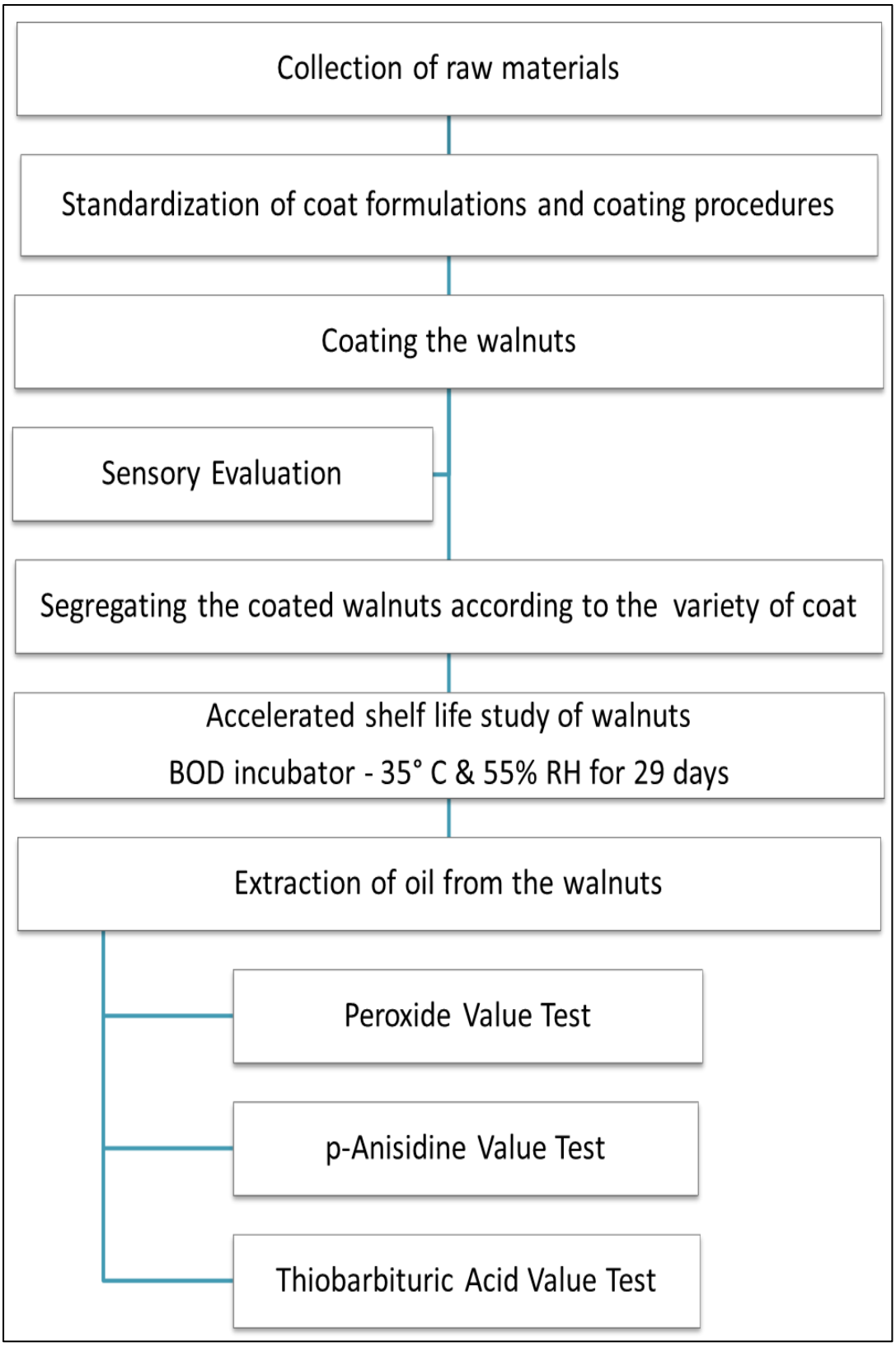

Figure 4.3: *Overall experimental Design*

were coated by dipping into the coating prepared solution for 3 seconds and then allowed to dry for 1 hour. A thin coat was thus prepared on the kernel surface. A brief procedure for coating development is given in figure 4.7.

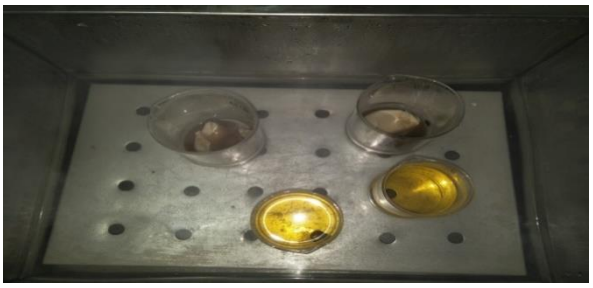

Figure 4.4 & 4.5: *Dipping of coat to check the appearance; formulation of coat*

4.3 Sensory Profile Analysis

The sensory qualities are essentially to be measured subjectively. With the development of sensory evaluation techniques on scientific lines, the experts were replaced by semi trained panel members. The panel members analyse food products through properly plant experiments and their judgement were quantified appropriately.

Sensory analysis was carried out according to Amerine et al. (1965) [269] with slight modifications in the method. A panel of 10 semi-trained members evaluated the products using a 9 point hedonic scale of 1 to 9 scores with 9 for like extremely and 1 for dislike extremely. Descriptive sensory analysis for all the formulations was conducted to determine: Skin colour ©, Glossiness (G), Flavour (F), Taste (T), Aroma (A), Crunchiness (CH), After taste (AT) and Overall acceptability (OA). Formulation with highest average scores was pursued for further analytical tests. Samples were given random numbers to minimise perception based biasness in evaluation. Figure 4.6 shows the format used for sensory analysis of coated walnuts and a comparative evaluation with respect to the uncoated walnut sample. Codes i.e. 526 denote trial 1, 259 denotes uncoated reference and 642 denote trial 2. This information was not shared with the panellists.

Sensory Evaluation Form

Sample Name: Coated walnuts

Directions: Give a rating for each of the following

Sample Number	Skin colour	Glossiness	Flavour	Taste	Aroma	Crunchiness	After taste	Overall Acceptability
526								
259								
642								

Instructions: Please give ratings to the sample based on the scale presented below.

9 Point Hedonic Scale: 9-Like Extremely; 8-Like Very Much; 7-Like moderately; 6-Like Slightly; 5-Neither Like or Dislike; 4-Dislike Slightly; 3-Dislike moderately; 2-Dislike Very Much; 1-Extremely Dislike .

Suggestions for Improvement:

Panellist Name: Date:

Figure 4.6: *Sensory evaluation format*

4.4 Accelerated Shelf Life Study

The coated walnuts were segregated into a batch size of 40gms each into 8 batches for each trial. The batches were marked as control batch, trial 1 and trial 2. The accelerated shelf life study was aimed to continue for a period of 29 days, with testing the measurable oxidation products for profiling the rancidity (analytical tests) after every 4^{th} day. The batches of walnuts were placed inside a BOD incubator with the set value of temperature at 35° C and 55% RH.

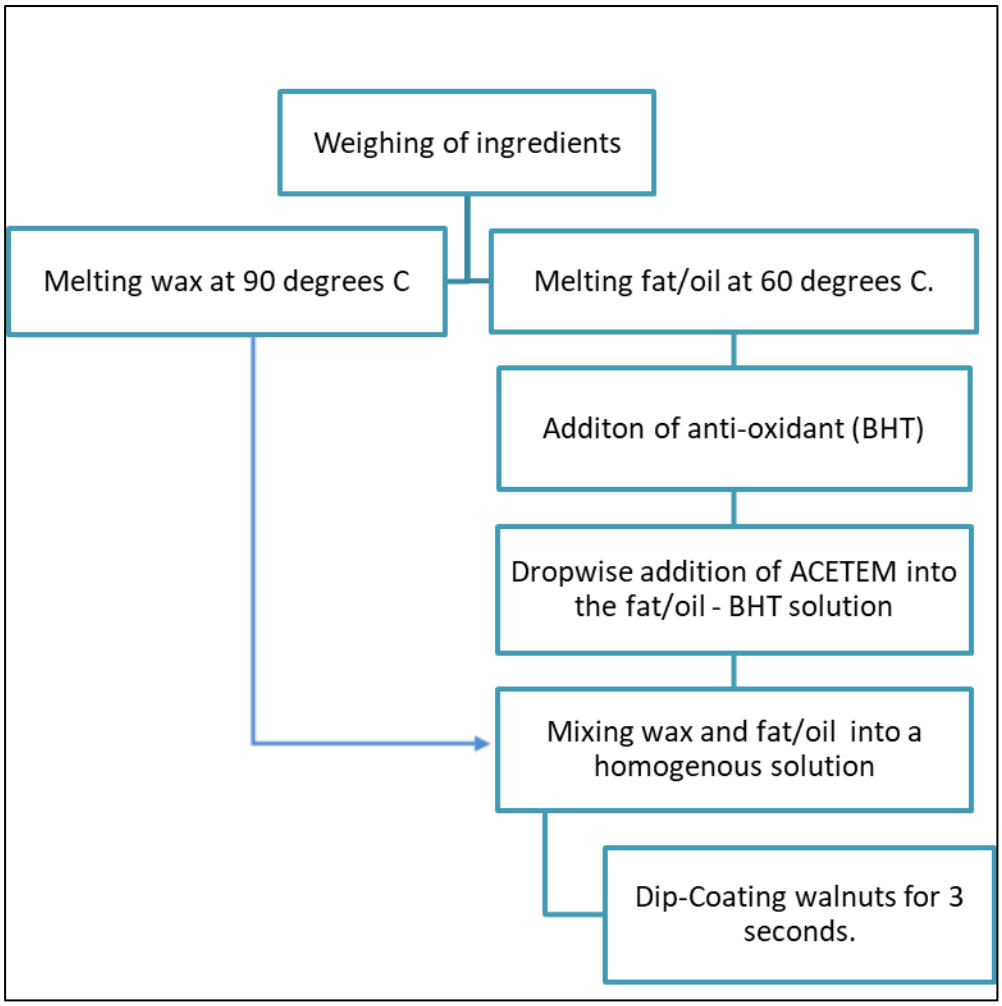

Figure 4.7: *Coat formulation and coating procedure*

Figure 4.8: *Storage under accelerated conditions in BOD Incubator*

4.5 Extraction of walnut oil

Chemicals and Reagents:

Diethyl ether (Thermo Fisher Scientific, USA)

Instruments and Apparatus:

Mortar pestle, Vortex shaker, Vacuum dryer

Protocol:

A brief explanation of extraction procedure is given in figure 4.9. The method used was a simple alteration of traditional solvent extraction method. Diethyl ether having a boiling point of 35° C was most suitable solvent to be used for extraction of oil from walnut kernels, because of the ease of vaporisation of the solvent without causing much hindrance to the set test parameters for the quality of walnut oil. The walnut kernels were taken out from the incubator chamber and were immediately crushed in a mortar pestle batch wise (40gm) into a finely mashed paste. 90 ml of diethyl ether was poured into this paste and mixed to collect the solution into 3 different 50 ml bottles with proper closures. The solution was then vortexed for 5 minutes and then stored in dark for 24 hours at room temperature (25° C). This was done to allow the diethyl ether to seep into the paste of walnuts to solubilise the walnut oil completely. The solution was then centrifuged at 10000 rpm for 15 minutes at 15° C. The supernatant containing the oil fraction was then poured into petri-plates without disturbing the retentate. The solvent (diethyl ether) was then evaporated in a vacuum oven at leaving the oil fraction behind. Vacuum oven was used so as to maintain a lower temperature (32° C) so as to not affect the chemical properties of the oil. The collected oil is then stored at -20° C (deep freezer) until used for further analysis.

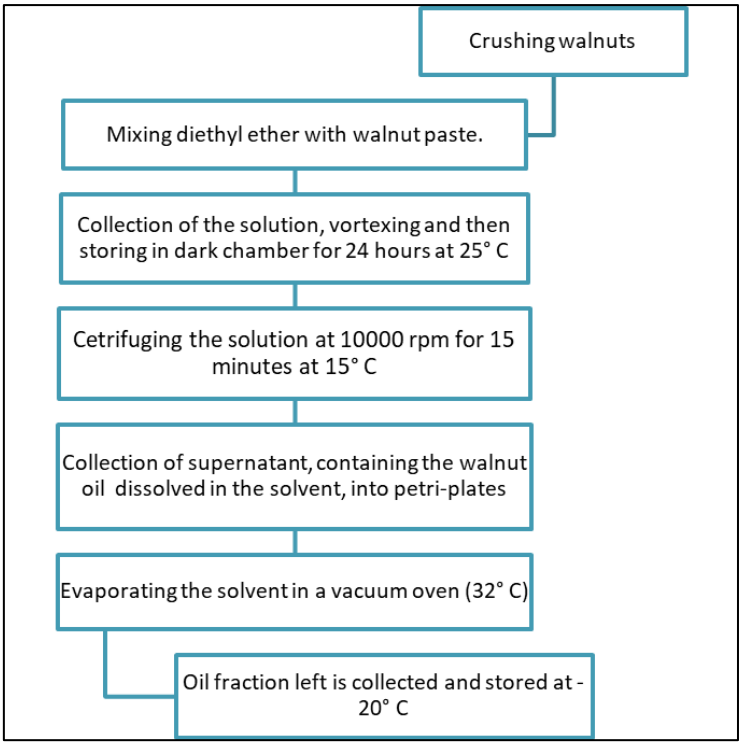

Figure 4.9: *Oil extraction procedure*

4.6 Analytical Rancidity Profiling

The oils extracted from walnut samples were then chemically tested for accessing the quality parameters. The procedures helped profiling the extent of oxidative degradation and rancidity development in the coated and uncoated samples. The three analytical tests performed were peroxide value test, p-anisidine value test and thiobarbituric acid reactive substances assay. The procedural aspects for every test are explained below.

Chemicals and Reagents:

Chloroform, Glacial Acetic Acid**,** Sodium thio-sulphate solution (0.01N)**,** Saturated potassium iodide solution**,** Starch indicator (1% w/v soluble starch solution), Iso-Octane, Anisidine reagent (0.25% w/v in glacial acetic acid) – freshly prepared, Tri-chloroacetic acid (TCA) reagents (20% & 0.1% w/v), Thiobarbituric Acid (TBA) reagent (0.5% TBA diluted in 20% TCA), Double distilled water,

Instruments and Apparatus:

UV-Vis spectrophotometer, Hot water bath, Burette and pipette system, Precision balance (Sortorius), Iodine flask

4.6.1 Peroxide Value Test

Principle:

It is used for determining the rancidity in fats and oil, which gives a measure of the extent to which an oil sample has undergone primary oxidation. In oil and fats, unsaturated bonds play a role in auto-oxidation as oils which have high degree of unsaturation are most susceptible to auto-oxidation as peroxides are formed as intermediates in the auto-oxidation. This test determines this amount of peroxides in the lipids. PV is most commonly quantified using iodometric titration and is expressed as milli-equivalents of active oxygen (peroxide) per kg of lipid (meq/kg) (Shahidi and Wanasundara, 1998). The peroxide value is determined by measuring the amount of iodine which is formed by the reaction of peroxides (formed in fat or oil) with iodide ion. The indicator used in this reaction is a starch solution where amylose forms a blue to black solution with iodine and turns the solution colourless where iodine is titrated with the solution of sodium thio-sulphate. Figure 4.10 describes the reaction mechanism for the peroxide value determination.

Generation of hydroperoxides:

$$R\text{-}H + O_2 \rightarrow ROOH \qquad \text{(Reaction I)}$$

Generation of iodine:

$$KI + CH_3COOH \rightarrow HI + CH_3COO^-K^+ \qquad \text{(Reaction II)}$$

$$ROOH + 2\,HI \rightarrow ROH + H_2O + I_2 + \text{starch indicator} \qquad \text{(Reaction III)}$$

Titration step:

$$I_2(\text{purple}) + 2\,Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\,NaI\,(\text{colourless}) \qquad \text{(ReactionIV)}$$

Figure 4.10: *Reaction mechanism – Peroxide Value (Ref- International Fragrance Association)*

Protocol:

1.5 to 2 g of oil sample was weighed in an iodine flask. 14 ml Chloroform was added and shaken vigorously and then 21 ml of acetic acid was added to solution containing the sample. 1 ml of saturated potassium iodide solution was added to the flask, vigorously shaken for 1 min and then put in dark for 5 minutes with continuous shaking. 75 ml of distilled water was then added to the sample mix and was shaken magnetically. The sample mix was then treated with 1 ml of starch indicator (1% w/v) after which the colour of the sample mix turned to purple-violet. The sample mix was then titrated against 0.01 N Sodium thio-sulphate solutions until disappearance of purple colour which marked the end point of the titration.

Calculation:

$$\mathrm{POV}\left(\frac{\mathrm{milliequevalentofactiveoxygen}}{\mathrm{kgofsample}}\right) = \frac{(\mathrm{S}-\mathrm{B}) \times \mathrm{N} \times 1000}{\mathrm{W}}$$

Where,

S – Volume of titrant for sample

B – Volume of titrant for blank

N – Normality of Sodium Thiosulphate (0.01)

W – Weight of sample in grams

4.6.2 p-Anisidine Value Test

Principle:

It estimates the amount of –α and –β unsaturated aldehydes (mainly 2-alkenals and 2,4-dienals) formed as a result of secondary oxidation which is mainly imputable to aldehydes and ketones, and therefore able to tell the oxidation history of an oil or a fat. The aldehyde reacts with p-anisidine to form a chromogen that is measured spectrophotometrically.

Protocol:

Sample Preparation: 1 g of walnut oil sample was taken in a 25 ml volumetric flask and the volume was made up with iso-octane and it was accompanied with vigorous shaking.

Chromogen Development: 5 ml of sample was pipette out into a clean test tube and 1 ml of Anisidine reagent was added to it. It was run on vortex for 10 seconds and was stored in a dark chamber (10 minutes) for development of chromogen.

Standard Preparation: It was prepared using 5 ml of pure Iso-Octane and 1 ml of Anisidine reagent and shaking it vigorously on a vortex for 10 seconds followed by 10 minutes of storage in dark.

Spectrophotometry: The absorbance values of the samples were measured at 350 nm.

Calculations:

$$p - Anisidine\ Value\ (AnV) = \frac{25 \times (1.2 \times \mathrm{Eb} - \mathrm{Ea})}{\mathrm{W}}$$

Where,

Ea = (Absorbance of Oil sample mixture in Iso-octane) – (Absorbance of Pure iso-octane)

Eb = (Absorbance of Solution A) – (Absorbance of Solution B)

W = Weight of the sample taken for analysis

4.6.3 Thiobarbituric Acid Reactive Substances (TBARS) Assay

Principle:

The TBA value is a method to investigate secondary oxidative aldehyde products. The thiobarbituric acid (TBA) test measures malonaldehyde (MDA) which is produced due to the oxidation of fatty acids with three or more double bonds, and it measures other TBA reactive substances such as 2-alkenals and 2,4-alkadienals. Therefore, TBA is also referred to as TBARs (TBA reactive substances). For standard preparation, Thiobarbituric acid (TBA) is reacted with MDA, which is resulting in a colour compound, which can be determined by spectrophotometrically

(Xiaoqing Yang).Malondialdehyde (MDA) is a major end-product of oxidation of omega-3 and omega-6 PUFAs (Arnis Kuksis, Waldemar Pruzanski, 2014).

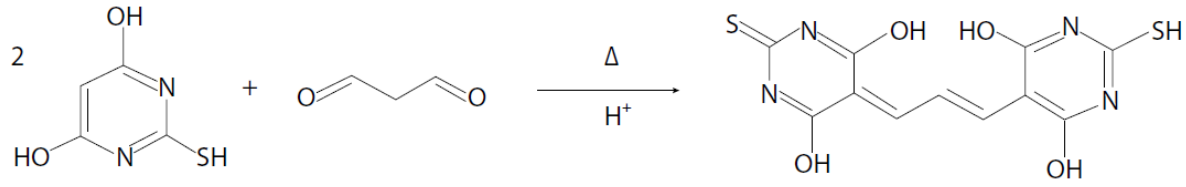

Figure 4.11: *Reaction mechanism – TBARS Assay*

Protocol:

Sample Preparation: 1 g of walnut oil sample was taken in a test tube and 5 ml of distilled water was added to it and the solution was run on vortex for 5 minutes. It was then centrifuged at 5000 rpm for 5 minutes and the aqueous layer was collected. The procedure was repeated twice to collect sufficient water washed sample fraction for further analysis.

Chromogen development: 2 ml of sample was mixed with 2 ml of TBA reagent and heated at 95° C for 45 minutes. This was accompanied by rapid cooling (-18° C) of the reaction mixture. This led to the development of pink coloured chromogen due to presence of malondialdehyde.

Spectrophotometry: The solution was centrifuged at 10000 rpm for 5 minutes to get a clear solution. This was viewed under spectrophotometer at 532 nm and 600 nm respectively.

Calculation:

$$MDA\ content\ \left(\frac{\text{nmol MDA}}{\text{goil}}\right) = \frac{\Delta A_{\text{Corrected}} \times x \times DF \times 1000}{\varepsilon \times b \times y}$$

Where,

$\Delta A_{\text{Corrected}}$ = (Abs at 532nm – Abs at 600nm)

x = (ml) TCA 0.1 % used for extraction (Here not applicable)

DF = Dilution factor (1g oil – 10ml water extract – 2ml extract mixed with 2ml TBA)

ε = Milli-molar extinction coefficient (155 mM^{-1} cm^{-1})

b = light path length (1cm)

y = weight of sample used (of walnut)

1000 = conversion factor (nM to µM)

4.6.4 Total Oxidation (TOTOX) Value

Principle:

It tends to indicate the total oxidation of a sample using both peroxide and p-anisidine values.

Calculation:

$$Totox\ Value = p - Anisidine\ Value + (2 \times Peroxide\ Value)$$

4.7 Statistical Analysis

Calculation of mean values, standard deviation values and graphs were plotted using Microsoft's M.S. Excel. All data were expressed as mean ± standard deviation (Std. Dev.) from triplicates for each experiment. Means of treatments were calculated from triplicate samples for each test time (n = 3). Data were subjected to two-tailed student's T-test using the software SPSS 16 for Windows. A significance level of $P \leq 0.05$ was selected for the difference test.

CHAPTER 5 – RESULTS AND DISCUSSIONS

The results for final coating characteristics and textural properties, oil extraction procedures, and analytical tests done for the determination of oil quality, are presented in this section with a brief discussion for the same.

5.1 Coating Characteristics

In the presented study, 2 types of lipid based edible coating for walnuts were prepared with different carrier viz., palm superolein (Trial 1) and palm stearin (Trial 2). Some physical characteristics that were defined and measured were-

- **Weight of the coats:** There was an increase in about 6.5% in the weight of the walnut kernels after being coated with Coating Trial 1 which had palm superolein as carrier and an increase of 6.9% with Coating Trial 2 which had palm stearin as carrier.
- **Appearance:** Coating Trial 1 made a close to transparent coat on the walnuts, with slight milkiness only evident on the grooves of the kernel because of the non-uniform surface of the kernel which led to higher deposition of the coating in the grooves. Coating Trial 2 however had a slight translucence due to saturated fat (palm stearin) being used at a greater amount with respect to the carrier used in Coating Trial 1.
- **Textural Characteristics:** The coatings developed on walnut kernels exhibited varied textural characteristics owing to the difference in physico-chemical profile of the carriers as well as difference in compositions.
    - *Trial 1*: The coat developed was rigid and stable and did not easily scrap off the surface of the kernels when exposed within the ambient temperature range of 25-32 degrees C. However at an elevated temperature i.e., above 35 degrees C, slight greasiness could be felt on the kernels upon being held. It however retained its physical integrity over the accelerated conditions.
    - *Trial 2*: The coat developed was stable but with respect to Coating Trial 1, scrapping off the surface of the kernel was a possibility. At a temperature range between 35-40 degrees C the greasiness was comparatively lesser

than as observed in Trial 1. It however retained its physical integrity over the accelerated conditions.

Figure 5.1: *Drying of coated samples*

5.2 Sensory Profile Analysis

Both coating formulations were successfully evaluated with respect to uncoated control samples on the basis of sensory attributes by a panel of 10 semi-trained members on a 9 point hedonic scale. The mean scores of sensory panel were analysed for Skin colour ©, Glossiness (G), Flavour (F), Taste (T), Aroma (A), Crunchiness (CH), after taste (AT) and Overall acceptability (OA). The results are depicted in Table 5.1.

Samples	Skin Colour	Glossiness	Flavour	Taste	Aroma	Crunchiness	After Taste	Overall acceptability
526 (Trial 1)	7.1	8.4	8.3	8.8	8.1	8.8	9.0	8.4
259 (Uncoated)	7.9	7.7	9.1	9.3	8.8	9.4	9.3	8.8
642 (Trial 2)	6.6	8.8	7.7	8.1	7.9	8.3	8.7	8.0

Table 5.1: *Sensory evaluation scores for coated and uncoated samples on 9 point hedonic scale.*

From this sensory data it can be easily inferred that all three samples had high overall acceptability with insignificant difference among them. Overall acceptability for Trial 2 was a little less in comparison to uncoated samples due to translucent appearance and milkiness on the surface. This is evident from the skin colour rating of the samples. Glossiness was comparatively high in both the trial samples thus improving the desirability. There was no significant difference found in terms of taste, aroma and crunchiness. According to this data, coated samples were highly desirable in sensory perspective and thus the application of these coatings can be commercialised easily.

Figure 5.2: *coated walnuts*

5.3 Oil Extraction yield

The prescribed method for oil extraction was very efficient in terms of quantity of oil extracted (% yield). The suggested method is relatively simple to perform and did not require any specialised oil pressing equipment. Also as for the qualitative analysis of oil to be performed, it was not at all required to extract total oil content from the kernel but just a sufficient amount so as to have more than required oil sample for chemical analysis. The % yield is given below for different samples.

- *Control:*An effective extraction of 69.2 % (mean value) of the total lipids present in the walnuts could be done.
- *Trial 1:* An effective extraction of 72.6 %(mean value) of the total lipids present in the walnuts could be done.The oil extracted had a substantial

portion of lipids from the coating (majorly superolein) over the surface of the kernel.

- *Trial 2:* An effective extraction of 70.7 %(mean value) of the total lipids present in the walnuts could be done.The oil extracted had a substantial portion of lipids from the coating (majorly stearin) over the surface of the kernel.

5.4 Analytical Rancidity Profiling

Certain analytical chemical tests were performed to determine the total quality of walnuts over an extended course of 29 days at an elevated temperature of 35° C. The tests results showed the different aspects of rancidity development in nuts and thus helped building a Total Rancidity Profile for nuts.

5.4.1 Peroxide Value Test

It is the initial product of auto-oxidation of the oil sample, which is given by the ability of the iodine to be liberated from saturated potassium iodide solution. The content is expressed in terms of milli-equivalents of Oxygen per kilogram of fat/oil. The peroxide value is directly dependent upon the degree of unsaturation of the lipid used as double bonds increase the susceptibility to form free radicals on exposure to heat, light and metals. Also the presence of lipid peroxidation biocatalysts which are present in walnuts as depicted by A.L. Tappel et al. (1956) enhances the rate of reaction.

The peroxide value for uncoated samples increased linearly over the entire period of observation (refer figure 5.3). As for the coated samples, the peroxide value for both Trial 1 and Trial 2 increased linearly with time overlapping at various points. However, in the interval between 25^{th} to 29^{th} days, Trial 1 gave a slightly increased peroxide value as compared to Trial 2. This attributed to the fact that Trial 2 would have undergone a slower rate of auto-oxidation due to the usage of saturated fat (palm stearin) in the coating as compared to palm superolein as carrier in Trial 1 which is more susceptible to auto-oxidation.

The difference in the values of uncoated and coated samples was substantial to mark the effectiveness of applying the coat on the kernel. The peroxide thus formed in

the oil samples further react with alkyl free radicals to form saturated and unsaturated aldehydes as secondary oxidation products.

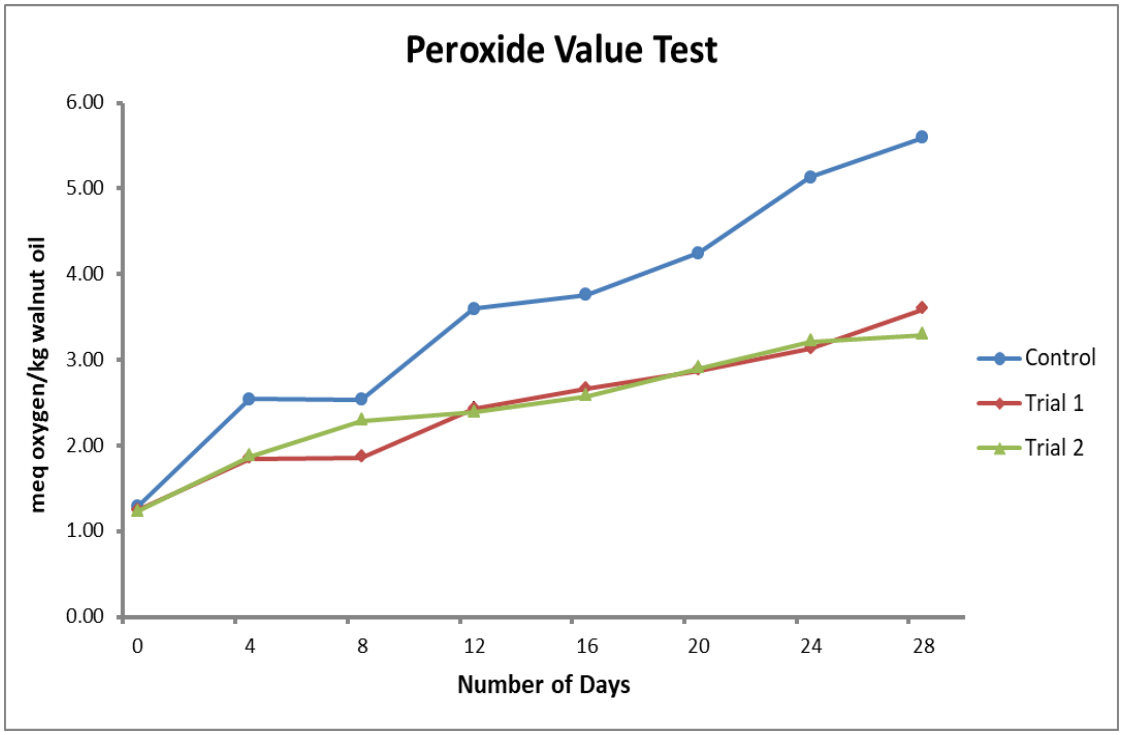

Figure 5.3: *Peroxide values (meq oxygen/kg oil) for control and coated samples over a course of 29 days.*

5.4.2 p-Anisidine Value Test

The p-Anisidine value is indicative of the amount of secondary oxidation products such as high molecular weight saturated and unsaturated carbonyl compounds present in the walnut oil samples (Diana Moigradean et al).

The test showed a linear increase in the anisidine value for the uncoated samples over 29 days (refer figure 5.4). The sample coated with Trial 1 had a steady increase in the value till 13th day after which the rate slowed down but there was again a substantial increase in the values after 25th day. The samples coated with Trial 2 also showed an increase in the anisidine values which initially overlapped with that of coating Trial 1 values till the 9th day. There was an observable drop in the values on the 13th day for Trial 2. However the rate of increase paced up rapidly from 21st day onwards in Trial 2.

This attributes for the rapid rise in the concentration of peroxyl radicals decomposing to form secondary oxidation products. The slight drop and decrease in the rate of reaction for all the three trials during 13th day could be attributed to the lag phase and minor experimental errors. The overall data however indicated the major difference in the anisidine values due to coating which was far less than the uncoated samples. The difference in the values between the coatings could be due to the iodine values of the carriers used, as that is directly related to the degree of unsaturation which is the key factor affecting the rate of oxidative rancidity. Oils with an anisidine value of less than 10 were considered good (Rossell), while Subramanium et al considered good quality oil as one having an anisidine value of less than 2.

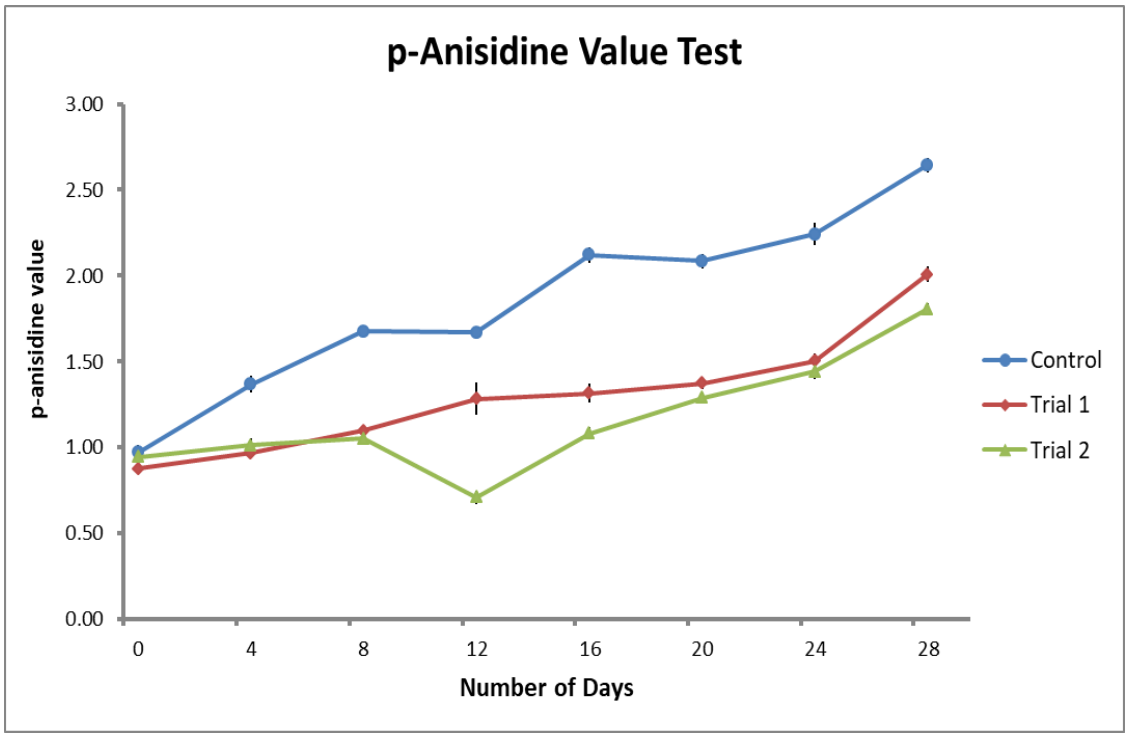

Figure 5.4: *p-Anisidine values for control and coated samples over a course of 29 days.*

5.4.3 Total Oxidation (TOTOX) Value

The total oxidation value gives the measure of the total products of oxidation due to primary and secondary oxidation. It is obtained from the summation of peroxide value which indicates primary oxidation products and p-anisidine value which indicates secondary oxidation products like unsaturated aldehydes formed as a

result of decomposition of hydroperoxides. Often calculating only hydroperoxide content for determination of rancidity could lead to erroneous results as, over time it decomposes to form secondary oxidation products which leads to a decrease in the amount of hydroperoxides (after a peak value has reached over a span of few months) thus remaining in the oil sample.

Hence to get the accurate measure of rancidity TOTOX value is calculated (refer figure 5.5). The lower the TOTOX value, the better the quality of oil (Matt Miller). The value for uncoated samples increased linearly till 25th day after which there was a drastic increase till 29th day. As for the coated samples, in accordance with the peroxide value and anisidine value, the TOTOX value for both Trial 1 and Trial 2 increased linearly coinciding at various point, however the slope of the curve for Trial 1 was slightly higher than that of Trial 2 indicating the higher effectiveness of coating Trial 2 over the observed period of time.

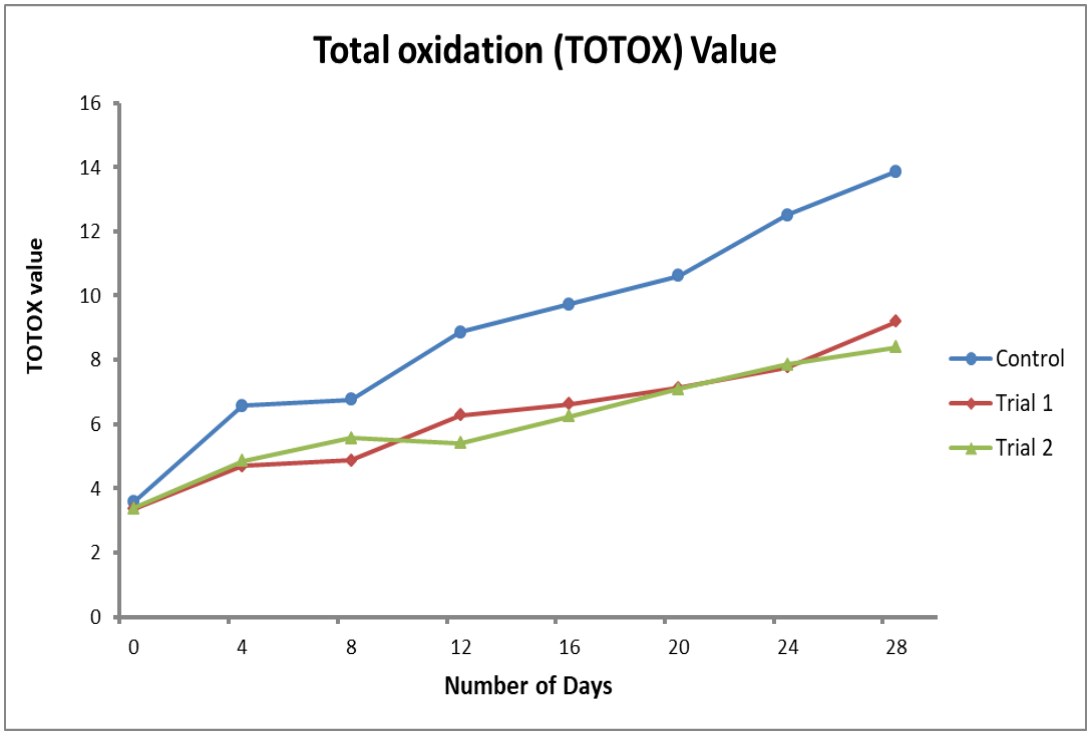

Figure 5.5: *TOTOX values for control and coated samples over a course of 29 days.*

5.4.4 Thiobarbituric acid Reactive Substances Assay

The thiobarbituric acid reactive substances assay or simply TBARS assay was done to measure the amount of malondialdehyde which is a product of secondary oxidation of walnut oil. This is a potent carcinogen hence it is essential to control the rate of rancidity so as to lower its concentration in oil.

The TBA value in uncoated sample showed a linear increase over the entire time span (refer figure 5.6). As for Trial 1, the TBA value initially increased linearly when it reached a peak on the 13th day, further there was stagnancy in the rate till the 21st day, after which the TBA value rose subsequently over the entire time of evaluation till the 29th day. As for Trial 2, the increase in the TBA value initially coincided with that of Trial 1 till the 9th day. Further the rate of the reaction remained stagnant till the 21st day, after which it progressed linearly with time.

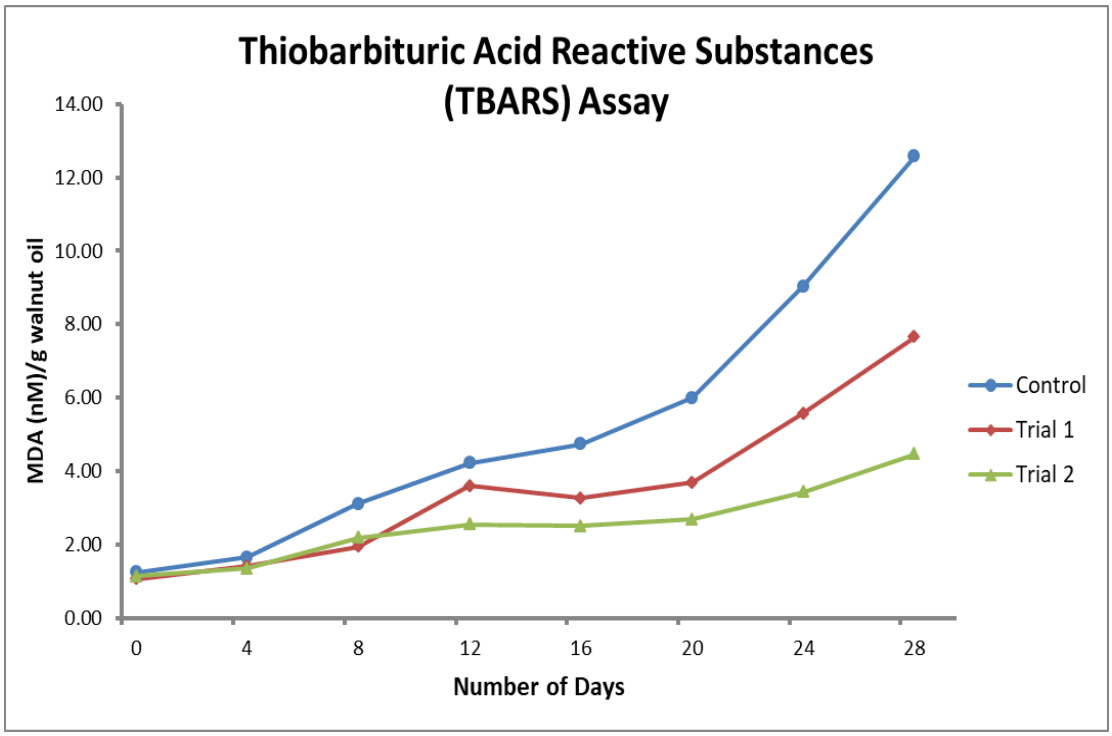

Figure 5.6: *Increase in malondialdehyde content (nM/g oil) for control and coated samples over a course of 29 days.*

The basic observation was that the curve for uncoated sample depicted a higher slope than the rest of the coated sample justifying for the functionality of the coat and the resulting effectiveness. The curves between Trial 1 and Trial 2 also indicated that Trial 2 had a lesser value of slope due to the usage of saturated fat

(palm stearin) as carrier in the coating which has a higher prohibitive impact on the rate of oxidation. Hence, it could be stated that coating Trial 2 worked better than coating Trial 1 in reducing the rate of formation of malondialdehyde and thereby its quantity.

CHAPTER 6 - CONCLUSION

Nuts are high value products imparting a major chunk of nutrition with multifarious health benefits. They provide a considerable amount of energy and are rich in unsaturated fatty acids such as mono-unsaturated fatty acids and poly-unsaturated fatty acids.

Walnuts specifically have a significant quantity of lipids (65%) of which the poly-unsaturated fatty acid content is over 70%, making it highly susceptible to oxidative rancidity. Presence of certain lipid peroxidation biocatalysts further is suggestive of the enhanced rate of oxidation reaction in walnuts. Accumulation of all the external parameters along with the presence of bio-catalysts conjures up to accelerate the rate of reaction, ensuing in the reduction of the lag phase and a faster propagation of alkyl free radicals to form hydroperoxides which further decomposes to form saturated and unsaturated carbonyl compounds. These carbonyl compounds are responsible for 'flavour fade' due to masking of the actual flavour of walnuts by these secondary short chain carbonyl compounds and flavour deterioration which is caused to excessive accumulation of secondary products of oxidation, imparting the walnuts unacceptable. Edible coating on walnuts is the most recent and eco-friendly approach directed at safeguarding the properties of walnut. The coatings can be majorly segregated into polysaccharide based, protein based and lipid based which could be conglomerated to form composite coatings as well.

Our study was focussed on the development of lipid based coating (carrier + bee wax + ACETEM + anti-oxidant) for walnuts to enhance its shelf life which is often impeded by the products of auto-oxidation as a result of rancidity. Lipid was chosen over polysaccharide and protein for coat formation due to its hydrophobic characteristic rendering the water vapour impermeable. This application was enhanced by usage of bee wax, which is 100% organic and is considered as GRAS has excellent barrier properties towards gases, prohibiting the massive gaseous exchange thus posing a hindrance to oxidation. Acetylated monoglyceride further substantially improved the barrier properties towards oxygen and water vapour, and its characteristic film forming abilities added to stability of the coating. To understand the best carrier for the key components, various formulations were devised using saturated fat (palm stearin with chemical properties close to cocoa butter, with a

melting temperature of 35 degrees C and solidifying at 25 degrees C) and unsaturated fat (palm superolein with Iodine Value between 60 and 63).

The coats thus developed depicted differences in their physical structure as well as their core functionality of preventing or slowing down auto-oxidation of lipids. Further addition of anti-oxidants played the role of free radical scavenging which also facilitated a reduction in oxidation.

To corroborate the hypothesis, certain tests such as Peroxide value, p-Anisidine value, TOTOX value and TBARS were conducted. The results validated the catastrophic fall in the rates of oxidation upon application of coating. A reduced peroxide value accounted for a slower rate of auto-oxidation reaction which further reduced the amount of secondary oxidation products thereby a slowing down the rate of deterioration. The effect was further elaborated using TBARS for determining malondialdehyde content and p-Anisidine value to measure the secondary oxidation products responsible for the distinct rancid flavour. The total oxidation value was the standard parameter to judge the overall quality of the walnut kernels. Upon analysis, it was found that application of coating explains for the improved shelf stability of the walnut kernels. The TOTOX value for the coated samples over a period of 29 days remained within 10, which is within the acceptable range and is considered as good. Also the sensory data further validated the results obtained by analytical methods. Application of coating increased the glossiness as well as the retained flavour and crunchiness. The application of palm stearin as a carrier having better stability than palm superolein as described by the results, could be attributed by the fact that an increase in the degree of unsaturation would provide favourable condition for external parameters such as light, heat etc to act on, leading to an increased rate of formation of alkyl free radicals resulting in an accelerated rate of propagation of the reaction. However palm superolein as a carrier in coating gave a superior physical appearance. Hence the formulations have been devised such that, they could be used in accordance with the utility desired, for example, for better appearance and slightly lesser shelf stability coating Trial 1 (with palm superolein) would be more desirable than coating Trial 2 (with palm stearin). Keeping any of the parameters constant, the right kind of coating could be selected, which would result in significant enhancement in shelf stability.

CHAPTER 7 - FUTURE SCOPE AND DIRECTIONS

The effect of moisture migration through walnut kernels before and after coating and its effect on rancidity could be enunciated. This could be done under controlled RH condition, to study the effect better.

Free fatty acid profiling before coating and after coating over the entire duration of accelerated shelf life study could be done by FTIR and GC-MS to study the effects on each individual fatty acid due to oxidative reactions better. This could facilitate the exact chemical properties of the carrier in the coat which would foster optimum effectiveness.

Walnuts have some inherent tocopherol content as they are rich in Vitamin E which could be exactly estimated for the variety to be used and the effect of the inherent tocopherol content of the walnut against additional anti-oxidant added as top-up in the coating could be studied. Also the prospects of addition of various natural anti-oxidants as top-up in the coating could be studied. The results could be compared with that of chemical anti-oxidants.

The effective rheological properties of the coatings developed are yet to be standardised to better understand and devise the most stable coating formulation and to ensure reproducibility and precision.

Various statistical models of correlation are yet to be developed to study the effect of each individual ingredient in the formulation towards rancidity and identifying the best combination of ingredients which inhibit oxidative rancidity to the maximal extent.

Microbial studies throughout the period of observation under accelerated shelf life condition could be done to innumerate the growth of most susceptible micro-organism and study its physiological and metabolic characteristics to determine further addition of the most effective anti-microbial in accordance with the microbe to stimulate microbial stability to the coat.

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