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Development of Whey Protein Concentrate Edible Films - A Review

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Abstract

A Bioactive/Biodegradable film is typically produced from food-derived ingredients using certain manufacturing process. WPC (Whey Protein Concentrate) exhibit excellent nutritional and functional properties that are most important for the formation of edible films. WPC based films are water based coatings showing certain characteristics that is these are flavor less, tasteless and flexible materials. Some antimicrobial agents are used as food preservatives that have been used to inhibit food borne diseases and also increases the shelf life of food products. The mechanism of developing Whey Protein Concentrate Edible Films involves use of certain types of essential oils.

Keywords: Whey Protein, biodegradable films, preservatives, essential oils.

Introduction

Bioactive/Biodegradable Film

An edible/biodegradable film is one which is typically produced from food-derived ingredients using wet or dry manufacturing process. The resulting edible film (EF) should be a free-standing sheet that can be placed on or between food components (McHugh, 2000). For up keeping and maintaining the quality of food, coatings and bioactive films have been used since ages. The ultimate functionality of edible film is related to its bioactivity such as antioxidant, antimicrobial and antibrowning and its functional attributes such as its ability to serve as a barrier to water vapour, oxygen, carbon dioxide and Ultraviolet light. In addition they can preserve and enhance the sensorial properties and may have ability to modify the internal atmosphere of the food. As early as twelfth century, citrus fruits from Southern China

were preserved for Emperor's table by placing them in boxes, pouring molten wax over them, and sending them to North (Hardenburg, 1967). In Europe, the process was known as larding- storing various fruits in wax or fats for later consumptions. Since 1980s, biodegradable film has evoked an interest among various researchers and various food industries due to plastic films which causes serious threat to environment. These films not only enhance the quality of foods but also act as barriers (moisture, gas and aroma) and provide protection to a food product after the primary package is opened (Kim and Ustunol, 2001). The biodegradable films have impact on overall packaging requirements of foods, which has great potential for reducing and eliminating the complexity of the packaging, thereby reducing packaging waste (Krochta, 1997).

Biodegradable natural polymer films offer alternative packaging with lower environmental costs. The main renewable and natural biopolymer films are obtained from polysaccharides, lipids and proteins. An intense search for new renewable sources to produce edible and biodegradable materials is observed and can be prepared from various components such as proteins, polysaccharides, lipids or the combination of these. Among them, protein based biodegradable films are found to be attractive with better mechanical and gas barrier properties than lipids and polysaccharides (Ouet *al*, 2004). The formation of edible films using proteins from plant sources has been limited, but may be advantageous to those from animal sources, because of their low cost, and perceived safety concerns by consumers or dietary restrictions over consuming animal-derived products (Gennadios, 2002). Films have been prepared previously using proteins from plant sources, such sunflower (Orliac *et al*, 2002), lentil (Bamdad *et al*, 2006), pea (Kowalczyk and Baraniak, 2011), and rapeseed (Jang *et al*, 2011). Animal and vegetable proteins are of great interest for the production of food packaging films because of their relatively low cost and high availability as by product of food industry and agriculture and inherent biodegradability. Soy protein is an interesting alternative for obtaining environmental friendly materials as these proteins are not only abundant but also renewable resource with high biodegradability. Soy protein produces more flexible, smooth, and clear films compared to those from other plant protein sources (Guilbert, 1986).

Whey Protein Concentrate

Milk proteins can be readily separated into casein and whey protein fractions. Casein represents 80% and Whey protein comprises 20% of the milk protein. Whey proteins are the byproduct protein that remains soluble after casein has been precipitated at pH 4.6 during cheese making process. The major fractions of whey protein are α -lactoglobulin (MW/ molecular weight = 18 KDa, PI/ isoelectric point = 5.3), β -Lactalbumin (MW = 14 KDa, PI 4.8), bovine serum albumin (MW = 66 KDa, PI = 5.1), immunoglobulins (MW = 150-960 KDa, PI = 5.5-6.8), and proteose-peptones. α -Lactoglobulin is the major component and constituting 50-60% of whey protein (Dybing and Smith, 1991).

Whey protein also constitutes other proteins such as lactoferrin, immunoglobulins, ceruloplasmin, and such milk enzymes such as lysozyme, lipase, and xanthine

oxidase, which are present in low concentrations. Whey protein concentrate (WPC) is produced by an industrial fractionation process involving ultrafiltration and diafiltration of pasteurized liquid whey. This is followed by vacuum concentration and spray drying, with a protein concentration ranging from 35% to 80% (e.g. WPC35, WPC80), dry matter basis. Contrary to this, Whey protein isolate (WPI) is a highly purified whey protein product (>95% protein) obtained by ultrafiltration and ion exchange (Dybing and Smith, 1991). Depending on the type and sequence of membrane processing, WPI does not preserve the proportions of whey proteins, as it is the case for WPC. The WPI is generally richer in α -lactoglobulin and β -lactalbumin, but proportionally poorer in Ig, lactoferrin, lactoperoxidase and glycomacropeptide (Cryan, 2001).

Besides their intrinsically nutritious properties, whey proteins exhibit several functional properties that are essential for the formation of edible films (McHugh and Krochta, 1994). On the other hand, the presence of triglycerides in the milk protein network significantly improves water vapour barrier properties, due to their low polarity. However when present, they also lead to more opaque and relatively inflexible films and coatings (Guilbert *et al*, 1996).

Film Forming Properties of WPC

Whey protein concentrate has excellent nutritional and functional properties and can be made into edible films and coatings, both in the denatured and the native state (McHugh *et al*, 1994 and Perez-Gago *et al*, 1999). Moreover these have demonstrated better mechanical and barrier properties than competitive proteins or polysaccharides and comparable to the best synthetic polymer films in the market (Khwaldia *et al*, 2004). Whey proteins are most distinctive than other film-forming biopolymers as these undergo conformational denaturation, presence of electrostatic charges, and amphiphilic nature.

The formation of film requires gelation of the said proteins. Gelation is the result destabilization of proteins in form of both physical (electrostatic and hydrophobic) and chemical (disulphide) interactions among whey protein molecules. Destabilization of the soluble proteins in whey can be induced via addition of chemicals, change in net charge, increase in hydrostatic pressure, heating, cooling, or partial enzymatic hydrolysis. Each of these processes induces partial or total unfolding of the initial proteins, thus

resulting in protein aggregation and eventual gel formation. The resulting film properties are affected by the amino acid composition, distribution and polarity, conditions affecting formation of ionic cross linking between amino and carboxyl groups, presence of hydrogen bonding, intra-molecular and intermolecular disulfide bonds (Gennadios and Weller, 1991).

The methods employed for the film formation are heating, -irradiation, cross linking agents such as enzymes, addition of chemicals, change in net charge and increase in hydrostatic pressure, cooling, or partial enzymatic hydrolysis. Each of these processes induces partial (or total) unfolding of the initial proteins, thus resulting in protein aggregation and eventual gel formation.

Irradiation affects proteins by causing conformational changes, oxidation of amino acids and rupture of covalent bonds and the formation of protein free radicals (Cheftel *et al*, 1985). -irradiation modifies the conformation of proteins due to the formation of bityrosine bridges between the protein chains. Ouattara *et al* (2002) used gamma irradiation cross-linking to improve the water vapour permeability and the chemical stability of milk protein films. The results showed that gamma irradiation significantly ($P < 0.05$) reduced water vapour permeability and increased resistance to microbial and enzymatic biodegradation. Some enzymes that have been used for cross linking proteins include transglutaminase, lipoxygenase, lysyl oxidase, polyphenol oxidase and peroxidase. However, transglutaminase is a kind of enzyme which can catalyze the covalent cross linking reactions between proteins to form high MW biopolymers. De Jong and Koppelman (2002), reported that transglutaminase catalyzes acyl transfer reactions between -carboxamide groups of glutamine residues and -amino groups of lysine residues. The increase in gel strength of proteins submitted to the action of transglutaminase was dependent on the order and intensity by which the enzyme produced cross links. In some cases, such as with isolated soy protein and deamidated gluten films, the transglutaminase treatment also significantly increased the surface hydrophobicity of films (Tang *et al*, 2005).

However, the formation of whey protein-based films has mainly accomplished by heat denaturation in aqueous solution at 75-100°C. The heat treatment produces intermolecular disulfide bonds, which are responsible for film structure. The formation of whey

protein films normally involves heat denaturation of said proteins in aqueous solutions and in the absence of thermal processing, such films would readily crack into small pieces upon drying, owing to food intermolecular interactions (McHugh *et al*, 1993). Further heat treatment promotes water insolubility, which may be beneficial to maintain film and food integrity (Perez-Gago *et al*, 1999). Protein films are finally obtained from whey protein gels via dehydration after heat- or cold-set gel formation (Vliet *et al*, 2004). A common practice in film formation is to dry at room conditions, typically 21–23°C and 50% relative humidity. However, control of this drying process is crucial as faster drying results in stiffer, less flexible films. The compression molding of WPI films has also been conducted by using a Carver Press at 0.8-2.2 MPa at 104-140°C for up to 2 min and this being the first step toward a continuous extrusion process (Sothornvit *et al*, 2003).

Characteristics of WPC Based Films

Whey protein based films and coatings are water based and generally flavourless, tasteless and flexible materials. The films vary from transparent to translucent depending on formulation, purity of protein sources and composition. Ramos *et al* (2013) developed the edible films from Whey Protein Isolate (WPI) and Whey Protein Concentrate (WPC) and observed that WPI films have lower moisture content, film solubility, water activity, water vapour permeability, oxygen and carbon dioxide permeability and colour change values as well as statistically higher density, surface hydrophobicity, mechanical resistance, elasticity, extensibility and transparency values than their WPC counterparts.

Ramos *et al* (2012) developed WPI films incorporated with organic acids and lactic acids and chito-oligosaccharides that exhibited different degrees of effectiveness against target bacteria. Zinoviadou *et al* (2010) studied physical and thermo mechanical properties of WPI films containing antimicrobials and their effects on spoilage flora of fresh beef. Films were prepared by incorporation of different levels of Sodium lactate (NaL), -Polylysine (-PL) into Sorbitol plasticized WPI films. NaL affects moisture uptake behaviour and water vapour permeability (WVP) at all concentrations used, while antimicrobial films made from 0.75% w/w -PL films reduced the growth rate of microbial flora significantly and growth of lactic acid bacteria (LAB) is completely inhibited. Lacroix *et al* (2002) worked on combination of

radiative and thermal treatments of the films based on calcium caseinate and whey proteins that resulted in an increase in the puncture strength of the films.

While considering the edibility, the whey protein film forming process should be appropriate for food handling in terms of pH change, salt addition, heating, enzymatic modification, drying, use of organic solvents, and application of other chemicals. Additionally, plasticizers and any other additives used should be compatible with the biopolymer (Han, 2002 and Nussinovitch, 2003). Such compatible WPC based edible coatings have been used on various foodstuffs such as dried strawberries (Huang *et al*, 2009), eggs (Alleoni *et al*, 2004), cheese (Cerqueira *et al*, 2009a), chocolates (Lee *et al*, 2002a), peanuts (Lee *et al*, 2002b) and apples (Perez Gago *et al*, 2006).

Antimicrobial Activity

For the prevention of the growth of spoilage and pathogenic microorganisms, several preservation techniques had been employed. Antimicrobial agents including food preservatives have been used to inhibit food borne bacteria and for extending the shelf life of processed food particularly meat (Yin and Cheng, 2003). These are the group of synthetic compounds which have their own ill health effects. Some of them have also been accused for possessing carcinogenic and toxic properties. This increased the consumer concerns toward healthier meat and meat products and the demand for natural food additives over the years which led researchers to examine natural alternatives to synthetic food additives (Mariutti *et al*, 2011). These natural additives should improve meat quality without leaving residues in the product or in the environment (Simitzis *et al*, 2008).

Essential oils are aromatic and volatile oily extracts obtained from medicinal plant materials, including flowers, buds, roots, bark, and leaves and possess strong antimicrobial and antioxidant activity (Hyldgaard *et al*, 2012). The antimicrobial action is attributed to their phenolic compounds. A number of essential oils like rosemary, thyme, ginger, coriander, oregano etc. have been tried successfully. Lemongrass essential oils (EOs) of diverse origin are mainly characterized by the presence of citral (geranial and neral) which accounts for 75-80% of total. It has medicinal properties and is safe as well that makes it a potential multi-functional food additive (Abd-Ei *et al*, 2010). Cinnamon essential oil contains high cinnamaldehyde content; which is the main

component in cinnamon and possesses antibiotic quality (Wong *et al*, 2014).

Mechanism of action of Essential oils (EOs)

EOs have been found to possess potent antibacterial and antifungal activities against several microorganisms associated with meat, including Gram positive and Gram negative bacteria. Generally they consist of more than 60 organic compounds with low molecular weight and large differences in antimicrobial capacity. The major active components of essential oils can be classified in three classes, namely phenols, terpenes, and aldehydes (Ceylan and Fung, 2004). Moreover, several works reported that all three classes of components principally act against the cell cytoplasmic membrane (Sikkema *et al*, 1995; Ultee *et al*, 2002; Ceylan and Fung, 2004), especially because of their hydrophobic nature, which can affect the percentage of unsaturated fatty acid on the membrane and thus alter its structure Burt (2004).

The mechanism of action of phenolic compounds, which among the essential oil constituents exhibit the highest antimicrobial activity (Burt, 2004), is apparently related to the interaction of the hydroxyl group characteristic of their molecules with the cell membrane, causing leakage of cellular components, alteration of fatty acids and phospholipids profiles and impairment of the energy metabolism and synthesis of genetic material (Ceylan and Fung, 2004; Di Pasquet *et al*, 2007).

Carvacrol, a phenolic compound, likely acts as protonophore that is a carrier of protons across the lipid bilayers, therefore causing the dissipation of the proton motive force (Lambert, 2001; Ultee *et al*, 2002). In particular, the carvacrol molecule is supposed to insert into the membrane, disrupting its structure and exchanging its hydroxyl group for another ion, such as potassium ion (Ultee *et al*, 2002). Moreover, acting on the permeability barrier of cytoplasmic membrane, carvacrol could also cause leakage of various other substances, such as ions, ATP, nucleic acids and amino acids (Lambert *et al*, 2001). More specifically, on *Escherichia coli* carvacrol caused the decrease of intracellular ATP as well as the increase of extracellular ATP, suggesting a disruptive action on the cytoplasmic membrane (Helander *et al*, 1998). While the site of action of terpenes, such as limonene, is also the cytoplasmic membrane, their mechanism of action is likely the accumulation in the cellular membrane, causing a loss

of membrane integrity, the inhibition of respiratory enzymes and dissipation of the proton-motive force (Sikkema *et al*, 1995).

The hypothesized mechanism of action of cinnamaldehyde, which is the aldehyde with highest antimicrobial activity, is also based on the dissipation of the proton motive force. Differently from phenols, in this case the antimicrobial action is related to the leakage of small ions rather than large molecules such as ATP as observed by Gill and Holley (2004), due to the only partial disruption of the outer cell membrane, which is not disintegrated by the access of cinnamaldehyde molecules to the periplasm and to the deeper parts of the cells.

Lemongrass Essential oil

The genus *Cymbopogon* possesses a large number of odoriferous species of the grass family (Poaceae) and is characterized by plants bearing aromatic essential oils in all parts. All the grasses were first categorized under *Andropogon* and only five species were recognized. Out of total 30 taxa were reported from Indian sub-continent. Currently we have 21 taxa in India and majority of *Cymbopogon* species can be distinguished from the related genera by the aromatic smell.

Lemongrass is widely used in traditional medicine in many countries. Among its attributable popular properties are those related to analgesic and anti-inflammatory actions (Ortiz *et al*, 2002). Besides the medicinal use, the lemongrass essential oil is also used in the food (flavouring), perfume and cosmetics industries (Bhattacharyya, 1970; Thapa *et al*, 1971; Oliveira *et al*, 1997b). *C. citratus* and *C. flexuosus* have been cultivated in several countries given the high citral content in its essential oil nearly 70-80% (Robbins, 1983). A wide number of terpenes have been identified in the essential oil of *C. citratus*, using analysis by GC or GC-MS. Limonene, one of the most frequent monoterpenes in essential oils, is also found in the lemongrass oil. This was isolated in concentrations between 0.3 and 5% (Zheng *et al*, 1993; Faruq, 1994; Chalchat *et al*, 1997 and Schaneberg and Khan, 2002). The predominant compound (30 to 93.74%) of the lemongrass essential oil obtained is the citral; a mixture of the aldehydes neral and geranial. (Schaneberg and Khan, 2002). Among the several alcohols and esters obtained from the essential oil of lemongrass, the geraniol is the most frequently compound found, regardless the plant

origin (Schaneberg and Khan, 2002) reported Limonene is one of the most familiar mono-terpenes in essential oils present in lemongrass. This mono-terpene was separated in concentration between 0.3 and 5%.

Lemongrass oil is reported to have potent antimicrobial action against number of organisms. The oil obtained from the *C. citratus* leaves exhibited antimicrobial activity when tested against 42 microorganisms (20 bacteria, 7 yeasts and 15 fungi). The isolated bacteria presented a superior susceptibility compared to the fungi (Ibrahim, 1992). This plant extracts and/or essential oil, especially the oil for its citral content, presented positive antibacterial activity for *Escherichia coli* (Ogulana *et al*, 1987), *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *S. pyogenes*, *Neisseria gonorrhoeae*, *Clostridium perfringens* (Syed *et al*, 1995; El-Kamali *et al*, 1998 and Ahnet *et al*, 1998); *Acinetobacter baumannii*, *Aeromonas veronii* biogroup *sober*, *Enterobacter faecalis*, *Klebsiella pneumoniae*, *Salmonella enterica* subsp. *Entericasorotipo typhimurium*, *Serratia marcescens* (Hamer *et al*, 1998); *Proteus mirabilis*, *Shigella flexneri* and *Salmonella typhi* (Syed *et al*, 1995; Chalchat *et al*, 1997). Hammer *et al* (1999) reported that the essential oils derived from lemongrass had shown the antimicrobial activity against some pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*.

The antioxidant activity of lemongrass oil has also been highlighted in many studies (Baratta *et al*, 1998) registered that the lemon grass oil had shown anti-oxidizing capacity comparable to that of tocopherol and butylated hydroxyl toluene (BHT). Cheah *et al*, 2001 reported that the dichloromethane and methanolic extracts of this plant showed powerful antioxidant activity.

Cinnamaldehyde and its Structure

Cinnamaldehyde is an organic, pale yellow viscous liquid which occurs naturally in the bark of cinnamon trees and other species of the genus *Cinnamomum*. The essential oil of cinnamon bark is about 90% cinnamaldehyde and natural product is trans-cinnamaldehyde consisting of a phenyl group attached to an unsaturated aldehyde. Cinnamon is reported to have many desirable medicinal and soothing effects and is used frequently in herbal medicines, as it is claimed that cinnamon can be used to treat diarrhoea

and arthritis. Cinnamon is also widely used in cooking due to its intense aroma and flavour. It can be used in its bark state (cinnamon sticks) or as fine powder. As with many components of essential oils cinnamaldehyde displays antiviral, antibacterial and antifungal properties. Eugenol is minor component of cinnamon oil, which constitutes upto 10% of the oil.

Chang *et al* (2001) worked on the antibacterial activities of the essential oils from leaves of two *Cinnamomum osmophloeum* clones (A and B) and their chemical constituents against nine strains of bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Salmonella* sp., and *Vibrio parahaemolyticus*. It was found that cinnamaldehyde possessed the strongest antibacterial activity compared to the other constituents of the essential oils. The MICs of cinnamaldehyde measured against the *E. coli*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *S. epidermidis*, MRSA, *K. pneumoniae*, *Salmonella* sp., and *V. parahemolyticus* were 500, 1000, 250, 250, 250, 250, 1000, 500, and 250 g/ml, respectively.

Singh *et al* (2007) studied the antioxidant, antifungal and antibacterial potentials of volatile oils and oleoresin of *Cinnamomum zeylanicum*. They analysed the bark of cinnamon and showed the presence of 13 components and cinnamaldehyde being the major component. The leaf and bark volatile oils have been found to be highly effective against all the tested fungi except *Aspergillus ochraceus*.

Cinnamaldehyde was also utilized as preservative in food products directly or through the carrier in packaging films. Ravishankar *et al* (2009) developed apple-based edible films containing cinnamaldehyde or carvacrol as antimicrobials in concentration 0.5%, 1.5%, and 3% and evaluated their activity against *Salmonella enteric* or *E. coli* O157:H7 on meat and poultry products and found that carvacrol films induced greater reductions than cinnamaldehyde films at all concentrations. Ouattara *et al* (2000) compared the antimicrobial activity of films based on chitosan matrix incorporating acetic or propionic acid with or without lauric acid or cinnamaldehyde and observed strongest inhibition with films containing cinnamaldehyde. They demonstrated the improvements to be done to find better antimicrobial agents which would be active against a large spectrum of bacteria and a better way to control the release of the incorporated antimicrobial agents.

Rojas Grau *et al* (2007) detailed the mechanical, barrier and antimicrobial properties of alginate-apple puree edible film, incorporated with 0.1–0.5% suspensions of the essential oils namely: oregano oil (carvacrol), cinnamon oil/cinnamaldehyde; and lemongrass oil/citral and evaluated their activity against the food borne pathogen *Escherichia coli* O157:H7 using solutions used to prepare edible films. They found that the presence of plant essential oils did not significantly affect water vapor and oxygen permeabilities of the films, but significantly modified tensile properties. Few studies involving incorporation of antimicrobial agents such as citric, lactic, malic, and tartaric acids and nisin (Eswaranandam *et al*, 2006), oregano, rosemary and garlic essential oils (Seydim and Sarikus, 2007) into whey protein films and coatings.

Eugenol or Clove oil

Eugenol is used in perfumes, flavourings, and also used as a local antiseptic and anaesthetic. Eugenol can be combined with zinc oxide to form zinc oxide eugenol which has restorative and prosthodontic applications in dentistry. For example, zinc oxide eugenol is used for root canal sealing. Attempts have been made to develop eugenol derivatives as intravenous anesthetics, as an alternative to propomid which produces unacceptable side effects around the site of injection in many patients. It can be used to reduce the presence of *Listeria monocytogenes* and *Lactobacillus sakei* in food (Kouidhi *et al*, 2010).

Eugenol is an allyl chain-substituted guaiacol, i. e. 2-methoxy-4-(2-propenyl)phenol. Eugenol is a member of the allylbenzene class of chemical compounds. It is a clear to pale yellow oily liquid extracted from clove oil, nutmeg, cinnamon and bay leaf. It is slightly soluble in water and soluble in organic solvents. It has a pleasant, spicy, clove-like odour. Eugenol is used in making perfumes, flavours, essential oils and in medicines as it has antiseptic and analgesic properties. It is widely used in the production of isoeugenol for the manufacture of vanillin. Eugenol has wide application in dentistry. For example, oil of cloves or eugenol is commonly used by dentists because it is antiseptic and anti-inflammatory (Fujisawa *et al*, 2002). Oftenly, it is applied to the gums to kill the germs and relieve the pain of dental surgery such as tooth extractions, fillings, and root canals. Moreover, clove oil, as well as cinnamon, basil and nutmeg oils; each of which contain eugenol, are some common ingredients used for mouth washes, toothpastes, soaps,

insect repellents, perfumes, foods, and various veterinary medications. Some studies showed that eugenol fights bacteria and inhibits the growth of many fungi, including *Candida albicans*, the pathogen which is responsible for most human yeast infections. In cell culture, eugenol has been between 1.4 and 2.3 times as effective against *C. albicans* and *C. tropicalis*, respectively, as equivalent doses of the antifungal pharmaceutical nystatin. Eugenol or oil of clove is already used to fight fungal infections of the skin, ears, and vagina in some non-western countries, but this treatment can cause irritation and dermatitis so this should not be tried without the guidance of a health-care practitioner. Various researches also show that eugenol is a very powerful fat-soluble antioxidant, inhibiting the accumulation of fat peroxide products in red blood cells and maintaining the activities of the body's antioxidant enzymes at normal levels. Overall results of laboratory studies using animals indicate that only small amounts of eugenol are required for a significant protective effect because eugenol can be directly incorporated into cell membranes, which prevents lipid peroxidation right in place. In one study, rats were poisoned with carbon tetrachloride, which strongly damages tissues by oxidation. Rats who were given eugenol along with the carbon tetrachloride were strongly protected from its toxic effects, according to the researchers.

Peppermint oil

It is derived from the peppermint plant - a cross between water mint and spearmint that thrives in Europe and North America. Peppermint oil is commonly used as flavouring in foods and beverages and as a fragrance in soaps and cosmetics. Peppermint oil is used for a variety of health conditions and can be taken orally in dietary supplements or topically as a skin cream or ointment. The main chemical component in Peppermint essential oil is menthol (Alankar, 2009).

Menthol has a monoterpene backbone with an alcohol functional group. Menthol contains energizing properties that contribute to the overall energizing effect of Peppermint oil. Menthol is known to soothe the smooth muscle that lines the colon. This relaxant property occurs due to menthol's ability to keep calcium channels working optimally (Zivanovic, 2005).

The relaxing of colon smooth muscle reduces the movement of bowels, which eases occasional bowel

looseness. Because of this effect, menthol is a main contributor to peppermint essential oil's ability to promote digestive health. Peppermint oil also contains omega-3-fatty acids, iron, magnesium, calcium, vitamins A and C, minerals, potassium, manganese and copper. Clinical evidence suggests that peppermint oil likely can help with symptoms of irritable bowel syndrome. It may also help in digestion and prevent spasms in the GI tract caused by endoscopy or barium enema. Some studies show that used topically it may help soothe tension headaches and cracked nipples from breastfeeding – but more research is needed to confirm these studies. When used as directed, dietary supplements and skin preparations containing peppermint oil are likely safe for most adults. Peppermint oil is sensitive to light and heat damage, and should only be stored in a cool, dark place in tightly sealed bottles. Peppermint essential oil is a natural antibacterial, antiviral, anti-fungal and analgesic (pain reliever) and is an excellent anti-parasitic. All over the world it has powerful medicinal properties; modern research today attributes these qualities to menthone, menthyl esters, and most importantly to the active compound, menthol, which is found in high concentrations in peppermint oil. Peppermint oil is used widely in the manufacturing of products we use daily such as shampoo, chewing gum, toothpaste, ice cream, soap, tea and more.

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