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Antimicrobial activity of active packaging film to prevent bread spoilage

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Abstract
Essential oils were only used as aromatherapy and fragrances but they can be exploited for antimicrobial effects for the prevention of food spoilage from several microorganisms. Their antimicrobial and antioxidant properties destroy the cell membrane of microbes. By combining these essential oils into the structure of food packaging, moisture can be repelled, reducing spoilage of food across the board. This study aimed at producing carbohydrate based film consisting of active component i.e. cinnamaldehyde (cinnamon oil) and eugenol (clove oil). The films were prepared by using essential oils as a contributor of antimicrobial effect. The amounts for different constituents were optimized, standardized and homogenised and then dried on acrylic plates. Tests done showed the mechanical and physical properties of the film by which it was concluded that the film was biodegradable and completely soluble in water. The development of bioactive packaging systems through the incorporation of antimicrobial agents into biopolymer-based coatings could make a significant contribution toward shelf-life extension and food safety preservation. This study describes the main methods for elaborating pectin edible films, principal characterization techniques for determining their physical-mechanical properties, and applications of pectin edible films as antimicrobial food packaging. The active packaging thus made can be substantially used for the shelf life extension of many food products.

Keywords: active packaging, phytochemicals, cinnamon oil, clove oil

1. Introduction
Active packaging is defined as a packaging that changes the condition of the packaged foodstuff to extend its shelf life or improve its safety while maintaining its quality and it has become a popular alternative to control undesirable moulds in foods. A new tailor-made active paper packaging using natural essential oils as antimicrobial solutions was introduced. In active packaging, some essential oils (clove EO and cinnamon EO) were used to manufacture edible film using pectin [1]. *Rhizopus stolonifer* fungus (black bread mould), together with *Aspergillus* and *Penicillium* genera are the most prevalent spoiler of bread and bakery products. This fungus is responsible for the production of mycotoxins, off-flavour formation, and disgusting appearance in bread. *Rhizopus stolonifer* usually grows on bread and fruit and, because its spores are ubiquitous in the air, infests the product within a short time when bread is stored in an enclosed, humid environment [2].

Essential Oils
Essential oils are concentrated volatile aromatic compounds produced by plants - the easily evaporated essences that give plants their wonderful scents. Each of these complex precious liquids are extracted from a particular species of plant life. Each plant species originates in certain region of the world, with particular environmental conditions and neighbouring flora and fauna. Essential oils are frequently referred to as the “life force” of plants [3]. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds [4]. The amount of essential oils found in these plants can vary from 0.01 percent to 10 percent of the total. That's why tonnes of plant material is required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavour and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Essential oils cannot be substituted with synthetics [5]. Natural essential oils or spices are exploited as food preservatives. Essential oils are rich sources of terpenes and phenols, and for this reason, they have strong antimicrobial properties. Another interesting feature is that these natural compounds do not have any significant medical or environmental impact, so they constitute effective alternatives to conventional antimicrobial agents [6].

Properties of essential oils
Essential oils have various biological activities such as antioxidant, insecticidal, bactericidal and antifungal properties [7]. Reports of essential oil use date back thousands of years as effective stimulants, sedatives and treatments of countless ailments, today they are frequently used in food industry, medicine and cosmetics [8].

Clove oil (*Syzygium aromaticum*)
Clove represents one of the Mother Nature’s premier antiseptic. Approximately 16-18% of clove is essential oils. Clove oil consists of 70-90% eugenol. Eugenol is a phytochemical that causes cytoplasm membrane and active transport imbalance [9]. Clove oil contains high tannin content of 1-19% providing antimicrobial activity. The other constituents of clove oil (*Syzygium aromaticum*) are eugenyl acetate, caryophyllene and iocaryophyllen. Eugenol is the active ingredient and has considerable anti-fungal activity against clinically relevant fungi [5]. Clove oil has antioxidant, anti-diabetic, antiviral, anti-inflammatory properties [10]. Clove EO is proved to have inhibitory activity against fungi- *Aspergillus* and *Candida*. It has fungicidal effect resulted from an extensive lesion of cell

membrane. It also caused a considerable reduction in quantities of ergosterol, a specific fungal cell membrane component [11]. Antimicrobial activity of this oil can be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with -SH groups in the active sites of target enzymes, resulting in the deactivation of the enzymes in fungi [12].

Cinnamon oil (*Cinnamomum zeylanicum*)

The essential oil of this spice is known to have antimicrobial compounds. Cinnamon barks and leaves are widely used as spice and flavouring agent in foods and for various applications [13]. The essential oil from its bark is rich in trans-cinnamaldehyde with antimicrobial effects against animal, plant pathogens, food poisoning and spoilage causing bacteria and fungi. The bark and leaves of Cinnamomum *sp* are commonly used as spices in home kitchens and their distilled essential oils are used as flavouring agent in the food and beverage industries [14]. Chemical components of cinnamon EO (0.51%) are eugenol, benzaldehyde and other terpenes.

The main constituent of cinnamon EO (*cinnamomum zeylanicum*) is cinnamaldehyde, which is the compound containing an aldehyde group and conjugated double bond outside the ring. This compound possesses much stronger antifungal activity [15]. Cinnamaldehyde is proven to inhibit spore formation in fungi and it may be a potential lead compound for the development of antifungal drugs through the control β-(1, 3) - glucan and chitin synthesis in yeasts and moulds [16].

Polysaccharides based active film

Active films and coatings have received considerable attention in recent years because of their advantages including use as edible packaging materials over synthetic films. This could contribute to the reduction of environmental pollution [17]. Active films can enhance the organoleptic properties of packaged foods provided they contain various components (flavourings, colourings, sweeteners). Their use as based on natural polymers and food grade additives has been constantly increasing in the food industry [18].

The coatings/films can be produced with a variety of natural products such as polysaccharides, proteins and lipids with the addition of plasticizers and surfactants. The functionality and performance of edible films mainly depend on their barrier, mechanical and colour properties, which in turn depend on film composition and its formation process [19]. In the case of active coatings, the method of application on the product, and the capacity of the coating to adhere to the surface are the most important parameters. Food products are usually coated by dipping or spraying, forming a thin film on the food surface that acts as a semi-permeable membrane, which in turn control the moisture loss or/and suppress the gas transfer [20]. The films also function as carriers for antimicrobial and antioxidant agents.

Pectin

Pectin is a white, amorphous and colloidal carbohydrate of high molecular weight occurring in ripe fruits, especially in apples, currants, etc. and used in fruit jellies, pharmaceuticals and cosmetics for its thickening and emulsifying properties and ability to form to a gel [21]. All these properties and applications have put pectin in the market of the biopolymers with great potential and possibilities for future developments.

Pectin is one of the main components of the plant cell wall chemically constituted by poly α1–4-galacturonic acids. According to its degree of esterification with methanol, pectin can be classified as high methoxyl pectin or low methoxyl pectin. In food industry, pectin is listed as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) and is used as gelling, stabilizing or thickening agent in food products such as jams, yoghurt drinks, fruity milk drinks and ice-creams [22]. Due to its biodegradability, biocompatibility, edibility and versatile chemical and physical properties (such as gelation, selective gas permeability, etc) pectin is a suitable polymeric matrix for the elaboration of edible films intended as active food packaging. Active packaging is a packaging system which possesses attributes beyond basic barrier properties that are achieved by adding active ingredients in the packaging material and/or using functionally active polymers [23]. When the packaging system has antimicrobial activity, the packaging limits or prevents the microbial growth by extending the lag period and reducing the growth rate of microorganisms [24].

Surfactants and Plasticizers

Plasticizers are widely used to improve the process ability, flexibility and ductility of polymers. In the case of a polymer like pectin, an efficient plasticizer has to not only reduce the brittleness but also depress the heterogeneity and insolubility [25]. In this context, surfactants are interesting ampiphilic substances in which the balance between the hydrophilic and hydrophobic fractions determine their applications. Polyoxy ethylene sorbitan mono oleate (Tween 80) has a high hydrophilic/lipophilic balance (HLB) value (>10) and is used for oil-in-water-type applications. A reduction in the water absorption capacity of pectin based films has been observed with increasing HLB of the mixture of surfactants. Surfactants with higher HLB allow a greater association of their hydrophilic fraction with the hydrophilic film matrix which in turn, may reduce the amount of water binding sites while the hydrophobic fraction may act as a water vapour permeability (WVP) barrier [26].

Plasticizers are added to polymers to modify their physical properties and to improve their film forming characteristics. Plasticizers can change the viscoelastic behaviour of polymers significantly. In particular, plasticizers can turn a hard, brittle polymer into a softer, more pliable material, and possibly make it more resistant to mechanical stress [27]. To be effective, a plasticizer must interpose itself between the polymer chains and interact with them thereby extending and softening the polymer matrix. Plasticization in general, refers to a change in the thermal and mechanical properties of a given polymer which involves (a) lowering of rigidity at low temperature; (b) lowering of transition temperature, at which substantial deformation can be affected with not too large forces; (c) increase in the elongation of polymers. These changes in the mechanical properties also affect the permeability of polymer films [28]

***Aspergillus* and *Penicillium* Moulds**

The green mould, with the chain producing structures appears to be a deuteromycete of the genus *Penicillium*. *Penicillium* is a common fungus of food and is characterized by brush shaped conidiophores. The spores of these fungi are produced in chains from specialized hyphae called sterigmata [29]. The black mould is almost certainly a species of the genus *Aspergillus*. The members of *Aspergillus* produce black moulds and their spores are radically arranged blastospores. These members are also

some of the most abundant food moulds found [30].

2. Materials and methods

Phase 1

Procurement of Raw Materials

Essential oils (cinnamon and clove) were procured from local market of Rani Bagh and bread samples were procured from local market of Hauz Khas, New Delhi, India. The emulsifying agent, Polyoxyethylene (20) sorbitan monooleate, commercially known as Tween80, food grade pectin were used from Food Technology Laboratory, Institute of Home Economics, University of Delhi.

Phase 2

i) Film preparation

Method of Emulsion preparation

Direct oil-in-water (o/w) emulsions were prepared by adding 2% (w/v) of Cinnamon EO, 1.5% (w/v) of Clove EO and 1.5 % (w/v) of tween 80 to distilled water, followed by complete mixing.

Film Formation

Solutions of pectin 2.3% (w/v) were prepared by dissolving powdered pectin in distilled water and mixing until complete solubilisation, the solutions were also incorporated with previously formed emulsions, produced by complete homogenization. In this technique, a portion of the film suspension is poured onto acrylic plates, and then, dried at room temperature. Dried films were cut, peeled from the casting surface, and stored folded in butter paper within air-tight sealed plastic bags [31]. With slight alterations of respective components of film the procedure was followed:

Cinnamon EO	- 0.5% (w/v)
Clove EO	- 0.3% (w/v)
Tween 80	- 2% (w/v)
Pectin	- 2.5% (w/v)
Polyethylene Glycol 400	- 8% (v/v)

Phase 3

Mechanical tests

i) Tensile Strength: Tensile strength was found in accordance with ipc-tm-650 test methods manual. A paramount digi strength tensile strength tester (capacity: 250 kg, sensitivity: 100 gm) was used to determine tensile strength. The test specimen was 120 mm x 80 mm. The specimen was placed in the grips of the testing machine, taking care to align the long axis of the specimen with an imaginary line joining the points of attachment of the grips to the machine. The specimen should be aligned as perfectly as possible with the direction of pull so that no rotary motion that may induce slippage will occur in the grips. One end of test specimen was tightly clamped in the upper jaw and the lower end in the lower jaw. The grips were tightened evenly and firmly to the degree necessary to minimize slipping of the specimen during testing. The machine was started by pushing load button and stopped when the film breaks or maximum load is reached, the load versus extension was noted. Triplicate readings were taken for each film and tensile strength was expressed as kg/cm2 [32].

$$Tensile\ Strength = \frac{Force(kg)}{Area\ of\ sample(cm\ sq.)}$$

ii) Tear strength

Tear strength of the film samples were evaluated using the adopted method from sample was uniformly placed within the clamps. The pendulum was raised which resulted in the release of sector stop and the specimen was torn. As the sector completed its return swing, it was caught with the thumb and fore- finger of the left hand. The reading was recorded from the scale indicated by the pointer. Triplicate readings were taken for each film and the results were expressed in grams (g) [32].

$$Tear\ Resistance = \frac{s \times 6400}{n}$$

Where,

S = mean of sample reading N = number of readings taken 6400 = machine capacity

iii) Bursting strength

Bursting strength was evaluated using method given in [32]. Micronix bursting strength tester mil-0301-sd was used to determine the bursting strength. One film section of 6.3 cm x 6.3 cm (2.5 in. X 2.5 in.) Was cut from each of the test films produced. The specimen was clamped securely and the pressure was applied until the specimen ruptured. The maximum pressure registered by the gauge was recorded. Triplicate readings were taken for each film and the results were expressed in kg/cm2.

$$Tear\ Resistance = \frac{s \times 6400}{n}$$

iv) Thickness

Thickness was evaluated from the method given in (ASTM D 6988 – 03) (American Standards Test Method) [33]. Thickness was measured using manually operated thickness gauge. Samples measuring 10 x 10 cm were used. The sample to be measured was kept on an anvil. The press foot was raised and then gently lowered on to the sample. The reading on the dial gauge was recorded as the thickness of the sample. The above procedure was repeated to obtain the values of thickness at 20 different locations on the sample. Readings were taken in triplicates for each sample.

v) Water vapour transmission rate (WVTR)

Water vapour transmission rate was evaluated from the method given in [32]. Aluminium dishes were cleaned and weighed (w1). The dishes were filled with desiccant anhydrous calcium chloride and weighed again (w2). The desiccant should be filled to within 6 mm of the specimen and enough space was left so that shaking of the dish could mix the desiccant at the time of weighing. Specimen was cut and the dishes with calcium chloride were packed inside the specimen and sealed using wax. This assembly was weighed (w3) and was placed inside the desiccator for 24 hours. After 24 hours, the dishes were weighed again (w4). Triplicate readings were taken for each film type.

$$WVTR = \frac{g(gm) \times 24hrs}{t(hr) \times a(m\ sq.)}$$

Where,

g = w4-w3 (w4-weight after 24 hrs, w3-weight of the complete assembly)

t = Time for which the sample was kept in desiccator (24 hours)

a = Area of the aluminium dish

Physical Tests

i) Film Solubility in water

A modified method from [34] was used to measure film solubility. Film portions measuring 1×3 cm2 were cut and were dried at 110 ˚C in oven for 24 h and then weighed to the nearest 0.085 g for the initial dry weight. Then films were placed in glass beaker with 50 mL of distilled water and shaken gently at 25 ˚C for 24 h. The solution was then filtered through Whitman No. 1 filter paper to recover the remaining undissolved film. The remaining pieces of film after immersion were dried at 110 ˚C to constant weight (Final dry weight). Tests for each type of film were carried out in three replicates. Solubility in water (%) was calculated by using the following equation:

$$Solubility\ in\ water(\%) = \frac{Initial\ dry\ weight - Final\ dry\ weight}{Initial\ dry\ weight}$$

ii) Flame test

Hold the sample to the edge of a flame until it ignites. If no flame is produced quickly, hold the sample in the flame for about 10 seconds. If the material burns, note the colour of the flame, the nature of the smoke, the presence of soot in the air and whether, while burning, the sample drips. Next, extinguish the flame and cautiously smell the fumes. To identify the odour, samples of known plastic samples for comparison can be most helpful. Finally, check your observations against the known characteristics of each plastic. The test was repeated thrice for each sample [35].

iii) Biodegradability Field test

Film samples were buried in soil, or performing a full-scale composting process with the biodegradable plastic, represent the ideal practical environmental conditions [36].

Microbiological test

i) Antimicrobial Disc Plate Assay

Three samples of each clove enriched film, cinnamon enriched film, and control (Without any enrichment of essential oils) were cut into 1/4th of the diameter of petriplate.

The petriplate was filled with bread on wet filter paper inoculated with cultures of *Aspergillus* and *Penicillium*. The petriplate was incubated at 30˚ C for 48 hours (incubation period for fungi and moulds) was followed by checking it until the growth of moulds (*Aspergillus* and *Penicillium*) is observed [37].

3. Results and Discussion

Phase 1

Procurement of Raw Materials

The raw materials were procured, as discussed in methods section previously.

Phase 2

Film formation

A 2.5% pectin concentration was selected as the optimum polysaccharide concentration in the film forming solution. The composite films formed using clove EO and cinnamon EO were visually homogeneous without signs of phase separation between the components. There were no brittle areas or bubbles and they could be easily peeled from the casting plates. The addition of essential oils to pectin films may induce a plasticizing effect that can improve the mechanical integrity of resulting films [38].

Fig 1: Visual appearance of film (Cinnamon EO enriched)

Phase 3

Mechanical Properties

i) Tensile Strength

Tensile strength (TS) is the key indicators of films strength [38]. It expresses the maximum stress developed in a film specimen during tensile testing. The incorporation of plant EOs in pectin based edible films caused a significant increase in tensile strength of the film. These differences could be related to their different polarities [39].

The effect of incorporating clove and cinnamon EO on mechanical properties of active polysaccharide films is presented in Table 1. Control film had tensile strength value 0.0113kg/cm sq. Incorporation of EO into films increased tensile strength value.

This is because of a strong interaction between the polymer and the EO produced a cross linker effect, which decreases the free volume and the molecular mobility of the polymer. This phenomenon led to a sheet like structure [24].

ii) Tear resistance

Tearing resistance is the force perpendicular to the plane of the packaging material required to tear multiple plies through a specified distance after the tear has been started. Higher tear values may be needed machine operations or for package strength while low tear values are necessary and useful for easy opening of some package types. It usually takes more energy to pull the fibres out of the sheet than it does to fracture the fibres. The ratio of fibre fracture to fibre pull out changes as a function of the bonding. At higher bonding levels, there is a higher degree of fibre structure and at lower bonding levels; there is more fibre pull out [40].

Film enriched with clove oil had the highest tear resistance of clove being 13653.33g followed by cinnamon enriched and control films.

iii) Bursting strength

Bursting Strength is the hydrostatic pressure in kg/cm^2 required to rupture the material. It is used as a measure of resistance to rupture and primarily as an indication of the suitability of certain fibre material and the extent of processing. The bursting strength is dependent on the type, proportion, preparation and amount of fibres present and their formation, internal sizing and the surface treatment. Bursting strength is measured by means of a Mullen tester which has clamps for holding the sample over a rubber

diaphragm and a motor that forces a liquid into a pressure chamber under the diaphragm at a rate of 95ml per minute [40].
Film enriched with clove oil had the highest bursting strength of 0.8 kg/cm sq followed by others.

iv) Thickness

Thickness of a material is the perpendicular distance between the two outer surfaces of the materials. The films thickness is an important parameter in determining the workability of active films as packaging materials for food products as the thickness of the films affects other characteristics of the films, such as tensile strength, elongation, and water vapour permeability and gas transmission rate etc. Gas transmission rate is inversely proportional to the thickness. The films thickness is dependent on both the composition of film and processing conditions. Thickness was measured by means of a manually operated thickness gauge [40].
The thickness ranges between 10.45 µm to 11.15 µm.

v) Water vapour transmission rate (WVTR)

WVP (Water Vapour Permeability) is a measure of the ability of a material to be penetrated by water vapours [41], Water vapour permeability decreased with addition of essential oils in films and the results were in agreement with those obtained by [39].
Carvacrol (Component of clove EO) addition to films resulted in significant decrease in film water vapour permeability. Water vapour transfer generally occurs through the hydrophilic portion of the film; thus, water vapour permeability depends on the hydrophilic-hydrophobic ratio of the film components [42].
Water vapour permeability increases with polarity, unsaturation, and branching degree of the lipid, depending also on the water absorption properties of the polar part of the film [43]. Essential oil's chemical nature also plays an important role in the barrier properties of edible films. Differences observed by addition of different plant EOs can be explained by their hydrophobicity. In this way, carvacrol, a phenolic compound containing an alcohol group in its chemical structure seems to be a good barrier compared to aldehyde compounds (e.g., cinnamaldehyde) because the hydroxyl group has less affinity for water than for the carbonyl groups. Carvacrol then offers the possibility not only to enhance antimicrobial efficiency but also to improve barrier properties of active films [39].

Table 1: Mechanical Properties

Sample	*Tensile Strength (kg/cm sq)*	*Tear Resistance (g)*	*Bursting Strength (kg/cm sq)*	*Thickness (µm)*	*WVTR (g/m^2/24hr)*
Clove film	0.0301±0.001	13653.33±0.000	0.8±0.26	10.45±0.25	0.125±0.02
Cinnamon film	0.0214±0.002	11306.67±0.000	0.63±0.30	10.75±0.27	0.372±0.01
Control film	0.0113±0.003	9813.33±0.000	0.46±0.25	11.15±0.31	0.660±0.05

** Results are expressed as Mean ± SD for triplicate readings*

Physical Tests

i) Film Solubility in water

All the three samples that were tested for solubility in water were found to be 100% soluble as no insoluble matter was left after 24 hours. Hydrophilic nature and plasticizing effect of PEG 400 and Tween 80 increases the solubility of the films [44].

ii) Flame test

The flammability test is used to determine the relative rate of burning of self-supporting plastics. This test is mainly used for quality control, production control and material comparisons. It cannot be used as a criterion for fire hazard.
The flame was of orange colour with no floatability and high speed of drip with good scratch able properties for films enriched with cinnamon and clove essential oils.
For the control sample with no essential oil the flame was of yellow colour with slow drip speed with good scratch able properties.
The odour was same like paraffin for the three samples and average time taken for a film of 14.8 cm *5.5cm was 24.08 sec. The parameters were checked in accordance with [45]. The residue of films after burning was black in color.

Table 2: Observations for Flame Test

Type	Odour	Colour of flame	Drip	Speed of drip	Floatability	Scratchability	Time
Clove	Paraffin	Orange	Yes	Fast	No	Yes	24 sec
Cinnamon	Paraffin	Orange	Yes	Fast	No	Yes	24.05 sec
Control	Paraffin	Yellow	Yes	Slow	No	Yes	24.08 sec

*The readings were taken in triplicate.

iii) Biodegradability

Biodegradable plastics, as novel materials, make claims to be environmentally friendly. Consequently, it must be proved by using scientifically based and generally accepted methods that this is indeed the case. A first generation of biodegradable plastics consisted simply of polyethylene blended with starch. Initially, these were sold as biodegradable plastics, but in practice they did not fulfil the expectations of the users. Arguments for claiming these blends as biodegradable included the growth of microorganisms on the material's surface, or a certain loss in mechanical properties (e.g., tensile strength) when they were exposed to the environment.
The term biodegradable plastics normally refer to an attack by microorganisms on no wate rsoluble polymer-based materials (plastics). This implies that the biodegradation of plastics is usually a heterogeneous process. Because of a lack of water-solubility and the size of the polymer molecules, microorganisms are unable to transport the polymeric material directly into the cells where most biochemical processes take place; rather, they must first excrete extracellular enzymes which depolymerize the polymers outside the cells [36].
The film made was degraded in 3 days and no residue was seen.

iv) Microbiological test

Food products are highly susceptible to microbial contamination that may affect their quality attributes and reduce their

nutritional value. Moreover, the possible presence of microbial toxins or pathogenic microorganisms such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens*, and *Aspergillus niger* may even endanger consumer safety and contribute to foodborne illness [46].

Although synthetic fungicides, mainly nitrite and sulphites, proved to be highly effective against a wide variety of pathogenic microorganisms in foods, their potential negative impact on human health has prompted research on the use of naturally occurring antimicrobial agents to inhibit the growth of foodborne pathogens and prevent food spoilage. *A.niger* was selected from several fungi because it is well known as an opportunistic human pathogen and as a microorganism commonly encountered in food contamination cases. *A.niger* strains produce mycotoxins such as aflatoxin, which is one of the most potent carcinogens known to man and has been linked to a wide array of human health problems [47].

Livings beings including humans, when come into with *A.niger* and mycotoxins usually through consumption may cause many negative effects, i.e., immunotoxicity, carcinogenicity and hepatotoxicity. The effects on animals include decrease in antibody resposes, size reduction in immune organs and an alteration in the production of cytokine which are proteins and peptides specifically used in signalling [48].

A wide variety of natural antimicrobial agents, including essential oils (EOs) derived from plants have been tested for their antimicrobial potential against pathogens and spoilage bacteria in various food products [49].

With disc plate antimicrobial assay method the shelf life was increased by 4 days. With visual inspection after the incubation of moist bread with film at 30 degree Celsius, the samples with cinnamon and clove essential oil enriched film showed good antifungal effects against *Penicillium* and *Aspergillus* (main causative agents of bread spoilage) [38].

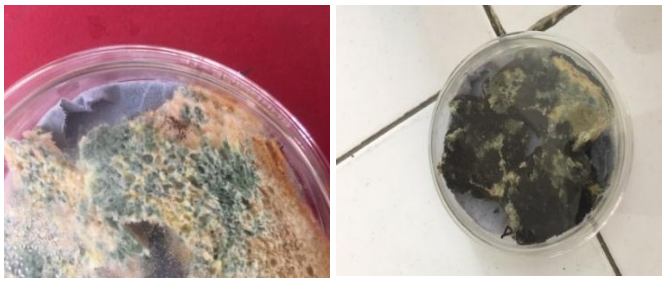

Fig 2: Control sample (without film addition)

Fig 3: Samples in contact with film (Observation as on 8th day)
*C-Cinnamon film, CL-Clove film, Control film

Fig 4: Samples in contact with film (Observation as on 9th day)
*C-Cinnamon film, CL-Clove film, Control film

4. Conclusion

Over recent times essential oils have become very well-known for their safe and natural healing properties. While their antiseptic and anti-inflammatory properties are widely known, their anti-fungal abilities are often overlooked. Increasing concerns about human health risks and environmental contamination, restricted commercial channels for conventional production, and the proliferation of resistant strains of pathogenic fungi are important problems related to the use of conventional chemical fungicides. Much work has been recently carried out to propose new formulations of active coatings based on pectin to be applied in the protection of fresh food, improving organoleptic and nutritional characteristics and extending shelf-life. The films produced were transparent, homogenous and showcased good physical properties. Transparency of the films was influenced by plasticizer and surfactant concentration due to the dilution effect of Poly Ethylene Glycol (PEG) and TWEEN80.Cinnamon EO enriched films were slightly yellowish in colour as compared with clove EO enriched film and control (without any incorporation of EO). Mechanical properties were best demonstrated by the films made of essential oils than control. Films studied were completely biodegradable. Despite the fact that recent studies have reported significant improvements in specific applications of these formulations, there is still a large amount of research to be performed since some of the most remarkable improvements are not yet experimentally attained or reproducible at large scale. In general terms, there is still a need for a better understanding of the composition-structure-processing-properties relationships in active films based on pectin for food packaging, both at the laboratory and industrial scale. Moreover, since many of the studies related to this issue have been carried out using some basic pectin already in the market, there is still a lot of room for variation and maturation in the development of active coatings for application in food packaging. Essential oils can be exploited further as an elixir for antimicrobial effects in food industry.

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