chapter ten

Natural nontoxic insect repellent packaging materials

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Contents

10.1 Introduction

Materials such as polymers, paper or paperboard, textiles, and metal foils are all used in producing packaging material for packaging foods. A major drawback of such packaging materials is that pests leading to infestation of the packaged foods can penetrate them. The degree of pest infestation of packaged foods depends upon the pest species involved, the time of exposure to invading pests, and the prevailing environmental conditions.

In many instances, synthetic pesticides have been the only effective measure available for controlling pest infestation of stored foods. However, most synthetic pesticides have significant adverse effects on humans and the environment, and accordingly, their use has been substantially excluded from packaged foodstuffs.

The use of nontoxic crude extracts of neem or turmeric, capable of repelling insects, to protect packaged foodstuffs from insect infestation was proposed and a patent was granted (Navarro et al., 1998). This approach was developed based on the assumption that many plants inherently produce various chemicals that protect them against insects, and extracts from these plants may affect the metabolism of insect species other than those attacking the plant from which the chemical was derived. The search for naturally occurring substances is an important approach for the development of an ecologically sound, plant protection strategy suitable for adoption by the food industry.

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In this chapter the authors delineated effective substances of plant extracts that could be used to develop improved food packaging materials that are insect repellent and can prevent insect penetration into the packages. The insect-repelling substances that were developed are nontoxic to humans, environmentally compatible, and suitable for protecting food and the like from insect infestation.

10.2 *Insect damage and types of penetration into packaging materials*

10.2.1 *Insects that Cause damage to food packages*

There are two types of insects that attack packaged products: penetrators, insects that can bore holes through packaging materials, and invaders, insects that enter packages through existing holes, such as folds and seams and air vents (Highland, 1984; Newton, 1988). *Sitophilus* spp., *Rhyzopertha dominica* (F.), *Plodia interpunctella* (Hubner), *Lasioderma serricorne* (F.), and *Stegobium paniceum* (L.) are some of the stored product insects that are capable of penetrating food packaging. However, *Tribolium* spp., *Cryptolestes ferrugineus* (Stephens), and *Oryzaephilus* spp. cannot penetrate intact packages but enter through existing holes in the package (Highland, 1991).

Beetles and moths comprise the majority of stored grain insect pests. Ambient temperature and moisture content of the commodity have a major influence on the rate of insect development. The rate of beetle development is generally more affected by temperature than by commodity moisture content (Hagstrum and Milliken, 1988). Moth development is more dependent on ambient humidity above the grain and moisture in the grain.

Stored product insects are mainly of tropical and subtropical origin and have spread to temperate areas via international trade. Because insects cannot control their body temperature, their rates of development and reproduction increase with rising temperature. Consequently, most of them become inactive at low temperatures (10 to 15°C) and will die after prolonged periods at very low temperatures (0 to 5°C). Most species are unable to hibernate or enter an inactive phase termed diapause, though some such as *P. interpunctella* and *Trogoderma granarium* do hibernate.

For each insect species there is a minimum and maximum temperature at which they are able to develop (at certain low temperatures, oviposition and larval growth ceases; at specific high temperatures, egg sterility occurs and mortality increases). Conversely, there is a temperature range at which oviposition and insect development are optimal. The lower and upper limits and optimal temperatures of most of the important stored product species have been studied and are well known.

Survival of *Tribolium castaneum* from egg to adult is highest between 25 and 27.5°C and decreases rapidly below and above this temperature (Howe, 1960). Temperatures below 15°C generally arrest all insect development sufficiently to prevent damage, though not to cause mortality. For most insects, sustained temperatures above 40°C and below 5°C are lethal.

Each stored product pest species has different food requirements. Studies have been made to identify the nutritional requirements of different species in order to breed them on artificial diets. Clearly these requirements affect the ability of insects to develop on different stored products and their ability to compete with other species. Consequently, for each stored product, there is a range of insect pests.

All stored product insects are negatively phototrophic, which means that they stay away from sunlight. Because of their phototrophic behavior, they are generally not visible to the casual observer.

10.2.1.1 Order coleoptera (beetles)

Adults are typified by forewings modified to form rigid wing covers (elytra) that meet along the back and generally cover the abdomen. The hind wings are membranous, folded beneath the elytra, and used for flying. The beetles have biting mouth parts, and the upper plate of the first segment of the thorax covers the other segments to form a shield (pronotum). These are the tanks of the insect world. Metamorphosis is complete, and larvae may be active with well-developed or grub-like and sessile legs. Of about 250,000 species known to man, more than 200 are associated with stored products, but only a few constitute the major stored product pest species.

Stored product beetles are typified by being small enough to be able to penetrate between units of food products and live in the airspaces. Their armored bodies make them very adaptable to this environment. Some are primary pests capable of entering undamaged products. Generally their larvae are soft and sessile. Others are secondary pests that feed on broken or damaged grains, chaff, and dust, but cannot penetrate sound grain. Their larvae are active and well protected (wiry). A third group is scavengers of dead insects and mold feeders, but they do not attack grain kernels.

The most common beetles that penetrate flexible packages are *R. dominica*, *Sitophilus oryzae*, *L. serricorne*, and *S. paniceum*. The most common invaders are *T. castaneum*, *Tribolium confusum*, *Cryptolestes* spp., and *Oryzaephilus surinamensis*.

10.2.1.2 Order lepidoptera (moths)

The adults of Lepidoptera are typified by fragile wings covered with scales, which often have delicate colorings and markings. The head has sucking mouth parts (proboscis) or mouth parts are absent and long filiform antennae. Generally the adults are short-lived. The larvae are caterpillars with biting mouth parts that inflict damage. Young larvae are excellent invaders, and old larvae have the capability to penetrate packaging laminates.

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Most common moths that invade or penetrate flexible packages are larvae of *Ephestia cautella* and *P. interpunctella*.

10.2.2 Insect damage to plastic packages and types of damage

Insects infesting stored foods are one of the most common household problems. The many different kinds of insects that invade stored dried foods are often referred to as pantry pests. Insects contaminate more food than they consume, and most people find the contaminated products unfit for consumption. Stored food insect pests are often discovered when they leave an infested food to crawl or fly about the premises. They often accumulate in pots, pans, or dishes or on window sills.

Nearly all dried food products are susceptible to insect infestation; examples include cereals and their products (flour, cake mix, cornmeal, rice, spaghetti, crackers, and cookies), pulses, nuts, cocoa and coffee beans, confectionary-like chocolate, dried fruits, spices, powdered milk, seeds, and cured meats. Nonfood items that may be infested include all types of feed, dry pet food, ornamental seed and dried plant displays, ornamental corn, dried flowers and potpourri, garden seeds, and rodent baits.

The quality hazards to stored food due to insects are (1) package perforation (Figure 10.1), (2) devouring of the agricultural product (Figure 10.2), (3) substantial damage to the product, (4) contamination of the product (Figure 10.3), and (5) esthetical objections.

10.2.3 Propensity of damage by insects

A stored food product may become infested at the processing plant or warehouse, in transit, at the store, or in the consumer's home. Most of the insects attacking stored foods are also pests of stored grain or other commodities and may be relatively abundant outdoors. Food products that are left undisturbed on the shelves for long periods are particularly susceptible to infestation. However, foods of any age can become infested.

Stored food insects are capable of invading the food package or penetrating unopened paper, cardboard, and plastic-, foil-, or cellophane-wrapped packages. They may chew their way into packages or crawl in through folds and seams. Insects within an infested package begin multiplying and can spread to other stored foods or food debris that has accumulated in corners, cracks, and crevices, and eventually the entire cupboard.

Figure 10.1 A package damaged by the cigarette beetle, *L. serricorne*.

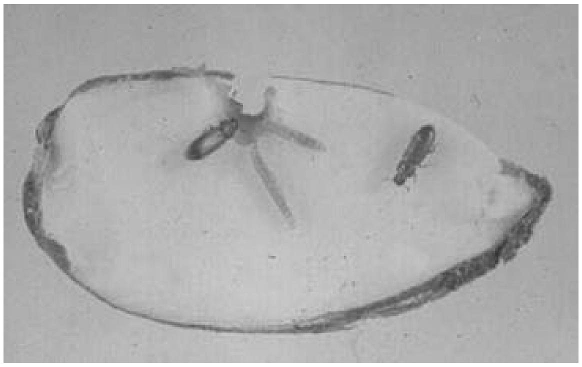

Figure 10.2 Product damaged by *T. castaneum*.

All stages (egg, larva, pupa, and adult) may be present simultaneously in infested products.

Most food infestations of storage pests maintain themselves on spills in the crevices of cupboards and drawers or in opened packages of food stored for long periods.

Stored food insects have been a major factor in food losses and the most difficult to combat. Insects are almost always present in food stores. The insects originate either from residual infestations hidden within the storage structural materials or from stored product insects that lay their eggs on the product.

The insect pests of stored grain have environmental requirements that greatly affect their abundance and consequently their potential danger for causing damage. The most important environmental factors are temperature

Figure 10.3 Chocolate damaged by tropical warehouse moth larvae.

and moisture (climate), food requirements, and competition with other living organisms.

For stored product pests the influence of external climate is reduced by the fact that the "climates" within a warehouse or grain storage silo may be very different from that outside. Thus, insects that are unable to withstand outdoor winter conditions in temperate climates may be able to survive and develop in relatively warm grain masses in warehouses, storages, or the heated buildings of food processing factories, even in relatively cool or cold climates.

10.2.4 Resistance of plastic films to insect penetration

Several factors need to be considered in developing a package for a product. The food package needs to be designed not only to maintain the quality of the product but also to attract the consumer. Cheap packaging can lead to infestation by insects and microorganisms. Food manufacturers are aware that if a consumer finds an insect in a package, it can cause a lasting and often irreversible impression, ultimately resulting in the loss of that customer. Although most insect pests under the category of invaders enter packages through existing openings that are a result of poor seals, the penetrators have the capacity to enter almost all existing packaging films. According to Mullen (1997), most infestations are the result of invasion through seams and improper closures. Minute openings in packaging materials due to sealing failures during manufacture or handling attract pests and are often large enough to permit entry of first instars of most stored product insects.

Most of the packaging materials for fresh fruits and vegetables as well as stored dry and semidry food commodities are cellulose or plastic or a combination of plastic and paper. Some plastic materials are rigid while others are flexible films.

Flexible packaging films vary in resistance to penetration. Bags made of laminated foil and paper were found to be more resistant than bags made of cellophane, polyethylene, multilayer paper, and fiberboard box (Kvenberg, 1975). The study was based on eight stored product insects, where the adult and larvae of the cadelle, *Tenebrioides mauritanicus*, and the cigarette beetle, *L. serricorne*, penetrated the test material.

In a method developed by Highland and Wilson (1981) to test insect penetration of flexible packaging films, 18 types of polymer films and fibers, used by 25 different companies, were exposed to adults of the lesser grain borer, *R. dominica*. Polyurethane and polyester films were most resistant. Polypropylene and fluorinated carbon polymer films varied in resistance to penetration. All Kraft papers, plasticized polyvinyl chloride (PVC), cellulose, polyvinyl alcohol, Saran, polyethylene, and ethyl vinyl acetate copolymer films were the most susceptible to penetration.

In a recent study Riudavets and Salas (2006) assessed the penetration ability of *R. dominica*, *S. oryzae*, and *O. surinamensis* to different plastic films. They studied polypropylene, polyethylene, and polyester, and a multilayer film (paper, polyethylene, aluminum, and polyethylene). Damages observed in each material were evaluated under binoculars. All three species were able to penetrate the films tested. *R. dominica* was the species with the highest penetration ability. The intensity of damage produced by all three species was higher in polyethylene than in polypropylene and polyester. In the multilayer film, *R. dominica* showed a similar penetration ability independently on the film side exposed to the insect, since the aluminum foil was the layer acting as a barrier to avoid the penetration of this species (Figure 10.4 to Figure 10.6).

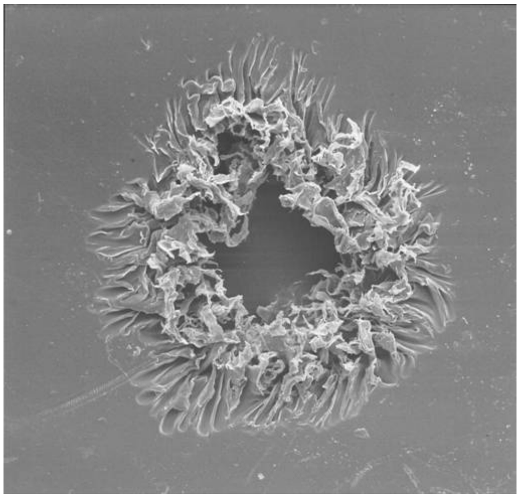

Figure 10.4 Typical penetration of *R. dominica* adults through polyethylene laminates under microscope.

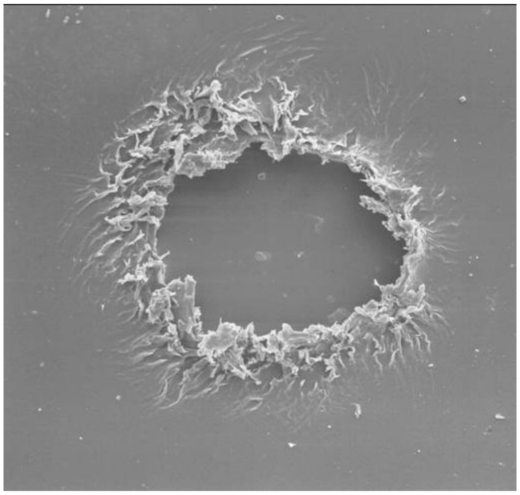

Figure 10.5 Typical penetration of *R. dominica* adults through polypropylene laminates under microscope.

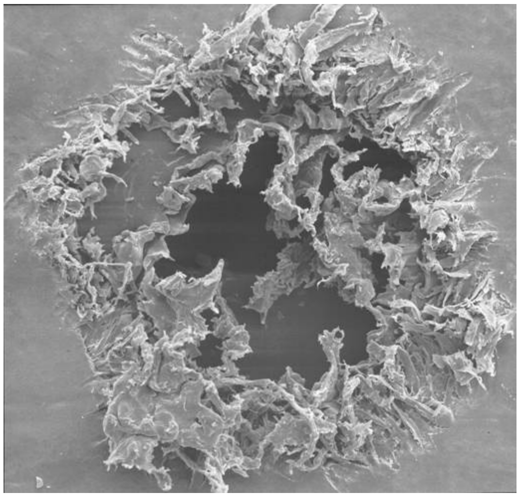

Figure 10.6 Typical penetration of *R. dominica* adults through multilayer paper–aluminum laminates under microscope.

10.2.5 Economic impact of insect damage to packaging material

Packaging of food products has an important role as a barrier for insect pest, and plastics films are among the most effective packaging materials. World expenses in packaging materials and equipment reach 240,000 million euros per year (Hanlon et al., 2000). Cardboard, paper, and plastic films are the most important materials used. However, during recent years there has been an increase in the use of plastic and a decrease in the use of paper and cardboard. Today there is also an increase in the use of new materials, such as bioplastics. World consumption of plastic films for packaging is approximately 100 million tons, with more than 30 different types of materials. The most common plastic materials used are polyethylene, polypropylene, and polyester. To combine the characteristics of these plastic films with other materials such as aluminum or cardboard, complex packaging materials and multilayer plastic films have been developed, and their use is increasing.

Food products are packaged to protect them against external attacks due to handling, hits, or other mechanical actions or against the effects of macro- and microorganisms. Among macroorganisms, insects are one of the most important affecting the packaged final products. In the marketing of durable raw and processed agricultural products, their quality and wholesomeness must be maintained from the time they are packaged until they reach the consumer. This is achieved by use of different kinds of packages whose purposes are to offer convenience, to render them attractive for promoting their sale, and above all to provide a physical barrier against external adverse influences, including the ingress of insect pests.

Control of insect pests of durable food commodities relies heavily upon hygiene and, to a limited extent, on the use of fumigants on raw materials, but almost only on hygiene and physical means after the food commodities are processed. Application of a selective and limited number of contact insecticides is permitted in the food processing plants, but their use directly on processed food is not permitted due to their toxicity. Therefore, the possibility of infestation and contamination of the commodity by insect pests during the postmanufacturing stages remains a major problem.

The introduction of insect pest management techniques into the food industry enables the integration of several chemicals and nonchemical preventative and control measures in the storage and processing plant to prevent contamination by insects of the commodity before it is packaged.

The extensive use of packaging in modern food distribution systems provides a potentially effective tool in the management of insect pests. New and modified food handling procedures, increasingly stringent sanitation standards, and increasing international trade impose a need for systems that will protect food from infestation from the time it is packaged until the package is opened by the consumer. Knowledgeable selection of packaging materials can help produce packages that resist infestation (Highland, 1991).

10.3 Natural substances for protection of food packages from insects

10.3.1 Traditional insect control practices

In today's food industry, one of the aims is to eliminate the use of noxious pesticides against food-infesting pests and to replace them with natural, nontoxic, environmentally friendly compounds.

The utilization of plant materials to protect field crops and stored commodities against insect attack has a long history. Many of the plant species concerned have also been used in traditional medicine by local communities and have been collected from the field or specifically cultivated for these purposes. Leaves, roots, twigs, and flowers have been admixed, as protectants, with various commodities in different parts of the world, particularly India, China, and Africa.

The neem tree (*Azardirachta indica*) is native to the Indo-Pakistan subcontinent and grows abundantly in this region. Neem trees are plentiful in South Asia and other developing countries where farmers are aware of its properties. In rural India an age-old practice is to mix dried neem leaves and turmeric powder with stored grain to keep away insects. To combat insect pests, food grain stored in gunny bags is mixed with dried neem leaves. Those who store wheat in mud bins rub fresh neem leaves on the inside walls of the bins. In the districts of Nawabshah and Khairpur, in Pakistan, Palli is commonly used for storage. Some farmers plaster its walls and top with mud having crushed neem leaves. In Rahim Yar Khan District, neem extract is sprinkled on the wheat straw packed at the bottom of Palli before pouring in the grain (InPho Newsletter, 2006)

Turmeric, *Curcuma longa* L., is a tropical herb of the Zingiberaceae family indigenous to southern Asia. The aromatic yellow powder from its mature rhizomes was used in Asian countries for many centuries as a yellow vegetable table dye for silks and cottons. It is still used in foods as a condiment, particularly as an essential ingredient of curry powder, in medicine as a stomachic, carminative, anthelmintic, laxative, and cure for liver ailment, and also as an ant repellent in India (Su et al., 1982).

10.3.2 Botanical insect repellent extracts

Over the last 30 years, intensive and pioneering research has been conducted on various plant materials, including neem and its derivatives, turmeric, etc. As a consequence, the potential role of botanicals in the fields of antifeedants, repellents, toxicants, and growth regulators has been established (Islam, 1986). Antifeedants inhibit insect boring, while repellents prevent insects from invading food packages through even the tiniest of openings, or even to approach packages containing repellent substances. Repellents and antifeedants act at some distance from the commodity (Gebbinck, 1999) and are not considered insecticides.

Numerous plant substances have been isolated and tested on stored product insects, and among these, azadirachtin (extracted from neem) appears to be particularly promising as a potential stored product protectant (Subramanyam and Hagstrum, 1996). Neem is listed as an approved pesticide for organic agriculture in the U.S. The active ingredient in neem, azadirachtin may be responsible for its insecticidal activity. However, neem is not an approved product in the U.S. or Europe for use in or in contact with food. Mixing neem extracts with other materials can boost their power.

Among these so-called promoters are sesame oil, pyrethrums, and piperonyl butoxide (National Research Council, 1992).

Pyrethrum is extracted from *Chrisanthamum cinerariafolium*. Pyrethrum and pyrethrins synergized with piperonyl butoxide were approved in the U.S. for use as an insect repellent on the outer layers of food packages or with adhesives (Highland, 1991). The repellency of pyrethrins was the primary mode of action against insect penetration and invasion (Laudani and Davis, 1955).

Methyl salicylate, an insect repellent, is registered in the U.S. for use in food packaging to control stored product insects (Radwan and Allin, 1997).

Insect repellents are used to prevent insects from entering packages by modifying their behavior (Highland, 1984; Mullen, 1994; Watson and Barson, 1996; Mullen and Mowery, 2000). DEET, neem, and protein-enriched pea flour are repellent to many stored product insects when tested by exposure on filter paper or in preference chambers (Khan and Wohlgemuth, 1980; Xie et al., 1995; Fields et al., 2001). Rajab (1988) has authored a number of publications describing the isolation identification and structure of limonoid insect antifeedants.

Whalon and Malloy (1998) used a mixture of plant extracts from eucalyptus, orange peel, cinnamon, neem, turmeric, and sweet flag to apply to lacquers on food packages and claimed that the coatings repelled Indian meal moths from invading the packages, over an 8-week period.

According to the FAO Compendium on Post Harvest Operations (InPho Newsletter, 2006), neem and pyrethrum are 2 of 130 plants known to control insects that have been used commercially to date. Both neem and pyrethrum are unstable and could not be used for long-term storage of grain (Cox, 2002).

Cox (2002) and Hou et al. (2004) reviewed the use of natural insect repellents and deterrents on stored food products. These reviews as well as various previous papers have pointed out that stored grain insect pests can be controlled using nontoxic plant-based repellent and antifeedant substances to prevent the insects from coming in contact with the food, not to kill them.

According to these reviews, although there has been considerable interest recently in the use of plant-derived repellents (Mordue and Blackwell, 1993; Ignatowicz and Wesolowska, 1994; Xie et al., 1995; Lui and Ho, 1999; Obeng-Ofori et al., 2000; Weaver and Subramanyam, 2000), no successful use was made of repellents to protect stored grain from insect attack on a commercial scale. Extracts from *Ocimum* spp. (Labiatae) have been shown to be repellent to *R. dominica*, *T. castaneum*, *S. paniceum*, and all three species of *Sitophilus* (Desphande and Tipnis, 1974; Bekele et al., 1996; Obeng-Ofori and Reichmuth, 1997). If suitable plant chemical repellents are identified, they could be used to provide protective bands around grain bulks or they could be incorporated into packaging materials, such as sacking and paper, to inhibit invasion by pests. Methyl salicylate has recently received regulatory approval in the U.S. for use as a repellent in food packaging (Mullen and Pedersen, 2000).

Wild angelic produces bisabolangelone, which is a good feeding deterrent for *Sitophilus granarius*, *T. confusum*, and *T. granarium*, and terpenoid lactones, secondary metabolites of many plants, have shown antifeedant properties. They could therefore find use in preventing insect damage in stored grain (Cox, 2002). Glucosinolates found mostly in the Cruciferae plant family are toxic to livestock but not to humans; they repel cabbage root fly. Ash tree foliage contains substances that can deter gypsy moth larvae. According to Cox (2002), to date, no tests have been carried out using oviposition or antifeedant inhibitors to repel grain storage insects.

Cox (2002) described a number of chemicals coined semiochemicals, produced by insects, which can act as insect repellents. When aphids are attacked by predators, they release a pheromone hormone, sesquiterpane hydrocarbon (E), beta farnasene, which causes dispersal of the aphids. However he stated that the production of repellents from plants would be less expensive than their synthesis from complex semiochemicals.

AU: Please check. Is sesquiterpane… the hormone, or are these three different things?

10.3.3 *Repellency and antifeedant tests on neem, pyrethrum, and turmeric oil*

There are several publications in the literature on the biological activity of turmeric rhizome extracts in repelling and penetration prevention of storage insects.

Su et al. (1982) isolated two compounds from *C. longa* and identified their spectral characteristics as *ar*-turmerone and turmerone. They gave class IV and class III repellency, respectively, to *T. castaneum*, after 8 weeks of study. Although *ar*-turmerone and turmerone evoked repellency, no mention was made of the ability of these compounds to prevent penetration or perforation by the insects. The importance of the concentration of the two compounds in the crude extracts was not investigated. Turmerone was reported to be unstable upon exposure to air and slowly aromatizes to *ar*-turmerone (Alexander and Rao, 1973). Turmeric oil extract at 680 μg/cm^2 applied to filter paper produced class IV (67%) repellency against *T. castaneum* 4 weeks after application (Jilani and Su, 1983). Studies by Jilani et al. (1988) indicated that turmeric oil, sweetflag oil, and neem oil not only repel *T. castaneum* (the red flour beetle), but also interfere with its normal reproduction and development. Jilani and Saxena (1990) reported that turmeric oil and sweetflag oil were significantly more repellent during the first 2 weeks than neem oil and neem-based insecticides, but thereafter, their repellency decreased more rapidly than that of neem oil and neem-based insecticides. *R. dominica* adults made significantly fewer and smaller punctures in filter paper discs (7 cm diameter) treated with the test materials than in the control discs. The paper described the extraction process to obtain oils from turmeric and sweetflag rhizomes, but a complete analysis of the extracts was not given.

May be Jilani etal 1988

Tripathi et al. (2002) found that *C. longa* oil reduced oviposition of *T. castaneum* by 72% using filter paper. They also found 81% antifeedant activity against *R. dominica*, *S. oryzae*, and *T. castaneum* at 40 μg/cm². In all the works cited above, no attempt was made to uncover a specific fraction or compound capable of repelling or inhibiting insect puncturing.

Navarro et al. (1998) tested the repellency and penetration prevention (antifeedant effect) of neem extracts, natural pyrethrum extracts (50%), and turmeric petrol ether extract on papers, against *R. dominica* and *T. castaneum* adults. The neem extracts included NeemAzal T/S (1% azadirachtin AI, other related limonoids, and neem oil), azadirachtin (30% purity). and neem oil.

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Repellency tests with both insects showed that the most effective neem extract was NeemAzal T/S. It was tested at a dose of 50 μg/cm² and resulted as class IV (of five classes). Azadirachtin, at the same dose, was substantially less effective (class II). Neem oil at a dose of 800 μg/cm² (class III) had the same activity as turmeric extract (at the same dose), both less active than NeemAzal T/S. The repellent effect of pyrethrum could not be detected, as it is actually an insecticide. At the low dose of 5 μg/cm², the *T. castaneum* insects showed substantial repellency (class III), but the *R. dominica* insects were moribund. For both insect species, there was no significant synergistic effect of piperonyl butoxide (PBO) on repellency in any of the tested extracts. The pyrethrum results were in accordance with other literature reported results, for example, McDonald et al. (1970), who reported that for a mixture of pyrethrum and PBO the integrated repellency effect is the sum of the two individual repellencies.

The penetration prevention (antifeedant effect on penetration) was tested in a way similar to that of the nonchoice test (see Section 10.4.2.3), with application of different dosages and testing after several time intervals, up to 75 days from application on the papers. Exposure times to *R. dominica* adults were 24 and 48 hours.

The penetration prevention results of neem extracts showed a protective effect in a dose-dependent manner. NeemAzal T/S at 31 to 500 μg/cm² reduced significantly the penetration at all the dosages for up to 75 days after treatment. It prevented any penetration at the highest dosage after 60 days. Neem oil and azadirachtin showed penetration prevention efficacy for short periods and were found to be less effective than NeemAzal T/S. Neem oil application resulted in at least partial penetration prevention at all the tested dosage levels of 160 to 2560 μg/cm² and up to 30 days after application. The complete residual (no penetration by insects) effect of neem oil was only obtained at the highest dosage after a 1-day delay. However, the tests showed that dosages of 1280 and 2560 μg/cm² gave significant protection until 30 days after treatment. Azadirachtin, at dosages of 31 to 500μg/cm², was tested 1 day after application on paper. Although penetration was significantly reduced, it was still apparent after confined exposure for 48 hours.

Pyrethrum extract at dosages of 2.5 to 160 μg/cm² after 1 and 15 days' time delay showed a reduced penetration at all the dosages except 2.5 and 5 μg/cm². High dosages that prevented all penetration also resulted in insect mortality. Turmeric extract showed a protective effect of up to 75 days using high dosages (1280 and 2560 μg/cm²) (see Section 10.4.2).

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Comparing the results of Navarro et al. (1998) to those reported in the literature (Jilani and Su, 1983; Jilani and Saxena, 1990; Malik and Naqvi, 1984) shows there is agreement about the basic findings that neem extracts and turmeric extracts have substantial repellency and antifeedant effects against storing insects. On the other hand, there are almost no records in the literature on the long-term residual penetration prevention effectiveness of those extracts, while the active compounds slowly degrade. In this context, the results of Navarro et al. (1998) showed that the most promising material tested was NeemAzal T/S, which gave complete protection at the highest concentration for 60 to 75 days. In storage conditions degradation processes caused by sunlight are less of a concern. This is supported by the observations of other investigators (National Research Council, 1992; Daniel and Smith, 1990; Makanjuola, 1989; Mordue and Blackwell, 1993).

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The diversity of results regarding the neem extracts may be explained by:

1. Differences in the experimental settings and test conditions.
2. Isman et al. (1990) reported that azadirachtin content varied widely between different neem oil samples. They showed that there is a clear trend in which bioactivity of neem oil is related to its azadirachtin content.
3. Recognition that azadirachtin is not the only neem component contributing to the repelling and penetration prevention efficacies of neem extracts. The combined effect of several components imparts their characteristic activity. For the neem extracts Isman et al. (1990) compared the bioactivity test results of pure azadirachtin to oil spiked with azadirachtin and showed that the bioactivity of azadirachtin is enhanced by the presence of the oil. They concluded that other potentially active constituents are present in these oils that can act as synergists to activate azadirachtin. They also pointed to the possibility that a botanical preparation may enhance the stability of azadirachtin and other active ingredients. Mordue and Blackwell (1993) reported that limonoid mixtures may be more effective than azadirachtin alone, that neem oil has insecticidal properties by itself, unrelated to its azadirachtin content, and that crude formulations may contain volatile repellent components. These results are in accordance with those of Navarro et al. (1998). These facts led Navarro et al. (1998) to the conclusion that the specific effectiveness of any particular neem oil or turmeric extract should be tested and confirmed before being applied in practice.

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10.3.4 *Insecticidal activity and toxicity of natural essential oils and their derivatives*

Besides the above plant extracts, which can be used as insect repellents or antifeedants, a number of plant extracts have been used as insecticides. Champon (2000) used mustard oil, lemon extract, and vegetable oil and others as a soil treatment, insecticide, fumigant, and structural fumigant. Coats et al. (2001) state that monoterpinoids, found in essential oils of mints, pine, cedar, citrus, eucalyptus, and spice compounds in this class, are commercially available to control fleas on pets and carpets, insects on house plants, and fumigation in honeybee colonies. Rajamannan and Okioga (1997) developed a pesticide from the plant *Tagetes minuta* that is claimed to be able to kill nematodes, wire worms, and insects. Hsu et al. (2001) claimed garlic oil extract combined with an essential oil has insecticidal effects on a number of insect pests as well as a fungicidal effect. Reeves and Shanker (1970) reported that a crude extract of garlic caused 100% mortality in five species of *Culex* and *Aedes* mosquito larvae when used in doses of 12 ppm or more. Borzatta et al. (2001) describes a process for the synthesis of alkylbenzodioxole derivatives from essential oils, especially sassafras oil, which contains 75% of such derivatives, which can be used to prepare insecticides such as piperonyl butoxide.

Although plant source repellents and antifeedants are not intended to kill the insects, curcuma oil, for example, if used at concentrations of 2000 $\mu g/cm^2$, can become nematocidal. Lee et al. (2001) found that concentrations of 1000 and 500 ppm *ar*-turmerone caused 100 and 64% mortality, respectively, in *Nilaparvata lugens* female adults. Against *Myzus persicae* female adults and *Spodoptera litura* larvae, *ar*-turmerone was insecticidal at 2000 ppm. At a concentration of 2.1 $\mu g/cm^2$ *ar*-turmerone was almost ineffective against *S. oryzae*, *Callosobruchus chinensis*, and *L. serricorne* as well as larva of *P. interpunctella*.

10.4 Development of a natural nontoxic insect repellent for insect packaging materials

10.4.1 Background

Turmeric oleoresins, or turmeric extractives, are obtained by solvent extraction of the turmeric (*C. longa* L.) dried powdered rhizome. Depending on the extraction solvent, the extraction proccess, and the turmeric type and cultivar, the oleoresin contains various proportions of curcuminoids (coloring matter), volatile essential oil (imparts the flavor to the product), and nonvolatile fatty and resinous materials. Different polar and nonpolar solvents can be used as extractors. For example, 21 CFR 73.615 has allowed the following solvents for the extraction: acetone, ethyl alcohol, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene. Since the curcuminoids are the main compounds of commercial interest in turmeric rhizome and the turmeric oleoresin is valued mainly for its curcminoids content, a polar solvent is usually prefered for the rhizome extraction. The commercial methods of extraction will vary by manufacturer and are proprietary information.

Turmeric essential oil is obtained by distillation or by CO_2 supercritical fluid extraction (Gopalan et al., 2000) of the powdered rhizome. Steam distillation appears to be the main commercial process. Commercial turmeric essential oil is also called crude oil. The essential oil can also be separated from oleoresins by extraction with hexane (Jayaprakasha et al., 2001) or other lipophilic solvents (as is sometimes done in the separation process of curcuminoids from oleoresins) and is called pure oil. Such separation and the liquid extraction of the rizhome may lead to the loss of volatile compounds of the oil during evaporation of the solvent. Furthermore, if methyl or ethyl alcohol is used as the solvent, artifacts may be produced by reactions of esterification or transesterification, etherification, and acetal formation. The solid residue that remains after the total removal of the essential oil from turmeric oleoresin by liquid extraction is defined as a solid residue of turmeric oleoresin (TOSR).

Navarro et al. (2005), in U.S. Patent 20050208157-A1 and European Patent Application 04101309.5, described the biological activity in repelling stored product insects and the chemical composition of turmeric oil, turmeric oleoresins, enriched turmeric oil fractions, and solid residue of turmeric oleoresin. The following section describes their main findings regarding the development of a natural, nontoxic insect repellent for insect packaging materials. Their work on turmeric oil fractions was carried out using crude essential oils from known sources that had similar biological activity.

Two main repelling effects — repellency and antifeedancy — were responsible for the repelling activity. Navarro et al. (2005) showed that several compounds are responsible for this biological activity. These effects are composed of partial contributions of several components, each with its own specific activity. Among these are components that have reverse effects — attraction or feedant. It was also established that components present at low concentrations could contribute substantially to the repellency and antifeedant effect. Their findings were the basis of two patents on pest-impervious packaging materials (Navarro et al., 1998, 2005).

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10.4.2 Bioassays

10.4.2.1 Insect repellency and penetration prevention bioassays

Three types of fast semiquantitative bioassays — repellency, nonchoice, and choice tests — were developed to evaluate the repellent and antifeedant effects of plant extracts on stored food insects.

In these tests, papers were impregnated with test samples of plant extracts at varying concentrations and representative insects were exposed to them. The effects on the insects were found to be nonlinear with concentration. It was concluded that the tests should be performed at a concentration range to which the insects would be most sensitive. At relatively high concentrations of the oils the biological activity could reach saturation and adversely affect insect sensitivity.

10.4.2.2 Repellency test

The repellency bioassay was devised to test the efficacy of preventing insects from moving onto paper treated with turmeric oil or other biologically active compounds or mixtures.

The propensity of the tested sample to repel insects was determined against adults of the red flour beetle, *T. castaneum*, using the methods by Laudani et al. (1955) and McDonald et al. (1970). Accordingly, filter paper strips were treated with acetone solutions of samples, usually at 50 μg/cm^2 (or any other predetermined concentration). The acetone was then completely evaporated. Two untreated strips were attached lengthwise, edge to edge, on either side of the treated paper strip. Four glass rings, 2.5 cm high and 6.4 cm i.d., were placed over the joined edges of the papers, on each side of the treated strips, where one half of each ring was found on the treated paper and the other half was found placed over the nontreated control paper. Ten adults of each species were placed into each glass ring. The numbers of insects found on the treated and untreated paper halves were recorded at 10 fixed times, from 1 to 24 hours after exposure. The average count of all four ring replicates, each containing 10 reading periods, was converted to percent repellency values (Navarro et al., 2005) (Figure 10.7).

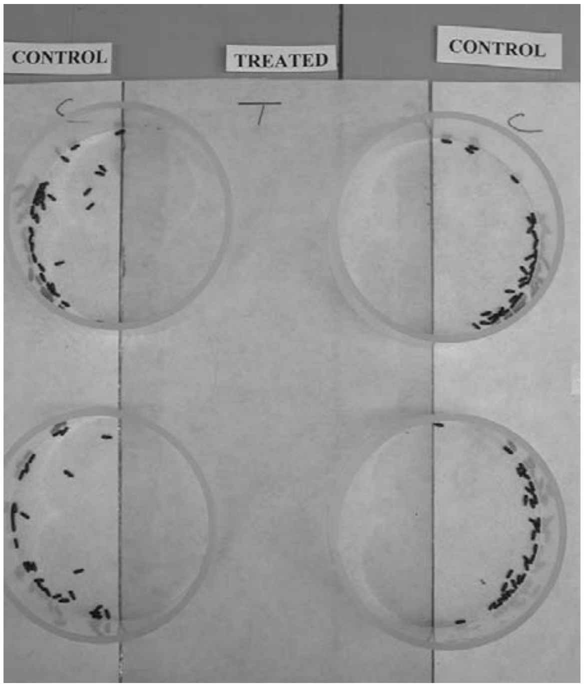

Figure 10.7 Adults of *T. castaneum* concentrated in the nontreated arena in a repellency test.

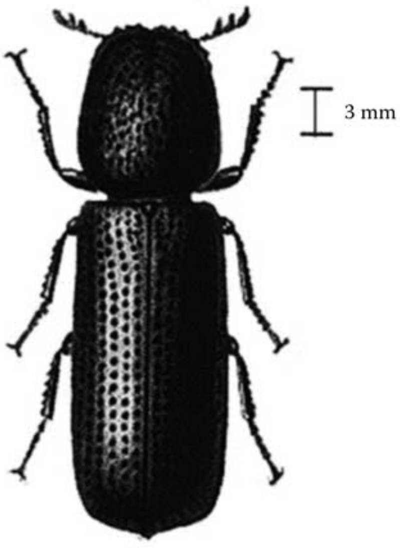

Figure 10.8 Adult *R. dominica* used in penetration tests.

10.4.2.3 *Nonchoice bioassay test for penetration*

Navarro et al. (1998, 2005) tested the efficacy of preventing adults of the lesser grain borer, *R. dominica* (Figure 10.8), from boring holes into white office printer paper discs treated with turmeric extracts. The average number of holes in the control compared to the average number of holes in the treated discs was expressed as penetration prevention efficacy (PPE).

In preparing the test, the paper discs were treated with acetone solutions of sample extracts at dosages of 50 and 640 μg/cm^2 in the routine evaluation tests (other dosages of up to 2560 μg/cm^2 were also tested in special experiments) and used in the bioassay the next day. The tested paper discs and the controls were pressed with a supportive piece of wire mesh between two open-ended glass cylinders (Figure 10.9). Ten insects were then placed inside each top cylinder. The tests were carried out in 10 replicates, usually for 24 hours, in the dark at 27°C and 65% RH (Navarro et al., 2005).

10.4.2.4 *Choice bioassay test for penetration*

Navarro et al. (2005) used the same experimental device (Figure 10.9) and conditions in the choice test as used in the nonchoice test. However, in the choice test, the paper discs were divided in half; one side was impregnated with the sample under test dissolved in acetone, while the other half treated with acetone alone was used as a control. In this test only a dosage of 50 μg/cm^2 of the test sample was applied on the paper. The test insects were entrapped on the paper and could choose to perforate the control, the impregnated part of the paper, or the border between the two halves of a

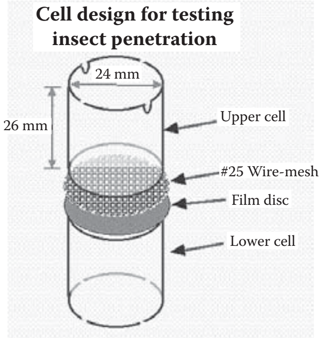

Figure 10.9 Device used in penetration tests.

AU: Please provide art for Fig. 10.10

Figure 10.10 Penetration holes made by *R. dominica* adults on the nontreated area in the choice test for penetration.

disc, or not to bore holes anywhere on the two disc halves (Figure 10.10). At the end of each exposure period, the number of perforations appearing in the two parts of the disc were counted, and a comparative analysis was performed using the Student's t test for residual effect, and the differences between control and dosages applied were determined using Dunnet's test (Anon., 1989).

10.4.2.5 Interpretation of results

The repellency test directly measures the ability of the tested sample to repel insects, while the other two bioassays revealed a combined effect of the antifeedant components and the repelling components in the tested sample on the insects. The choice test indicates the combined additive effects of both antifeedant and repelling components to prevent perforation, which differs from the nonchoice test where the repelling components enhance perforation and the antifeedant components prevent perforation. From the data of all three bioassays a conclusion could be drawn as to the antifeedant effect of the sample. For example, in the choice test, if the insects did not perforate the impregnated part of the paper but only the untreated part, and the results of repellency showed that the tested sample had low repellent efficiency, it was then concluded that the sample contained antifeedant components. When the sample had a high repellency, the choice test showed a high efficiency even in the presence of low antifeedant effect. In such a case, the nonchoice test would give a negative efficacy result. This negative efficacy could be reduced only when higher antifeedant components were present in the sample. When the effect of antifeedancy was higher than repellency, a positive efficacy value was obtained.

10.4.2.6 Tests for insect penetration of packaging films

The literature describes a number of methods devised for determining insect penetration. Higland and Wilson (1981) described a test device for penetration of *R. dominica* that consists of five pieces of machine assembly made of aluminum tubing. Wohlgemuth (1979) used for penetration tests a device consisting of several aluminum plates, the test foil, and a cover. Gerhardt and Lindgren (1954) studied two penetration test methods. One consisted of test bags exposed to insects. The second method consisted of two small plastic cups, with a 5-cm-diameter hole in their lids. One cup contained a small amount of food and the other 50 adult insects, and the film to be tested for penetration was inserted between the two cups. Mullen (1994) developed a rapid method to determine the effectiveness of insect-resistant packaging. This technique was based on exposing *P. interpunctella* larvae to plastic pouches. Other authors used pouches made of test films exposed to insects for penetration (Bowditch, 1997; Cline, 1978; Mullen, 1994).

AU: 1998b?

Navarro et al. (1998) developed a reliable and simple method, less expensive than testing with devices using metal sections. The test device (Figure 10.9) was the same as in the nonchoice test (Section 10.4.2.3) above, with the tested packaging film replacing the paper, and it enabled evaluation of the material following extremely short exposure times. Exposure periods of *R. dominica* adults (Figure 10.8) to the test films were 24, 48, or 72 hours or 7 days. Penetration by *R. dominica* adults increased with increasing exposure periods. The thickness of the films played an important role. The resistance of films used could usually be evaluated after 24 hours of exposure. A possible explanation for the speed at which insects penetrated could be the wire mesh used adjacent to the test film.

For long-exposure tests on samples of plastic laminates impregnated with repellents and antifeedants, adding of new food every 24 hours and removing dead insects and replacing them with live ones every few days was necessary.

10.4.3 Variability of the plant extracts in inducing insect repellence and penetration prevention

Navarro et al. (2005) tested several powdered turmeric rhizome extracts from various sources for repellency and penetration prevention in the repellency and nonchoice tests. The extracts included laboratory Soxhlet extracts using turmeric rhizomes from various sources, commercial oleoresin, and commercial essential oils prepared by steam distillation. Repellency tests were carried out on two test species, adults of *R. dominica* and *T. castaneum*, exposed for 24 and 48 hours separately. The applied turmeric extract dosage was 800 μg/cm^2 on the paper. The papers were kept for 4 days before exposure and the tests were run and insects counted for 5 days. In the nonchoice test, unlike the routine test, the turmeric extracts were applied at dosages of 50 to 2560 μg/cm^2 on the papers and the tests were carried out after 1, 15, 30, 45, 60, and 75 days of turmeric oil application on the papers. The petroleum ether extracts of turmeric rhizomes (oleoresins by definition, assumed to be essential oils by composition) induced repellency of 50 to 60% (averaged over 5 days) with both the test insects, thereby showing that the turmeric extracts contained highly effective insect-repelling substances. Such high repellency was not found in other turmeric essential oils tested from 20 other various origins.

The penetration prevention results showed a protective effect in a dose-dependent manner. The petrol ether extracts, applied at a dosage of 640 μg/cm^2, showed a substantially reduced penetration for up to 60 days. Higher turmeric extract dosages of 1280 and 2560 μg/cm^2 resulted in extended periods of protection of over 75 days. The nonchoice test shows that turmeric extractions (petroleum ether or steam distillation) resulted in a high penetration prevention efficacy (PPE) of 80 to 100% of these oils. The other turmeric extracts provided medium (40 to 80%) or low (0 to 40%) PPE in the nonchoice test.

Because the nonchoice test results are also influenced by the repellency effect, which acts in the reverse direction in this test, it is expected that all of the cited extracts have a rather strong antifeedant effect, as all of them showed a strong repellency. Nonetheless, in comparing the effect of different turmeric extracts on preventing insect penetration ability, it was discovered that the source of turmeric rhizomes, as well as the particular extraction method employed, substantially affected the biological activity of turmeric extracts. Rather high turmeric extract doses were initially applied on the papers in the various tests. This doubtless created saturation effects on the tested pests. High doses are impractical in most cases for use in pest-impervious packaging materials. Although these bioassays were run on paper, results point to the potential for long-term residual effectiveness of turmeric extracts in penetration prevention when integrated in packaging materials Navarro et al. (1998, 2005).

10.4.4 Conclusions

Navarro et al. (2005) demonstrated that turmeric essential oils and several turmeric oil fractions have both repellency and antifeedant effects on test storage insects. The solid residue of turmeric oleoresin has a strong antifeedant effect. It was also demonstrated that the integrated activity contributed by several compounds is responsible for the high activity of the essential oil and its fractions as a repellent and antifeedant. The turmeric oleoresin and its components provide a versatile system with varied components to become integrated in and compatible with food packaging materials and, as a result, lead to very effective and diverse pest-impervious packaging materials.

As a result of diverse rhizome sources and extraction methods, there is a tremendous variation in composition of commercial turmeric essential oils and oleoresins. It should be emphasized that each commercial batch of turmeric oleoresin, essential oil, TOSR, or oil fraction for the production of pest-impervious packaging material should be carefully and comprehensively checked for its biological activity and its chemical composition. Therefore, in U.S. Patent 20050208157-A1 and European Patent Application 04101309.5 (Navarro et al., 2005), among the 60 claims, essential claim 1 was on "a composition-of-matter comprising a substance usable in producing packaging material and at least one compound selected from the group consisting of *ar*-turmerone, sesquiterpene alcohols and a turmeric oleoresin solid residue."

10.5 Possibilities of impregnating packaging materials with repellents

10.5.1 Concepts associated with laminate composition

Various types of materials are used for dried food commodities packaging, including plastic polymers, paper, cardboard, textiles, and metal foil (usually aluminum). All of these are subject to boring attacks by insects.

Laminates are composed of several kinds of film layers, sandwiched together by adhesives under pressure, with heat. Some laminates can be produced without heat. Packaging materials composed of laminates are constantly gaining in popularity. These laminates are composed mostly of polymers, polymers and paper, or polymers and aluminum foil. Polymers used in producing laminates may include polyethylene, polypropylene, polyester, and many others. Coating substances to packaging materials include lacquers, varnishes, and paints, with or without an outer polymer film layer.

Usually, packaging materials selected are capable of conferring optimal mechanical and chemical protection to the packaged product, while being cost effective and therefore simple to produce. Laminate physical properties such as strength, elasticity, transparency, and permeability of water and gases are well determined. Additives in the laminate materials that come into contact with food must not migrate into the food or affect the organoleptic properties of the food they protect. The seams of plastic laminate bags must remain tightly closed for the intended shelf life of the laminate, and the laminate sheeting must be free of pinholes.

Considering the complexity of laminate compositions, there are potentially several options and a variety of methods for producing pest-impervious packaging materials by fabricating or modifying the packaging material to include suitable repellents, with or without carriers:

1. Coating ordinary packaging materials with a pest-impervious coating composition. The coating material can be produced by dissolving the active compounds in the solution, suspension, emulsion, or melt of the coating composition, by solvent compounding or by any other suitable method.
2. Adding, dissolving, or dispersing the active compounds in the adhesive, lacquer, or paint or any other additive between the layers of laminates used as ordinary packaging materials.
3. Polymer-based packaging materials can be generated by mixing the active compounds with the polymer as a melt, by solvent compounding, through processes such as extrusion, molding, foaming, casting, or dipping.
4. Paper-based packaging materials can be generated by adding active compounds to a paper pulp emulsion. Paper, paperboard, or textile substances can be generated by impregnation.
5. The margins of the packaging material that serve for welding or gluing of the packaged product are impregnated or coated with the active materials.

All these methods are well established and well described, for example, by Appendini and Hotchkiss (2002).

The level of the active compounds in the packaging material should be sufficiently high so as to effectively and reliably render resistance to insect pests. On the other hand, it should be sufficiently low so as not to substantially weaken or otherwise substantially alter the physical properties of the packaging material, such as its strength and its elasticity. The packaging materials should be safe and nontoxic. Ultimately, cost effectiveness considerations will determine whether these materials will be commercialized.

10.5.2 Coating applications

Whalon and Malloy (1998) in U.S. Patent 5,843.215 describe the use of mixtures of plastics with plant substances with proven capacity to repel insects and protect food packages from insect invasions. Whalon and Malloy (1998) prepared paint varnish coatings for food packaging materials to deter Indian meal moths. These coatings contained various mixtures of limonene from etheral oils, eucalyptal from eucalyptus, perillaldehyde from mandarin orange peel, loncadol from cinnamon oil, neem oil from neem leaves and seeds, turmerone from turmeric, aserone from sweet flag, and cinnamon oil from cinnamon. The coatings were applied to cartons and tested with surrounding moths over an 8-week period. The patent claimed that the treated varnishes repelled the insects while nontreated varnishes did not. The repellency was due to the volatility of the plant extracts in the surface varnishes. Volatile compounds in surface applications tended to lose their potency rather quickly with time. Whalon and Malloy's patent did not indicate the shelf life of these varnish applications as pest-impervious packaging material or the capability to deter other insects.

10.5.3 Pest-resistant laminates containing plant extracts

Feasibility studies and preliminary tests have shown that turmeric extracts can be effectively included in various conventional packaging materials without affecting their physical quality while conferring pest resistance (Navarro et al., 2005). In these experiments, turmeric oil extracts at various concentrations were successfully dissolved in lacquer, glue, and pigments used in industrial manufacturing of packaging films. The turmeric-amended lacquer remained smooth and well spread over the sheets and the turmeric-amended glue dried properly. In other experiments, turmeric essential oil was successfully embedded in commercial PVC sheets. The turmeric extract did not affect the physical appearance of PVC, and the tests resulted in substantial insect penetration prevention efficiency. In addition, turmeric extracts were dissolved in PSA lacquer and then brushed over bags and boxes of breakfast cereal. In this case, too, the tests resulted in substantially improved insect penetration prevention efficiency.

The feasibility studies and bioactivity tests for pest resistance in the preliminary experiments and in the development phase of laminates production were carried out at the Food Science Laboratory at the Agricultural Research Organization (ARO), Volcani Center during 2002 to 2004 (Navarro et al., 2005). The bioassays for repellency (exposure time of insects, 24 hours) and penetration (exposure time, 24 hours or a week), described earlier (Sections 10.4.2.2 and 10.4.2.6), were used to test the experimental impregnated laminates. The results were indicative and could guide the manufacturer in a comparable way for the development phase.

In the experimental laminate tests, good repellency results could be obtained, similar to the results obtained with tests on the paper. In the tests for penetration, the insects often made scratches in the plastic without puncturing even the outer layer of the laminate. At times, the outer layer of the laminate was scratched open but the insect failed to penetrate the laminate. This might have been due to the insect sensing the antifeedant compounds after uncovering the surface and then withdrawing.

10.5.4 Shelf life tests of treated laminates

Food shelf life is the length of time that corresponds to a guaranteed optimal quality or tolerable loss in quality of the packaged food. The extended-period expiration date of the packaged food relates to food safety. The shelf life of packaging materials, pest-impervious or regular untreated material, should be at least as long as the length of the food expiration date.

The aging of packaging materials refers to the variation and deterioration of their properties of interest, those related to efficacy, over time. Aging and shelf life studies are an indispensable tool to anticipate the behavior of the packaging materials throughout their commercial life under certain conditions of temperature and storing, and allow the manufacturer to detect and correct formulation problems not anticipated in the first stage of development and prior to release for use.

Determining the effects of aging on a packaging material or package/product with a long shelf life (typically 0.5 to 1 year) in real time is a lengthy process that could severely delay market introduction. The possibility of conducting an accelerated aging test that simulates the effects of real time is most welcomed. Data obtained from accelerated aging testing should represent a conservative estimate of shelf life and are tentative until real-time aging studies on the packaging material or product/package combination are completed.

Accelerated aging techniques are based on the assumption that for stored food packages the temperature is the main aging parameter and that the influence of other parameters, like humidity and light, is negligible. It is assumed that the chemical reactions involved in the deterioration of materials follow the well-known Arrhenius equation for reaction rate dependence on temperature. In a simplified protocol for accelerated aging and as a rule of thumb, it is stated that a 10°C increase or decrease in the temperature of a homogenous process results in an approximately two-time or half-time change in the rate of a chemical reaction (equivalent of saying that the aging factor is 2). Using this rule, it can be calculated, for example, that a shelf life of 38 days at 55°C simulates 1 year of shelf life at an ambient temperature of 22°C. However, since this formula is only an approximation and is based on rate kinetics of a single chemical reaction, with numerous assumptions regarding the reaction order kinetics and the activation energy, and not on real multicomponent reactions in packages or packaging materials, the direct extrapolation of this model to the aging of packaging materials must be used with caution (Hemmerich, 1998). Nolan (2006) stated that in research on homogeneous plastic materials, a higher aging factor (2.5 to 3.0) has been found. However, since package systems are usually made up of several different materials, a conservative aging factor of 1.8 to 2.0 is typically used. This aging factor is also used in the medical device package testing industry. This aging factor usually results in a built-in safety factor, ensuring that enough time under test has been achieved to satisfy the *estimate* of shelf life. Since the simplified protocol for accelerated aging enables testing at one elevated temperature, the selected temperature should be a temperature that avoids unrealistic failure conditions, such as deformation due to melting. For many packaging materials 55°C will be the highest suitable temperature for accelerated aging tests. It should be emphasized that real-time aging must be performed in conjunction with any accelerated aging study of a new packaging material to correlate the results found during accelerated aging.

The aging and shelf life testing of pest-impervious materials or food packages faces the problem of lack of information and experience on the behavior of such materials with regard to the deterioration with time of the repelling capability of insects. There are numerous possible combinations of using the turmeric oil, its fractions, or the solid residue of oleoresins in pest-impervious packaging materials, depending on the biologically active composition used and the way of application. Also, there are many potential mechanisms for deterioration of the repelling capability with time, as a result of any incompatibility between the components of the plastic packaging material and the additive composition. These mechanisms include physical mechanisms (diffusion and evaporation of biologically active components) and chemical mechanisms (induced thermal decomposition and reactions of biologically active components with laminate components). It was assumed by the manufacturer (and by now approved in several cases by real-time testing) that the simplified protocol for accelerated aging could be used for testing the shelf life of the various pest-impervious packaging materials with regard to the characteristic qualities and efficacies as packaging materials and packages (including welding quality).

Nonetheless, a simplified protocol for accelerated aging testing was considered unsafe in guaranteeing a conservative result for the shelf life of the packaging material's penetration prevention efficacy, even with an aging factor of 1.8 to 2.

For pest-impervious packaging materials or food packages designated for a relatively short storage time, the ultimate way of testing would be real-time shelf life testing. For long-shelf-life packages, fully accelerated aging testing was considered, which could also result in some information regarding the real mechanism of aging. Nonetheless, the implementation of full testing faced several difficulties and barriers:

1. A fully accelerated aging testing should enable the determination of the kinetics reaction order of the total aging processes and the dependence of the reaction rate constant on temperature. Therefore, the tests should be carried out at least at three elevated temperatures, with several time intervals at each temperature. At the end of each

time interval, the biological activity should be measured. As the penetration prevention test is destructive, the expected number of samples in the tests is rather high.

2. A need for a semiquantitative, reliable, and suitable test for the penetration prevention capability (antifeedant and repellency combined effects) of the pest-impervious packaging materials or the food packages. It is quite common for packaging materials for dry food for long storage, like flexible laminates, to be persistent for 2 weeks when food packages massively and constantly are exposed to storage insects, before any apparent penetration is revealed. As described in Section 10.6, a minimum testing time of 4 weeks is needed in order to get indicative and statistically reliable results for effective pest-impervious food packages. Each test or test series should be accompanied with comparable controls of regular food packaging and could take almost 2 months.
3. It was necessary to decide what percentage of penetration prevention would be the limit between success and failure of the pest-impervious packaging material, in the extreme conditions of the tests. No correlation has been established between the results of the tests with massive exposure conditions and the real storing conditions of food packages. Tentatively and as a command decision, it was decided that the shelf life tests would be carried out until the results show 50% deterioration in the high penetration prevention efficacy in freshly prepared pest-impervious packaging material.

Considering these difficulties and the enormous amount of work needed for executing a fully accelerated aging test, the accelerated aging tests have not been implemented. Instead, when time has permitted, real-time testing has been performed over lengthy time intervals, covering the practical shelf life needed for several food commodities. In other cases, dry food manufacturers were encouraged to test pest-impervious packaging materials by themselves, in order to be persuaded if such materials could solve the specific problems they face with their commodities.

10.5.5 *Safety and fragrance aspects of plant extract additives in food packaging materials*

Given the obvious importance of producing safe and wholesome food, it is important that food packaging not affect the food with which it comes into contact. The important issue is the potential migration of unsafe ingredients, monomers, or additives from plastic into food. The untreated regular packaging materials are obviously safe and certified materials.

Turmeric extracted from *C. longa* is a very well known food additive and is used as a spice, seasoning, and flavoring. As such, turmeric essential oil, oleoresins, and natural extractives (including distillates) appear on the FDA's generally recognized as safe (GRAS) list (21 CFR 582.20) and on the FEMA

(Flavor and Extracts Manufacturers Association in the U.S.) GRAS list (182.10, 182.20) without any limitation of use in foods. These lists of food additives are generally recognized as safe by a consensus of scientific opinion. Standard migration tests based on prescribed food stimulants, which included overall migration testing and specific migration tests, ensured the safeness of the pest-impervious laminates proposed for packaging dry food commodities. The migration tests were run by a certified laboratory, subject to national (Israel) provisions and according to EU and U.S. regulations.

Turmeric essential oil is the fragrant essence of turmeric rhizome. It has the same fresh, spicy-woody aroma, also characterized as musky earthy aroma, as the powdered turmeric spice. *ar*-turmerone, turmerone, and other main components of the essential oil have considerable contribution to the aroma. The inclusion of turmeric oil in packaging materials imparts a distinctive repelling efficacy, and at the same time imparts a characteristic odor to the packaging material. According to the composition of the pest-impervious packaging materials, it can be odorous with varying intensity on the outer and inner sides of the laminate that is in contact with the packaged food. As long as the penetration prevention efficacy is apparent (at least while the repellency effect exists), the packaging material is odorous on the outer side. The odor issue was taken into account as an important parameter during the development stage of the pest-impervious laminates and in the final pest-impervious laminates compositions. Using recommended laminates, the human olfactory system only perceived the odor at a very short distance from the food packages. This odor was considered pleasant or at least was not rejected in consumer tests. There is no such migration when a laminate with an outer active coating or paint varnish is used. When the turmeric oil was added to the adhesive or internal lacquer layer, the odor penetration could be diminished by a relatively thick plastic layer on the inner side of the laminate, or avoided by an aluminum foil layer on the inner side of the laminate. The accompanying odor problem could be solved by replacing turmeric oil with nonodorous turmeric oil fractions or the solid residue as the biologically active constituents in pest-impervious packaging materials.

10.6 Laboratory and field test results with nontoxic insect repellent packaging materials

10.6.1 Organoleptic test results with rice, pasta, nuts, and sunflower seeds exposed to packages constructed with treated laminates

The inclusion of turmeric oil in food packaging may cause customer objection should the integrity of the food product be influenced by the turmeric oil aroma. Since not all food products are consumed as they are stored in their package, initial tests were carried out with macaroni and polished rice inside treated packages and compared with untreated packages. All the treated and untreated packages were stored under controlled conditions of room temperature at 25°C for 3 months. At the end of the storage period, an official laboratory taste analysis test was performed. Test reports indicated that although the atmosphere inside the treated packages at the opening had a strong typical turmeric oil aroma, this was hardly detectable in the uncooked product in the package. Furthermore, the typical aroma of turmeric oil was not detectable by the test panels (with appropriate replicates) in any of the tests carried out following cooking of the macaroni and polished rice.

A series of commercial-scale tests was carried out with roasted groundnuts, almonds, and sunflower seeds. These roasted products were kept for 6 months in turmeric oil-treated packages and in untreated packages as control and then subjected to taste panels. The packages were kept in ordinary storage conditions from June to January 2005. On a careful examination before the opening of the packages, the typical turmeric oil aroma could be detected on the outside of the packages. This aroma was not detectable from a distance but only when the packages were held close to the nose. The same aroma could not be observed upon opening the package due to the interference of the strong roasted nuts and seeds aroma. The packages were tested by a team of the packaging company, who indicated that none of the roasted products (including the controls) had an objectionable aroma that would cause the product to be rejected.

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10.6.2 *Treated laminates as house fly repellents*

Field tests were carried out with a pest-impervious laminate sheet to evaluate the efficacy of repelling the house fly, *Musca domestica*. Treated laminate sheet was placed on a standard dry fruit box (80 cm long, 60 cm wide, 8 cm high). A petri dish (8.5 cm diameter) that contained a thin layer of a fly attractant liquid, Fly Buster, was placed on the center of the box. The fly attractant served to promote adult flies to approach the petri dish and to trap them in the liquid. Another petri dish with Fly Buster was placed on a box covered with an ordinary polyethylene liner (without treatment). Both boxes were laid on the ground under a fichus tree, 2 m apart, in August, when the daily temperatures fluctuated within the range of 22 and 30°C with ambient RH of 60 to 90%.

The Fly Buster remained in the petri dish for three successive weeks. Each test was run for 24 hours. At the end of each test, the insects in the petri dishes were counted and removed from the liquid. Results of four replicates showed that on the average, 19 adult flies were caught in the petri dish of control and only 5.5 flies caught in the petri dish of the treated laminate sheet. Flies were not observed most of the time during the day around the dish on the treated laminate, whereas around the dish on the nontreated (control) sheet flies were apparent most of the time. These preliminary results show clearly that the inclusion of turmeric oil in liners can provide a good solution to prevent flies from landing on surfaces.

10.6.3 Testing the repellency of plant volatile oils on other insect pests

10.6.3.1 ***Repellency of*** *volatile oils* ***against*** *three mosquito vectors*

Tawatsin et al. (2001) evaluated the effect of volatile oils extracted by steam distillation from four plant species (turmeric (*C. longa*), kaffir lime (*Citrus hystrix*), citronella grass (*Cymbopogon winterianus*), and hairy basil (*Ocimum americanum*)) in mosquito cages and in a large room for their repellency effects against three mosquito vectors, *Aedes aegypti*, *Anopheles dirus*, and *Culex quinquefasciatus*. The turmeric, citronella grass, and hairy basil oils, especially with the addition of 5% vanillin, repelled the three species under cage conditions for up to 8 hours. The oil from kaffir lime alone, as well as with added 5% vanillin, was effective for up to 3 hours. The standard repellent deet provided protection for at least 8 hours against *Ae. aegypti* and *Cx. quinquefasciatus* and only 6 hours against *An. dirus*. However, deet with the addition of 5% vanillin protected against the three mosquito species for at least 8 hours. The results of the large-room evaluation confirmed the results obtained under cage conditions. This study demonstrates the potential of turmeric, citronella grass, and hairy basil oils as topical repellents against both day- and night-biting mosquitoes. The three volatile oils can be formulated with vanillin as mosquito repellents in various forms to replace deet (N,N-diethyl-3-methylbenzamide), the most common chemical repellent currently available.

10.6.3.2 ***Repellency of*** *volatile oils* ***against*** *ants* ***and*** *clothing moths*

In our preliminary studies, Finkelman and Navarro tested several turmeric oils as insect repellents on a wide range of insect groups that are associated with food, stored products, processed and unprocessed packaged food products, and household pests. The groups of insects tested successfully were from among the Coleopteran Bostrichidae (*R. dominica*), Tenebrionidae (*T. castaneum*), and Anobiidae (*L. serricorne*), among the Lepidoptera Phycitidae (*Ephestia cautella* and *P. interpunctella*), and among the Dipterans (*Musca domestica*), and from among the Formicids were several house ant species.

The special characteristics of some botanical essential oils to specifically repel clothing moths are mentioned in the literature. This field is relatively new and attractive in view of the carcinogenic nature of naphthalene and para-dichlorobenzene. Their unpleasant smell has created significant interest in the scientific community.

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