This is to certify that the

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REMOVAL OF PATULIN FROM APPLE JUICE

BY CHARCOAL TREATMENT

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REMOVAL OF PATULIN FROM APPLE JUICE BY CHARCOAL TREATMENT

By

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ABSTRACT

REMOVAL OF PATULIN FROM APPLE JUICE BY CHARCOAL TREATMENT

By

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Removal of patulin from apple juice contaminated with the toxin was investigated by the treatment of commercial activated charcoals in complete mixed batch systems and fixed bed mini-columns. Four granular and five powdered activated charcoals were evaluated for the rate of adsorption, adsorption capacity and their effects on juice flavor and quality. The importance of apple juice's patulin concentration, pH, soluble solids, and temperature on the efficiency of toxin removal was also examined.

The adsorption of patulin was a very fast process for both types of charcoals. It reached equilibrium in 10 and 5 min for granular and powdered carbons, respectively. Granular charcoal exhibited a fast initial rate followed by a slower adsorption rate before reaching the adsorption plateau, whereas powdered charcoals showed no slow-down of patulin adsorption. Adsorption capacity of powdered charcoal, evaluated by Freundlich isotherms, was about ten times that of the granular charcoal.

The adsorption of patulin was concentration-gradient

driven. Increasing the initial patulin concentration increased the amount of toxin being removed by the same amount of charcoal. Increasing the soluble solids of juice, especially the sugar content, lowered the efficiency of adsorption. High temperature enhanced the rate of removal, but the total percentage of removal remained the same. This could be caused by the fact that desorption of the toxin was favored at high temperatures. Within juice pH 2.8 - 3.9, removal of patulin was not affected by the changes in the pH.

Charcoal treatments resulted in a decreased in titratable acidity, adsorption in visible and UV range, and the major flavor components. However, overall yellowness of juice was not significantly changed. Mini-column studies indicated that patulin could be effectively removed through continuous flow. The adverse effects of charcoal treatment on juice quality appeared only at the beginning of the effluent and should not be considered as a problem.

To my parents, husband and son

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INTRODUCTION

Patulin is a metabolite of numerous Aspergillus and Penicillium species. It is toxic to a wide range of biological systems including microorganisms, plants, and animals and has been considered as a potential public health hazard.

Apple juice is a major food product that has raised considerable concern regarding patulin contamination, because Penicillium expansum, the most common storage rot organism of apples, can produce patulin in contaminated apples even at low storage temperature. The thermal resistance properties of patulin makes the toxin even more likely to be present because it can survive normal heat processing. Indeed, levels of 5-1000 ppb of patulin have been detected in apple juice in countries around the world.

Activated charcoal has been reported to remove patulin from cider (Sands et al., 1976). The present study was designed to

(1) compare the adsorption efficiency of patulin of different commercial activated charcoals;

(2) determine fundamental kinetic adsorption parameters by examining the adsorption profile and adsorption isotherm;

(3) determine the effect of patulin concentration in juice on the removal of the toxin;

(4) evaluate the effects of juice pH, soluble solids and temperature on the removal of patulin;

(5) examine the effect of charcoal treatment on the pH, titratable acidity, soluble solids, color and flavor of the resulting apple juice.

The experiments were conducted in complete mixed batch system and/or in fixed bed mini-columns.

LITERATURE REVIEW

DISCOVERY OF PATULIN

The mycotoxin, patulin, has been the subject of considerable study since its discovery in 1941 (Glister, 1941). Patulin was of interest because of its antibiotic action and more recently because of its phytotoxic and carcinogenic properties. Patulin has been shown to be produced by many species of fungi found commonly in foods and feeds. So, the likelihood of contamination of foodstuffs with the toxin is a real possibility.

Because of the early controversy over the identity and structure of patulin, a number of synonyms may be found in the literature, all referring to the same compound. Some of these synonyms are clavicin (Anslow et al., 1943; Kent and Heatley, 1945; Karow and Foster, 1944), clavitin (Bergel et al., 1943), claviformin (Chain et al., 1942; Florey et al., 1944), expansin (Van Luijk, 1938), leucopin (Umezawa et al., 1947), mycoin c (DeRosnay, 1952), penicidin (Atkinson, 1942), gigantic acid (Philpot, 1943) and tercinin (Kent and Heatley, 1945).

This variety of names was generated by the wide diversity of fungal species from which patulin was isolated. Fungi capable of producing patulin include

Penicillium claviforme, P. cyclopium, P. equinum, P. expansum (P. leucopus), P. lanosum, P. lapidosum, P. granulatum (P. divergens), P. melinii, P. novae-zeelnadiae, P. urticae (P. patulum), Aspergillus clavatus, A. giganteus, A. terreus, and Byssochlamys nivea.

From the practical standpoint of contamination of foods and feedstuffs, the following species have been considered to pose potential health hazards (Ciegler, 1977): P. expansum, which can grow at 0°C and is the most important storage rot organism of fruits (Pierson et al., 1971; Harvey, et al., 1972), P. cyclopium, P. urticae, A. clavatus, and A. terreus, which have been isolated from flour by Graves and Hesseltine (1966), and B. nivea. Spores of B. nivea are thermotolerant and spoilage of heat processed fruits and fruit juices by this organism has been experienced in countries throughout the world (Put and Kruisvijk, 1964; Richardson, 1965; Splittstoesser, 1971; Yates, 1974).

Chemistry of Patulin

Patulin, 4-hydroxy-4H-furo [3,2-c] pyran -2(6H)-one, is a lactone and has an empirical formula $C_7H_6O_4$ with a molecular weight of 154. Its chemical structure as elucidated by Woodward and Singh (1949, 1950) is shown in Figure 1.

Patulin is a colorless to white crystal that melts at 110.5°C. It is stable in acid (Chain et al., 1942; Jefferys, 1952) but unstable in alkali. In alkali the toxin

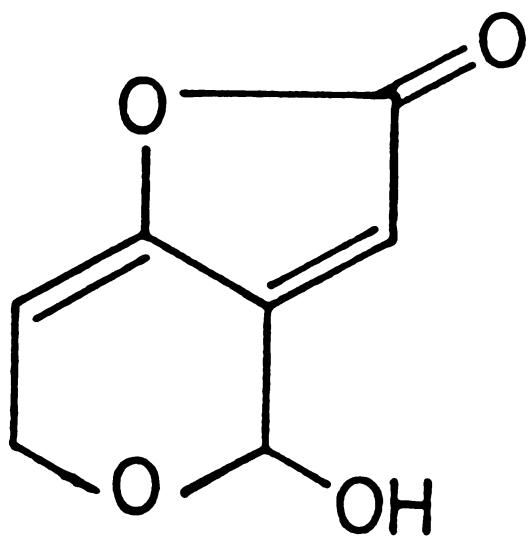

Figure 1. Structure of patulin.

loses its antibiotic activity (Atkinson, 1942; Hooper et al., 1944; Karow and Foster, 1944). Patulin is a neutral substance, which is soluble in water and in most polar organic solvents but insoluble in petroleum ether, pentane and hexane. It decomposes slowly in water and methanol, but it is stable in benzene, chloroform and methylene chloride.

TOXICITY

Patulin possesses moderately high acute toxicity for mice, rats, chicks, rabbits, and fish. Intravenous injection of patulin into mice and rats gave LD_{50} values varying from 15-25 mg patulin/Kg body weight (Yamamoto, 1954; Broom et al., 1944). LD_{50} for mice by intraperitoneal injection was reported by Broom et al. (1944) and Hofmann et al. (1971) to be 15 mg patulin/Kg, and by Andraud et al. (1964) to be 30 mg patulin/Kg body weight. Similar amounts used for intravenous injection have produced death in mice and rats by subcutaneous injection (Broom et al., 1944; Lochhead et al., 1946). Pathological changes included lungs edematous with hemorrhaging, capillary damage in the liver, spleen and kidney, and edema of the brain. A marked antidiuretic effect also was noted in rats (Broom et al., 1944).

The LD_{50} by oral administration to mice was 35 mg patulin, and 125 mg patulin/Kg was always fatal (Katzman et al., 1944; Broom et al., 1944). Pulmonary edema was a prominent feature on autopsy of dead animals. For rats

given comparable oral doses, if immediate death did not result from severe pathological changes, death took up to 4 days (Broom et al., 1944).

Several other species animals have also been observed to be sensitive to patulin. Sublethal dosages of patulin administered into the crop of chicks caused accumulation of liquid in the peritoneal cavity and extensive hemorrhage in the entire digestive tract, particularly in the preventriculus, gizzard, and intestine (Lovett, 1972). When ducks were treated orally with 0.2 mg patulin for 6 weeks, liver lesions were found (Mintzlaff and Leistner, 1971).

Studies have also shown 10-15 mg patulin/Kg to be fatal for cats, rabbits, and mice (Broom et al., 1944; Ernst, 1946). Chicken embryos (Hofmann et al., 1971; Mintzlaff and Christ, 1972), quail (Mintzlaff and Leistner, 1971), rabbit skin (Hofmann et al., 1971), guppies (Katzman et al., 1944), the crustacean Cyclops fuscus (Reiss, 1972a), and zebra fish larvae (Abedi and Scott, 1969) have also been found to be sensitive to patulin in various degrees.

In addition to the acute toxicity demonstrated by patulin to animals, teratogenicity, mutagenicity and carcinogenicity have also been observed with sublethal dosages. Gross teratogenic effects were reported in chick eggs injected before incubation (10 μg/egg) and in 4-day-old embryos (1 to 2 μg/egg). A variety of effects were noted, but the splayed foot and malrotated ankle predominated. The LD_{50} for 0- and 4-day-old chicken embryos was 68.7 and 2.35-2.4 μg/egg,

respectively (Ciegler et al., 1976; Ciegler et al., 1977).

Experiments by Dickens and Jones (1961) provided the earliest evidence of carcinogenic properties of patulin. These researchers injected male rats subcutaneously with a sublethal dose of 0.2 mg patulin twice weekly for 61 to 64 weeks. No signs of acute toxicity were seen, but 6 of the 8 test rats developed local fibroblastic tumors.

Animals, however, appear to be more resistant to the development of carcinogenicity when a sublethal dose of patulin is administered orally. No evident carcinogenic effect has been found in mice and rats following oral ingestion of the toxin (Enomoto and Saito, 1972). Daily oral feedings of 0.005-0.5 mg patulin/Kg body weight for a period of 4 wk to pig-tail monkeys decreased alkaline phosphate, but no statistically significant changes in physiological parameters, blood urea nitrogen, serum glutamate-oxalacetate transaminase, protein, cholesterol, glucose, sodium or potassium were noted (Garza et al., 1977).

Two other studies also suggested the lack of carcinogenicity of patulin to animals through oral administration. In the evaluation of the intermediate-duration toxicity of patulin, Dailey et al. (1977) found oral ingestion of patulin at a level of 1.5 mg/Kg failed to cause tumor in two generations of Sprague-Dawley rats over a period of 10 months. The only lesion found at necropsy that could be attributed to patulin administration was gaseous distension

of the gastrointestinal tract, which was believed to be a result of the antibiotic effect of this mycotoxin on the normal intestinal flora.

These researchers, however, did report a decreased weight gain in male rats that was dose-related. An impairment in growth rates of F1A and F2A progeny of both sexes was significant at the 1.5 mg/Kg level. Fetuses taken from patulin-treated females on day 20 of pregnancy were noticeably smaller than the control group and the difference was significant for F2A males.

Osswald et al. (1978) tested the long-term effect of patulin administered orally to Sprague-Dawley rats. Over a period of 64 weeks, a series of twice weekly doses totalling 358 mg patulin/Kg were administered. In spite of a high spontaneous incidence of benign tumors in the Sprague-Dawley strain, no carcinogenic action attributed to the intake of patulin was observed.

Transplacental carcinogenicity was also tested in the same study by feeding pregnant Swiss mice twice-daily 2 mg patulin/Kg on the 14th-19th day of pregnancy. The offspring of these mice reportedly showed no evidence of such activity. But during the neonatal period (2-6 days of parturition) eleven of the 52 females and eight of the 43 males born to the patulin-treated dams died, all showing similar toxicity. Gross examination of the dead mice showed hemorrhage in the skin (predominantly in the skin of the head) and to some extent in the brain and the lung.

Histological examination revealed massive hyperaemia in these organs without capillary bleeding. In contrast, no death occurred in the offspring of the control group within 6 weeks of parturition.

Apart from animal tests, carcinogenic effects of patulin have been investigated using a number of short-term assay systems. Mayer and Legator (1969) demonstrated the mutagenicity of patulin in an extrachromosomal mutation system of a haploid strain of S. cerevisiae. The yeast cells were exposed to 10-75 ppm toxin, and the colonies produced by the survivors of such treatment were tested for their ability to reduce 2,3,5-triphenyl-tetrazolium chloride to its red formazan. Results showed exposure to 50 ppm of patulin for 3 hr during exponential growth phase resulted in a mutation frequency of 60-80% with 1% survival, whereas similar treatment of stationary phase cells resulted in a mutation frequency of approximately 10% with 80% survival.

The mutagenicity of patulin was confirmed by Umeda et al. (1972) using mammalian cells. They found patulin induced DNA single and double-strand breaks of HeLa cell. The breakage was unrecoverable by further incubation after elimination of the toxin. It was suggested that the concentration at which DNA strand scissions took place was so high that the breakage could not be repaired and resulted in lethality.

Umeda et al. (1977) later also showed the mutagenicity of patulin with a FM3A mouse carcinoma cell line. The toxin

was found to induce 8-azaguanine-resistant mutations and DNA single-strand breaks. Severe breakage was noted to occur at higher concentrations than at the concentration where the mutation was produced. In addition, patulin also induced chromosome aberrations.

Lee and Roschenthaler (1986) used E. coli to study the DNA-damaging activity of patulin. Single- and double-strand DNA breaks in living cells of E. coli were observed at patulin concentrations of 10 and 50 ppm, respectively. Contrary to the findings of no repair of DNA damage caused by patulin in HeLa cells (Umeda et al., 1972), the repair of single-strand breaks was completed in the presence of 10 ppm patulin within 90 min. This discrepancy was considered to be the result of differences in the repair systems. In vitro systems with permeabilized E. coli cells, patulin at 10 ppm was found to induce DNA repair synthesis and inhibit DNA replicative synthesis. The in vitro induction of repair synthesis was observed to exhibit a correlation between concentration with the in vivo occurrence of DNA strand breaks and DNA repair.

RNA synthesis, however, was less affected and overall protein synthesis was not inhibited at 10 ppm. Only at higher concentrations of 250 and 500 ppm was inhibition of in vitro protein synthesis observed. As the correlation observed for DNA was not seem with RNA and protein syntheses, patulin was concluded to be a mycotoxin with selective DNA-damaging activity. The lack of inhibition of

protein and RNA syntheses was also reported by Rubin and Giarman (1947) in the multiplication of influenza virus in mice treated with patulin.

In the recombination-deficient mutant test of Bacillus subtilis, which detects the alteration of cellular DNA by potential mutagenic compounds, Ueno and Kubota (1976) and Lee Roschenthalter (1986) also proved that patulin possesses mutagenicity, whereas in the Ames test several authors reported negative results.

Wehner et al. (1978) showed that patulin was not mutagenic for Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537, with or without microsomal activation. The lack of mutagenic property was also observed by Wright and Lindroth (1978) using S. typhimurium strains TA98, TA100, TA1950, and TA1951, by Lindroth and Wright (1978) using E. coli pol A1, by Kuczuk et al. (1978) using S. typhimurium strains TA1535, TA1537 and TA1538 and S. cerevisiae strain D-3, and by Kangsadalampai et al. (1981) on using S. typhimurium tester strains TA98, TA100, TA1535 and TA1537.

In light of the reported carcinogenicity of patulin (Dickens and Jones, 1961), several researchers were concerned about the possible false negative tests by the Ames test. McCann and Ames (1977) proposed that any tester strain would fail to detect any toxic chemical, for reasons not related to their mutagenicity. Wehner et al. (1978) also pointed out the possibility of the antibiotic nature of

patulin, as manifested in the decreased spontaneous frequency of mutants, as well as complete inhibition of bacterial growth, may interfere with the manifestation of mutagenicity to tester strains. Kangsadalampai et al. (1981) further suggested that the Ames test is not suitable for the evaluation of mutagenicity of patulin because of the antibiotic property of the mycotoxin.

In addition to its effect on animals, the toxicity of patulin to plants has been demonstrated. The most significant observation of phytotoxicity was that exhibited by wheat. It was shown that a single exposure of growing wheat plants to 100 μg/mL patulin reduced plant vigor and grain yield (Ellis and McCalla, 1973). These problems were also noted in stubble-mulch farming, which produced an environment supporting extensive growth of patulin-producing fungi P. urticae, causing reduced wheat seed germination rate and plant size (Norstadt and McCalla, 1968; Ellis and McCalla, 1973). Ellis and McCalla (1970) also showed wheat seeds and seedlings were sensitive to patulin at a concentration as little as 20 ppm.

Iyengar and Starky (1953) reported that cucumber seeds were sensitive to patulin solutions and were unable to germinate or were stunted in root and stem length after exposure to patulin. Berestets'kyi and Synyts'kyi (1973) have found 90% of culture filtrates of 85 strains of Penicilliun urticae Banier to be toxic to seedlings of maize, peas, and flax, and patulin at 1 ppm to be toxic to

sugar beets.

Duckweed plant (Lemna minor) was inhibited by less than 1 μg patulin/g (Nickell and Finlay, 1954). Patulin at 59 μg/g wilted safflower seedlings (Gattani, 1957), and at 15 μg/g inhibited pea (Ascochyta pisi) seed germination (Wallen and Skolko, 1951), tomato seedlings (Miescher, 1950), and germinated corn (Norstadt and McCalla, 1969).

All bacterial species tested have been found to be sensitive to patulin to some degree, irrespective of Gram type. The inhibitory effect of patulin on several human pathogens was quantified by Chain et al. (1942). Bacterium coli (Escherichia coli) and Staphylococcus aureus were completely inhibited by a 0.1% concentration of patulin in 10 min. Chain et al. (1942) and Waksman et al. (1943) reported that patulin was a bactericidal compound. Indeed, over 75 species of bacteria have been demonstrated to be sensitive to patulin (Ciegler et al., 1971).

Patulin has also been shown to be an active yeast and mold antagonist, and can inhibit germination of mold spores (Reiss, 1973a). Several Pythium sp. have been observed to be quite sensitive to patulin, even at concentrations as low as 0.00025% (Anslow et al., 1943; Van Luijk, 1938). Katzman et al. (1944) noted a strong fungistatic activity by patulin against Rhizopus nigricans, Monilia albicans, Saccharomyces cerevisiae, and Sporotrichium schenkii but no effect upon Aspergillus clavatus, a patulin producer, even at concentrations up to 1 mg patulin/mL.

Patulin has also been evaluated for the control of several plant pathogens, including downy mildew of cucumbers (Ark and Thompson, 1957), damping off of safflower (Gattani, 1957), crown gall (Klemmer et al., 1955), and loose smut of wheat (Timonin, 1946). Similar results were found by Sanders (1946) who observed several human pathogenic fungi to be sensitive to concentrations of 0.0006% to 0.01% of patulin.

Using in vitro studies, acute toxicity of patulin to cells has been observed by many researchers. Toxicity to leucocytes at concentrations ranging from 0.00012 to 0.1% has been reported (Chain et al., 1942; Raistrick et al., 1943). Rabbit corneal cells have also been reported to be inhibited by patulin in concentrations of 0.02% (Vollmar, 1947).

Mouse leucocytes and rabbit epithelium cultures were stimulated at 20-40 μg of patulin/mL but inhibited at 100-200 μg patulin/mL, and a 50% inhibition of the multiplication of rat and mouse fibroblasts in culture has been observed in the presence of as little as 154 μg of patulin/mL (Perlman et al., 1959; Powell, 1966). In another study chick fibroblasts and heart cultures were inhibited by 100 and 10 μg patulin/mL, respectively (Abraham and Florey, 1949).

Cancer cells may also be affected by patulin. Ehrilch carcinoma cells and mouse ascites tumor cells were inhibited by 20-40 and 60 μg patulin/mL, respectively (Lettre et al.,

1954). Tissues described as malignant have also been observed to be inhibited by a 0.004% concentration of patulin (Vollmar, 1947).

BIOCHEMICAL ACTIVITY

Much work has been done to determine the precise mechanism of patulin toxic activity. Inhibition of aerobic respiration by patulin in several systems has been observed. Bacteria, fungi, guinea pig kidney slices, brain homogenates, and phagocytic cells have all been found susceptible to respiration inhibition by patulin solutions. Cell free extracts of Claviceps purpurea were found to be inhibited much more rapidly (40 min) than complete mycelia (3-6 hr) suggesting that a membrane barrier to patulin was present in whole cultures (Singh, 1967).

The effect of patulin on semipermeability of cell membranes has been studied. Potassium ion absorption by erythrocytes (Kahn, 1957) and glucose uptake by fungal mycelia (Singh, 1967) have been shown to be interrupted by patulin. However, no leakage of metabolites such as inorganic phosphorous, carbohydrates, amino acids from C. purpurea mycelium or of hemoglobin from bovine erythrocytes was noted in samples treated with patulin (Gottlieb and Singh, 1964). The lack of metabolite leakage in treated samples was suggested as an indication that the altering of membrane transport systems did not occur.

Since the effects of patulin on respiration appear to be

critical to its toxic activity, enzyme systems associated with respiration have been investigated. Dehydrogenase activities in mouse ascites tumor cells were found to be inhibited at 20 μg patulin/mL (Holscher, 1951).

Gottlieb and Singh (1964) studied the effect of patulin on the terminal electron transport system of C. purpurea. Succinate oxidase and dehydrogenase were inhibited up to 90% by 1155 μg patulin/mg protein. NADH oxidase, succinate cytochrome C reductase, and cytochrome oxidase were inhibited by approximately 30% at a much higher concentration, 7000 μg/mg protein.

Aldolase and lactic dehydrogenase from rabbit muscle and ATPase from human erythrocytes have also been shown to be inhibited by patulin (Andraud and Andraud, 1971; Ashoor and Chu, 1973a,b). Conversely, glucose oxidase and glyceraldehyde-3-phosphate dehydrogenase were not inhibited by patulin (Singh, 1967).

Because of the relative insensitivity of the terminal electron transport enzymes (NADH oxidase, cytochrome C reductase, and oxidase), the sensitivity of anaerobic bacteria to patulin, and the sensitive nature of oxygen consumption to patulin, Singh (1967) suggested that the site of action of patulin in biological systems is before the terminal stages of respiration.

The mechanism regarding the action of patulin is not clear. One proposed mode of action of patulin, once at the site of activity, has been the reported reactability of

patulin with sulfhydryl groups (Atkinson and Stanley, 1943a; Cavallito and Bailey, 1944; Cavallito and Haskell, 1945; Cavallito et al., 1945; Geiger and Conn, 1945). Patulin has been observed to react with cysteine, glutathionine, thioglycolate, and dimercaptopropanol (Rinderknecht et al., 1947; Hofmann et al., 1971; Geiger and Conn, 1945; Atkinson and Stanley, 1943a,b). It has been theorized that by reacting with critical sulfhydryl groups in the active sites of enzymes a toxic activity would be exhibited. Conversely, an excess of sulfhydryl groups would detoxify patulin, supposedly by binding all of the patulin molecules before they could react with a vital substrate.

Andraud and Andraud (1971) attributed the loss of ATPase activity to this sulfhydryl-patulin reaction. Ashoor and Chu (1973a) also assumed this reaction to be responsible for the non-inhibitory effect of patulin to lactic dehydrogenase by the mixtures of cysteine and patulin.

This sulfhydryl-patulin reaction theory, however, has been challenged by several findings. Ashoor and Chu (1973b) showed that patulin, bonded to cysteine, could still bond and inhibit muscle aldolase, and Singh (1967) observed that patulin did not react with glyceraldehyde-3-phosphate dehydrogenase, an enzyme containing sulfhydryl groups at its active site. Singh (1967) also reported that the cysteine-patulin reaction was a very slow one, even at high cysteine concentrations and suggested that a modified form of patulin may be the toxic form of the compound.

METABOLISM OF PATULIN

Studies of the distribution and metabolism of patulin are limited, and no metabolic products have been identified. Freerksen and Bonicke (1951) reported that patulin itself was not detected in the blood, urine, intestinal secretions, or lymph in rats or rabbits dosed orally with the toxin, although it appeared to be present in the stomach contents. Lovett (1971) also observed that when ^{14}C patulin was fed to laying hens, all edible tissue, particularly the liver, contained ^{14}C activity. Radioactivity also appeared in the eggs.

Excretion of 77% of ingested ^{14}C patulin occurred within 2 days following cessation of feeding (Lovett, 1971; Lovett, 1972). Using pig-tail monkeys as test animals Garza et al. (1977) found 30-50% of the orally ingested toxin was excreted within 24 hr. Broom et al. (1944) also reported that 5% of patulin was detected in urine within 48 hr following injection into the rabbit.

OCCURRENCE OF PATULIN IN FOODS

Since Penicillium expansum is the most important storage rot organism of apples, much of the concern of patulin contamination has been focused on apple and apple products.

1. Apple and apple products

The fungus P. expansum is a cause of the common storage

disorder of apples usually known as "blue mold". The fungus enters the fruit via wounds or lenticels and spreads rapidly through the apple fresh giving rise to a light-colored soft rot. This rotting is brought about by the action of extracellular pectin-degrading enzymes secreted by the fungus (Cole and Wood, 1961).

In addition to the ability to secret pectolytic enzymes, many strains of P. expansum can produce patulin. Harwig et al. (1973a) observed 42 of 61 (66%) strains of P. expansum isolated from rotted apples were capable of producing patulin in laboratory medium. Wilson and Nuovo (1973) further showed all of 60 isolates of P. expansum from apples decayed in refrigerated storage at 0°C produced the mycotoxin in inoculated apples.

In the sap of apples rotted by P. expansum, Brian et al. (1956) discovered patulin in concentrations of the order of 600-1000 mgm./l. Harwig et al. (1973b) also found 0.02 to 17.7 mg patulin per apple. Similar findings were noted by Walker (1969) and Lovett et al. (1975) that patulin was detected in naturally or experimentally contaminated apples.

Patulin may easily be incorporated in apple based foods if unsound fruits are used in processing. This was demonstrated by Beer (1974) who showed that patulin was not detected in fresh cider made from undecayed apples. However, the toxin was present in cider pressed from unhealthy fruit. Wilson and Nuovo (1973) also reported findings up to 45 ppm patulin in "organic apple cider"

obtained from local cider mills. Inspection of the mills revealed that the cider was either made from wild, unsprayed apples damaged and decayed by insects or from apples stored in large bins for extended periods of use.

Scott et al. (1972) were the first to report the presence of patulin in commercial apple products. They found levels of about 1000 μg/L in Canadian sweet apple cider. Since then numerous reports of the occurrence of patulin in apple products have been made in various countries.

In the United States, FDA reported that 37% of 136 juice samples surveyed were found to contain, on the average, 69 ng/mL juice. The toxin ranged from 40 to 440 ng/mL (Stoloff, 1975). Brackett and Marth (1979a) noted 23 of 40 samples of apple juice from roadside stands in Wisconsin contained 10 to 350 μg of patulin per liter. In the Washington D. C. area, levels of 49 to 309 ppb patulin were detected in 8 of 13 commercial apple cider samples (Ware et al., 1974).

In the United Kingdom, a survey indicated that of 249 fruit based products, 22 samples (8.8%), which were all apple and grape juices, contained patulin at low levels ranging from 1 to 38 μg/Kg (Ministry of Agriculture, Fisheries and Food, 1980). A recent study by Mortimer et al. (1985) revealed that of the 38 retail samples of apple, grape and other juice-based products, patulin was present in apple juice only. Six juices were reportedly contaminated with 5-10 μg patulin/L, and the remaining four positive

samples contained 16-56 μg/L.

In France, Jacquet et al. (1983) reported 6 of 20 samples of industrially prepared apple juice contained patulin (mean conc. 0.200 mg/L, max. 1.2 mg/L). Patulin was also detected in 4 of 19 samples of industrially and farm produced cider (mean conc. 0.250 mg/L, max. 2 mg/L), 16 of 18 samples of industrially prepared stewed apples (mean conc. 0.120 mg/Kg, max. 0.150 mg/Kg), and 11 of 42 packs of apple-containing foods for babies (mean conc. 0.02 mg/Kg, max. 0.1 mg/Kg).

In Finland, patulin was detected in apple juice, apple juice concentrates, apple jams, and apple flavor, but not in apple wine, apple cider and apple vinegar. Of the 64 lots (0.9 million Kg) of apple juice concentrates tested, 13 lots (0.2 million Kg) contained patulin at concentrations of 50-69 μg/L. Of 14 brands of apple flavors studied (0.2 million Kg), patulin was found in 3 lots (0.1 million Kg) at concentrations of 6-1770 μg/L. Of 20 samples of home-made apple juice examined, 8 were found to contain patulin with concentrations between 30 and 16400 μg/L. Of the 2 apple jams analyzed patulin was detected in 1 sample at a level of 1390 μg/Kg and the toxin was diffused to all levels of the jam. In this study Lindroth and Niskanen (1978) concluded that patulin risk in home-made apple products appeared to be greater than in commercial products.

In Poland, Lipowska et al. (1981) analyzed patulin in 88 apple juice concentrates in 1978, and 77 in 1979 taken from 8 industrial plants. Results showed 48% of the samples were

contaminated with patulin. The highest detected content was 253 µg/L and the average value was 30 µg/L.

In New Zealand, Wilson (1981) examined 20 samples of apple juice from 5 manufactures and found 3 positive samples with toxin concentrations ranging from 106 to 216 µg/L. In Belgium, 125-430 ppb patulin was detected in apple juice samples (Srebrnik-Friszman et al., 1983). In Sweden, Josefsson and Andersson (1976) tested 66 samples of single strength apple juice and found patulin levels exceeding 2.5 µg/L in 29 juices. In Norway, Stray (1978) found patulin in 136 of 140 apple juices from 11 Norwegian and 3 foreign producers. The toxin content ranged from less than 1 to 220 µg/L. In West Germany, Fritz and Engst (1981) detected patulin in 19 of 110 samples of fruit and fruit products, especially in commercial apple juice, in rotten parts of apples and in molded fruit products. Toxin concentrations ranged from 0.02 to 50 mg/Kg. In addition, researchers in Italy and Spain have also reported the detection of patulin in commercial apple products in the range of 5-610 µg/L (Mortimer et al., 1985).

The contamination of patulin in apple products appears to be a world-wide problem. However, only a few countries have established safe levels. Belgium declared a zero-tolerance level of patulin in all foods (Schuller et al., 1982). The Norwegian Ministry of Agriculture has provisionally decided that 50 µg/L is the highest acceptable content in apple juice (Stray, 1978). The Swedish National Food

Administration has used a tentative level of 50 μg patulin/L apple juice appropriately diluted for consumption since 1976 (Moller and Josefsson, 1980). Switzerland (Schuller et al., 1982) and the World Health Organization (Forbito and Babsky, 1985) have also adopted the same level.

2. Fruits other than apple

Patulin has been detected in fruits other than apples. Frank et al. (1977) demonstrated the production of patulin in tomatoes inoculated with P. expansum. Harwig et al. (1979) also showed the presence of patulin in tomatoes contaminated with the same species of mold originated from tomatoes. The patulin content ranged from 0.45 to 8.4 μg/g.

The occurrence of patulin in stone fruits was reported by Buchanan et al. (1974) and Burdaspal and Pinilla (1979). Buchanan et al. (1974) noted patulin in experimentally contaminated plums and cherries. Burdaspal and Pinilla (1979) discovered patulin in 50% of 12 samples of naturally rotted apricots at levels of 2-13 mg/Kg and in rotted peaches at levels of a 20 mg/Kg. Similar results were obtained for peaches and apricots by Frank (1977).

In the study by Burdaspal and Pinilla (1979), patulin was also found in naturally rotted pears in about 33% of 24 samples with concentrations between 0.9 and 10 mg/Kg. Gimeno and Martins (1983) reported patulin in one sample of naturally contaminated pears containing 1000 μg/Kg. An early study by Frank (1977) also showed patulin in different varieties of pears suffering from brown rot.

Blue mold rot of grapes due to Penicillium spp. is known to affect grapes before harvest (Harvey and Pentzer, 1960). Scott et al. (1977) demonstrated the potential of this fruit to support the growth of patulin-producing mold by detecting appreciable amounts of patulin (0.03 to 4.5 ppm) in juices prepared from four cultivars of naturally infected spoiled grapes using different processing methods.

Frank (1977) reported no detectable amount of patulin in spontaneously molded strawberries. Percebois et al. (1975), on the other hand, positively identified patulin in naturally contaminated strawberry juice. The difference in species of molds involved in spoilage may account for the contradictive observations.

In addition to fruits, patulin has also been reported in cereal, fermented meat and cheese products (Reiss, 1972b, 1975; Alperden et al., 1973; Stott and Bullerman, 1976; Jarvis et al., 1985). Generally, the presence of the toxin in these foods is temporary and the concentration is low (<100 ppb for cereal and <5 ppb for meat and cheese).

STABILITY OF PATULIN

Thermal stability of patulin has been studied in pure systems. Wiesner (1942) noted the resistance of patulin to boiling. Heatley and Philpot (1947) also reported patulin to be stable at 100°C for 15 min (pH 2.0). The toxin, however, became unstable at pH 9.5 when exposed to the same treatment. The decomposition of patulin was believed to be

caused by pH changes.

The effect of pH on the stability of patulin was investigated by Jefferys (1952). Results of his study showed that patulin in McIlvaine's buffer solutions was stable over a pH range of 3.3-6.3. Slower inactivation occurred at pH 6.8. Lovett and Peeler (1973) determined the thermal destruction kinetics of patulin in McIlvaine's buffer of pH 3.5, 4.5 and 5.5 heated to 105-125°C. Patulin was found to be resistant to thermal destruction at all pHs. However, both the thermal destruction parameters D and Z values increased as the pH decreased, indicating heat stability of patulin to acidic conditions.

Scott and Somers (1968) investigated the thermal stability of patulin in apple and grape juices. They found more than 50% of the 4 ppm added patulin remained after heating the juices for 10 min at 80°C. After heating the juices for 20 min, 45% of the toxin could still be detected. Patulin spiked in juices at the same concentration was observed to be stable up to 3 weeks at 22°C. This stability was also reported by Pohland and Allen (1970) where patulin was stable in apple juice for 5 weeks.

In another study by Kubacki (1986) patulin, however, was found to be stable at 80°C for 30 min. It slowly started to decompose at 90°C and only 20% of the toxin disappeared at 120 °C in 30 min.

In juice production heat treatment varied from flash pasteurization (90°C for 30 sec) to pasteurization at 70°C

for over 20 min (Tressler and Joslyn, 1961). From the results of the above studies, it appears that normal heat processing of juice will not destroy patulin.

The stability of patulin in apple as well as grape juices during storage has been attributed to their very low contents of sulfhydryl compounds. Sedlak and Kalocai (1957) found, in general, apples and black grapes contained negligible amounts of sulfhydryls. The SH content in the canned grape juices was less than 0.003 mmole per 100 mL juice (Jansen and Jang, 1952).

The levels of sulfhydryl compounds in fruits and vegetables were recommended by Scott and Somers (1968) as a guide to the stability of patulin in juices. These researchers pointed out that black currants contain high levels of SH compounds (0.1 to 0.4 mmole per 100 grams) and therefore would not be a good medium to support patulin stability. Indeed, instability of patulin was observed in black currant jams (Lindroth et al., 1978).

The sulfhydryl-reactive property of patulin made the toxin unstable in orange juice (Scott and Somers, 1968). Jansen and Jang (1952), and Miller and Rockland (1952) reported levels of 0.02 to 0.03 mmole of SH per 100 mL of juice. Nearly all the SH groups were present as glutathione and cysteine.

There are conflicting reports in the literature regarding the stability of patulin during the course of alcoholic fermentation. French researchers have reported the presence

of residual patulin following fermentation of apple juice (Drilleau and Bohoun, 1973). Patulin was detected in five of eight farm ciders and four of five industrial ciders. Of the 9 contaminated ciders, six contained 100 µg patulin/L and 3 contained 300 µg patulin/L. Harwig et al. (1973b), however, found patulin added at a level of 40 ppm to apple juice disappeared rapidly during alcoholic fermentation of cider. In another study Stinson et al. (1978) also observed the disappearance of 50 ppm added patulin during the alcoholic fermentation of apple juice by Saccharomyces cerevisiae.

Similar instability of patulin was observed for grape wine. Ough and Corison (1980) reported that over 99% of the 25 ppm patulin added to the Thompson seedless juice disappeared as a consequence of fermentation. Furthermore, Scott et al. (1977) demonstrated that patulin produced naturally from badly spoiled moldy grapes was detected in juices but not in partially fermented juices or in wine.

The presence of patulin in French ciders was believed to be related to the process of fermentation. Williams (1978) pointed out four possible factors for the contamination of French style ciders. First, sugar is generally not added in the making of French ciders. Thus apples are commonly harvested after they have fallen to achieve maximum sweetness. This would allow exposure of apples to infection by the ubiquitous P. expansum, which invaded through wounds and resulted in patulin production. Second, pulp is stored

up to 12 hr before being pressed and fermented, which would permit further fungal growth and patulin production. Third, fermentations are usually carried out by wild yeast naturally associated with fruit. As these organisms are often alcohol intolerant, arrested fermentations are common. Fourth, cider considered too dry is often blended with unfermented juice, which might contain patulin. None of these practices are allowed in the United States.

Considerable arguments have arisen regarding the cause of patulin disappearance during fermentation. Harwig et al. (1973b) suggested the disappearance was microbiologically mediated and might be due to the secretion of yeast metabolites. Sommer et al. (1974) also postulated the disappearance was due to metabolic destruction.

This assumption was later supported by the findings of Sumbu et al. (1983) in their study on the action of patulin on yeasts. Using radioactive precursor incorporation and gel electrophoresis of RNAs, these authors demonstrated that patulin blocked the syntheses of rRNA, tRNA, and probably mRNA of yeast cells after contacting the toxin for 10 min. The blockage, however, was recoverable. When the growth of yeast was resumed, patulin disappeared from the medium. These results suggested that two stages could have been involved in the disappearance of patulin from alcoholic fermentation of apple juice: first, the action of the toxin on yeast metabolism and the subsequent inhibition of growth; second, the resumption of growth. Sumbu and his coworkers

concluded that degradation of patulin during fermentation was an active process and was a consequence of yeast metabolism. It resulted from the activity of an inducible enzymatic system, and the induced cells resisted very high concentrations of patulin.

Pohland and Allen (1970), however, suggested the reaction of patulin with SO_2 was responsible for toxin disappearance. They showed that when patulin was added to a commercial apple juice, it was completely stable for at least 10 days, whereas the toxin disappeared rapidly from juice containing low concentrations of SO_2. Since the addition of SO_2 or its compounds is a standard practice in commercial wine making (Amerine and Joslyn, 1970), Pohland and Allen attributed SO_2 to patulin destruction during fermentation.

Scott et al. (1977) also considered the presence of SO_2 responsible for the absence of patulin in wine, cider and partially fermented juice. Since SO_2 could be formed naturally during fermentation from sulfates present in grape juice (Wurding and Schlotter, 1967; Eschenbruch, 1974), Scott et al. (1977) believed this natively formed SO_2 caused patulin destruction. Wurding and Schlotter (1967) reported that 13 to 114 ppm (average 47 ppm) of SO_2 was produced in 20 fermentations.

The effect of SO_2 on patulin stability during alcoholic fermentation was questioned by Burroughs (1977). He demonstrated that at concentrations of 2000 ppm SO_2 and 15 ppm patulin, SO_2 could indeed combine with 90% of the

patulin. However, at a SO_2 concentration commonly used in the processing of cider or wine (below 200 ppm), the affinity of patulin for SO_2 was much less. He concluded that this low level of SO_2 was of little significance for patulin destruction.

Little is known regarding the nature of substances resulting from patulin during alcoholic fermentation. With the use of ^{14}C-patulin Stinson et al. (1979) showed that little, if any, patulin was metabolized to CO_2. The compounds converted from patulin were water soluble and mostly non-volatile. By comparing the thin-layer chromatographic migration of the major patulin products from fermentation with the reaction products of patulin with cysteine, at least 58% of the added patulin was transformed to substances other than adducts of cysteine, peptides and proteins. The chemical or toxicological properties of the transformed products have not yet been characterized.

The stability of patulin has also been studied in meat, cheese and cereal products and is generally considered unstable in these systems. The instability has been related to the thio-reactive property of the toxin. It is known that patulin reacts readily with cysteine, glutathione, thio-glycollic acid and sulfhydryl-containing compounds (Stott and Bullerman, 1975; Hofmann et al., 1971; Katzman et al., 1944). Many researchers, therefore, suggested that the reaction of patulin with food components such as amino acids and proteins that contain sulfhydryl-groups caused the

instability of the toxin in cheese and meats (Lieu and Bullerman, 1977; Ciegler et al., 1972; Fiedler, 1972, Alperden et al., 1973).

CONTROL OF PATULIN

A. Prevention

Formation of patulin is closely linked to fungal growth. From a practical standpoint the best means of restricting patulin contamination is by prevention, in particular, by excluding or reducing toxigenic mold growth in the raw and processed products.

1. Controlled atmosphere

Mycotoxic molds are highly aerobic organisms and thus have a requirement for oxygen. A low oxygen concentration and/or high concentration of other gases may suppress mold growth and toxin formation (Hesseltine, 1976). The effectiveness of controlled atmospheres in retarding patulin has been studied by many researchers. Orth (1976), working with P. expansum, P. urticae and B. nivea, showed that low O_2 levels alone (2-0.5%) had scarcely any influence on the development and toxin production in Czapec and Kurtur nutrient media after 14 days at 25°C. Only higher CO_2 and lower O_2 concentrations reduced patulin production in all strains. Atmospheres with 90% CO_2 and 10% air as well as nitrogen atmospheres with 10% CO_2 also markedly inhibited the growth of molds and prevented patulin production. In a pure nitrogen atmosphere only minimum growth of mold

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without any patulin synthesis was observed.

Paster and Lisker (1985) found that an atmosphere containing extremely high levels of O_2 (60%) decreased patulin production by P. expansum on Czapec agar. The addition of 1% CO_2, however, restored toxin production to levels similar to those in 10% to 40% O_2 alone. These authors concluded that CO_2 also, in small amounts, was needed to allow the production of patulin when extremely low levels of O_2 were applied.

The effect of controlled atmospheres on the production of patulin on apple fruit was studied by Sommer et al. (1974) who inoculated apple tissues with P. expansum and found up to 300 μg of patulin per gram of tissue. Toxin production was substantially lowered to 2 μg/g tissue when apples were incubated in atmospheres modified to 2% O_2 + 7.5% CO_2.

Sommer and Buchanan (1976) studied the effectiveness of a controlled atmosphere in combination with temperature management. In their study at least two months of incubation was required for apples stored at 0°C with 3.1% O_2 + 4.8% CO_2 for traces of patulin (<0.3 ppm) to be detected, as compared to 5 days for those stored at 20°C with similar O_2 and CO_2 levels. The toxin concentration of apples stored in air at 20°C reached 17-35 ppm in 5 days. It was concluded that prompt cooling and storage at the lowest non-injurious temperature and modified atmospheres could maintain fruit vitality and avoid patulin production. In another study Beer and Woods (1977) also showed the

production of patulin was decreased by low storage temperature in controlled atmospheres containing 2-5% CO_2 and 3% O_2.

Moisture is another important factor governing the growth of mold and subsequent toxin production. The combined effects of water activity and temperature on patulin formation by Penicillium patulum were studied by Northolt et al. (1978). They found the minimum water activity value for toxin formation was 0.95 while the optimum temperatures for toxin production by the fungus varied with the strain tested ranged from 8 to 31°C.

The range of temperature over which microorganisms can grow is influenced not only by the water activity of host fruits but also by the moisture content of the storage environment. Unfortunately, information regarding the complex effects of storage temperature, relative humidity, and composition of a controlled atmosphere on the inhibition of patulin production is unavailable.

The effects of storage temperature, water activity and a CO_2 rich atmosphere on patulin production by P. expansum in black currant, blueberry and strawberry jams were studied over a storage period of 6 months (Lindroth et al., 1978). Reduction of storage temperature from 22 to 4°C resulted in decreases in hyphal growth and patulin production. An atmosphere containing 10% CO_2 had the effect of reducing patulin synthesis but did not significantly affect fungal growth. When the water activity of stored jam was reduced

by the addition of 20% and 44% sugar, toxin production in the jams was reduced to a level of 1/10-1/1250 with the maximum occurring in unsweetened jams, despite the fact that the addition of sugar stimulated hyphal growth. Maximum concentrations of patulin were usually observed after 1-2 months of storage, after which the toxin level fell significantly.

In addition to manipulating environmental conditions to control mycotoxin formation, other means may be applied to prevent mold growth and patulin formation. Some of these methods are the uses of insecticides and antimycotic agents.

2. Use of insecticide

The organophosphate insecticide, Naled (1,2-dibromo-2,2-dichloro-ethyl dimethyl phosphate), is used to control a wide variety of insects on vegetable crops, citrus, peaches, walnuts, cotton, hops, ornamentals and for adult fly control. Although Naled is presently not registered for use on apples and pears, its potential to inhibit the growth of patulin-producing fungi and toxin formation was demonstrated by Draughon et al. (1980). Results of their study showed Naled was effective when added before growth of the fungus. When 10 mg and 100 mg of Naled/L were applied to apples prior to inoculation with P. urticae and storage at 25°C for 40 days, production of patulin was inhibited by 64% and 76%, respectively. Naled also showed long term effectiveness, and was considered useful for long term storage of apples under conditions favorable to fungal growth was unavoidable.

Draughon and Ayres (1979) further evaluated the effect of insecticides from a wider chemical groups on growth and patulin production by P. urticae in potato dextrose agar. At a concentration of 100 mg/L Dichlorvos, a daughter compound of Naled, inhibited patulin production by 89%. Variable inhibitory activities were ascribed to the organophosphate insecticides Diazinon and Malathion, which inhibited patulin production by 5-42% at concentrations of 100 mg/L. Two carbamate insecticides, Landrin and Sevin, inhibited patulin synthesis by 85% and 100%, respectively. At 100 mg/L level chlorinated hydrocarbon Methoxychlor and plant pyrethroid Pyrethrum inhibited patulin formationn by 31 and 59%, respectively.

3. Use of antimycotic agents

Organic acids

The effects of potassium sorbate on growth and patulin production by strains of P. patulum and P. roqueforti isolated from cheese were studied by Bullerman (1984) in potato dextrose broth. Spore germination and the initiation of growth of P. patulum were delayed or prevented by potassium sorbate at 0.05 to 0.15% levels. Increasing concentrations of sorbate caused more variation in the amount of total mycelial growth and generally resulted in a decrease in total mycelial mass. Potassium sorbate also greatly reduced or prevented production of patulin by P. patulum for up to 70 days at 12°C. At 0.10% and 0.15% potassium sorbate, mycotoxin production was either

completely inhibited or restricted to very low levels. The effect of sorbate on P. roqueforti, on the other hand, was quite different. This organism, in comparison with P patulum, was only slightly inhibited and its production of patulin was actually enhanced.

In B. nivea, Roland et al. (1984) found that potassium sorbate at 50 µg/mL completely eliminated patulin production at 37°C. The effect of this sorbate concentration on growth was less striking. At 21°C, higher concentrations (75 to 100 µg/mL) of sorbate were required to inhibit growth of fungi, and even these levels had relatively little effect on patulin production.

A greater inhibitory effect on patulin formation than on mold growth was observed at 1.5% potassium sorbate level for P. expansum in culture at 16°C by Lennox and McElroy (1984). At this concentration sorbate caused an 83% reduction in mold growth but a 98% reduction in the synthesis of patulin. However, at a level of 0.15%, there was a 50% reduction in mold growth and only a 32% reduction in patulin formation. At sorbate concentrations of 0.015% through 0.00015%, there was little effect on either fungal growth or patulin production.

The effect of sodium propionate on growth and patulin synthesis was also evaluated in the same study. Propionate at 0.96% reduced mold growth by 43%, whereas, 98% of the patulin production was inhibited. Unlike potassium sorbate, more dilute propionate concentrations (e.g. 0.096% and

0.0096%) resulted in a less inhibitory effect on growth although marked inhibition on patulin production was observed.

These researchers also compared the inhibitory effects of sorbate and propionate. When used at similar concentrations (0.3% for sorbate and 0.32% for propionate), sorbate reduced mold growth by 57% and patulin production by 67% while propionate caused a 31% reduction in growth but a 91% reduction in patulin synthesis. It was concluded that at inhibitor concentrations commonly used in bakery products, propionate inhibited growth of P. expansum less efficiently than sorbate but was a more effective inhibitor of patulin synthesis.

Roland and Beuchat (1984) compared potassium sorbate, sodium benzoate and SO_2 for their effects on the production of biomass and patulin by B. nivea in apple and grape juices. Mycelia growth was retarded by 75 ppm SO_2, 150 ppm potassium sorbate and 500 ppm sodium benzoate over a 25-day incubation period. As in the biomass study, patulin production was also reduced. On the basis of concentration, SO_2 was found to have the most significant effect on the rate of patulin production followed by potassium sorbate and sodium benzoate.

Sodium diacetate is a derivative of acetic acid and consists of equimolar acetic acid and sodium acetate (Banwart, 1979). It contains 40% free acetic acid and in water the compound behaves as a buffered acetic acid

solution (Glabe and Maryanski, 1981). The inhibitory properties of sodium diacetate against bread molds were first reported by Glabe (1942). Glabe and Maryanski (1981) later tested its antimicrobial activity on the growth of common storage molds in plate culture and in whole kernel corn, corn silage and mixed poultry feed of corn and soybean. Results indicated that P. expansum was completely inhibited by sodium diacetae at levels above 0.05%.

Previous studies on the influence of preservatives in bread on the formation of mycotoxins revealed that an acidifying substance for dough, containing citric and lactic acid, suppressed the formation of aflatoxins and sterigmatocystin (Reiss, 1976b). The effectiveness of citric acid and lactic acid was later tested for their ability in controlling the development of colonies of P. expansum and the formation of patulin on whole wheat bread (Reiss, 1976a). However, results showed the addition of these two acids was ineffective in preventing patulin formation even at the 0.75% highest usage level.

Phenolic compounds

In addition to its antioxidant role in foods, butylated hydroxyanisole (BHA) has been shown to possess antimicrobial, in particular, antifungal activity (Ray and Bullerman, 1982). The inhibitory effect of BHA on the growth of two patulin-producers, P. expansum and P. urticae, was studied by Ahmad and Branen (1981). They found when BHA was directly incorporated into the products, 400 ppm in

cheese spread and 200 ppm in applesauce were required to retard growth of these two fungi. When applied as a surface emulsion, the growth of P. expansum on apples, applesauce and cheese spread was completely inhibited by 150-200 ppm BHA. While BHA seems promising in controlling the growth of patulin-producing fungi, the legality of adding BHA to applesauce or other non-lipid foods remains to be determined.

B. Removal

Once a product is contaminated with patulin, the toxin must be removed or be degraded in order to be used for human and animal consumption. Trimming of defective tissue from rotted apples has been demonstrated by Lovett el al. (1975) as an effective means of removing patulin from spoiled fruit. It was shown that 93-99% of total patulin could be eliminated by trimming, regardless of incubation temperature, fungus strain, or apple variety. The usefulness of trimming can be explained by the observation of Jacquet et al. (1983) and Frank (1976) that the toxin did not, to any noticeable degree, diffuse from decayed into healthy parts of apple tissues. Trimming, however, may not be as feasible in peaches, pears, and tomatoes as in apples. Frank (1977) reported significant diffusion of patulin in these fruits.

The use of ascorbate or a mixture of ascorbate and ascorbic acid in the removal of patulin was tested by Brackett and Marth (1979b). They showed that when vitamin C

was added at levels of 0.15-3% to a pH 3.5 buffer solution, which contained 5000 μg of patulin/L, all levels of vitamin C caused a decrease in the amount of patulin that could be detected after 8 days of storage at 25°C. The rates of disappearance of the mycotoxin increased with an increasing concentration of Vit C. When 3.0% of the vitamin was used, residual patulin after 8 days was only 15% of the initial amount.

In the same study an experiment was carried out using apple juice instead of buffer. Juice containing 300 μg of patulin/L was fortified with 5% Vit C and stored at 4°C. Results indicated that the addition of Vit C decreased the amount of patulin in the juice, although at a slower rate than it did when buffer was used. These researchers attributed the slower disappearance rate to the lower temperature used in the juice study and the greater stability of patulin in apple juice than in buffer solutions.

The mechanism by which patulin disappeared is not known. However, it was suggested that a metal-catalyzed oxidation of ascorbate might occur. This reaction yielded singlet oxygen or free radical in the form of a metal-ascorbate complex. The singlet oxygen generated could then attack the conjugated double bonds of patulin. This would change the structure of patulin and accounted for the decrease in the detectable patulin. If indeed the structure of patulin is changed, it is very likely that patulin is inactivated. If

Vit C does, in fact, cause inactivation of patulin, it might be of value in juices that contains the mycotoxin. However, before Vit C could be used to treat patulin contaminated juices, the toxicity of the reaction products of patulin and ascorbic acid must be evaluated.

Physical separation of patulin from juice is another possible means of removing the mycotoxin. In the concentration of an antibacterial substance, which later was identified as patulin, Wiesner (1942) showed that patulin could be removed from culture broth by absorbing it on charcoal. The use of activated charcoal as a filtration agent for the removal of patulin in apple cider was studied by Sands et al. (1976). They found cider spiked with ^{14}C-labeled patulin at 30 μg/mL was completely removed by either shaking or by eluting the cider through a 40- to 60-mesh charcoal column. Activated charcoal at 5 mg/mL reduced patulin in naturally contaminated cider to nondetectable levels.

ACTIVATED CHARCOAL

Activated charcoal is manufactured by controlling oxidation and carbonization of materials such as wood, coconut shells, and petroleum products. The resulting material has a large surface area which is capable of adsorbing a wide a variety of substances (Hassler, 1963). This unique property has led to the practical use of activated charcoal in the removal of organic contaminants

from drinking water (Weber and van Viliet, 1980) and waste water and to the clinical treatment of oral drug overdose (Levy, 1982; Buck and Bratich, 1986). The use of activated charcoal has also been reported in the removal of T-2 toxin (Romer, 1986; Galey et al, 1987).

Adsorption Kinetics

Information regarding the kinetics of patulin adsorption to activated charcoal was not found in literature. However, an understanding of the adsorption kinetics is important in determining the suitability of a particular carbon for application and in evaluating its adsorptive performance.

Adsorption of organics from aqueous solution by a porous adsorbent such as activated carbon involved three consecutive steps (Weber and van Vliet, 1980). The first step is the external or film diffusion. It is the transport of the adsorbate from bulk solution to the outer surface of the adsorbent by molecular diffusion. The concentration gradient in the liquid film around the adsorbent carbon is the driving force in film diffusion. The second step, termed internal or intraparticle diffusion, involves the transport of the adsorbate from the particle surface into interior sites by diffusion within the pore-filled liquid and migration along the solid surface of the pore. The third step is the adsorption of the solute on the active sites on the interior surfaces of the pores.

In general, the adsorption step is very rapid and does

not influence the overall kinetics (Weber, 1972). The overall rate of the adsorption process, therefore, is controlled by the slowest step, which would be either external or intraparticle diffusion. Several characteristics of the adsorbent, adsorbate, and solution phase are of importance in determining the rate limiting-step. These factors include the particle size of the adsorbent, concentration of the adsorbate, degree of mixing, affinity of adsorbate for adsorbent, and diffusion coefficients of the adsorbate in bulk solution and within the pores of adsorbent.

Helfferich (1962) concluded that external transport becomes the rate limiting step in systems which have (a) poor mixing; (b) dilute concentration of adsorbate; (c) small particle sizes of adsorbent; and (d) high affinity of adsorbate for adsorbent. In contrast, the intraparticle step limits the overall transfer for those systems which have (a) good mixing; (b) large particle sizes of adsorbent; (c) high concentration of the adsorbate; and (d) low affinity of adsorbate for adsorbent. In some systems, rate might be controlled by both external and intraparticle mechanisms (Weber, 1972; Zogorski et al., 1976).

Adsorption Equilibria

Adsorption equilibria provides information regarding the capacity of an activated carbon for adsorbing patulin from aqueous juice solution. It is important since, to a large

extent, capacity determines the useful life of the charcoal (Weber and Morris, 1964).

Several theoretical and empirical equations are available to describe the functional character of adsorption equilibria. The Langmuir and Freundlich equations are two isotherms that have been widely and successfully used to represent equilibrium data obtained from water treatment in the removal of organic pollutants from water treatment. These two equations provide means for mathematical description of experimentally observed dependencies of capacity (q_e) on equilibrium solution concentration (C_e).

Weber et al. (1964) examined the uses of Langmuir and Freundlich equations for adsorption process involving carbon. They concluded that in order to obtain a reliable capacity factor using Langmuir isotherm, four basic assumptions underlying Langmuir's model must be met. They are: (1) the molecules are adsorbed on definite sites on the surface of the adsorbent: (2) each site can accommodate only one molecule; (3) the area of each site is a fixed quantity determined solely by the geometry of the surface; and (4) the adsorption energy is the same at all sites. The Langmuir adsorption isotherm is expressed as:

$$q_e = (QbC_e) / (1 + bC_e)$$

where Q is the ultimate adsorption capacity and b is related to the energy of adsorption. The following linear form can be derived from the Langmuir equation and a plot of $1/q_e$ vs.

$1/C_e$ can be used to solve the capacity factor Q.

$$1/q_e = (1/Qb)(1/C_e) + 1/Q$$

The Freundlich adsorption equation is an empirical expression that encompasses the heterogeneity of the charcoal surface and the exponential distribution of adsorption sites (Faust and Aly, 1983). It is expressed as:

$$q_e = K_f C_e^{1/n}$$

where q_e is the amount of solute adsorbed per unit weight of adsorbent, C_e is the equilibrium concentration of the solute, and K_f and 1/n are empirical constants relating to the adsorption capacity and intensity, respectively (Weber, 1972). To determine the adsorption isotherm parameters, the Freundlich isotherm is linearized by taking the logarithm of the equation and a plot of log q_e vs. log C_e yields a straight line with slope 1/n and an intercept of log K_f.

FACTORS AFFECTING ADSORPTION OF ACTIVATED CHARCOAL

Weber.(1972) reviewed the factors affecting the adsorption of activated charcoal and concluded that adsorbent particle size, surface area, pore volume and pore volume distribution, adsorbate molecular size, adsorbate solubility, polarity, pH, temperature and adsorbate diffusion can all influence the kinetics and equilibria of an adsorption process.

The particle size of the adsorbent can affect the rate at

which adsorption occurs in a system. The larger the particle size, the slower the rate of adsorption. The adsorptive capacity of an adsorbent is a function of the total surface area available for adsorption (Snoeyink and Weber, 1967). The greater the available surface area, the higher the adsorption capacity of the adsorbent. Most of the surface area lies within the adsorbent particle and only a small fraction is present at the surface. Therefore, equal weights of the ground and the unground adsorbent from the previous example should have approximately the same adsorption capacity.

Pore volume is a measure of the total volume of micropores and macropores present within the adsorbent particles. The pore volume distribution curve of an adsorbent indicates how much of the total volume is present within a particular size range of the pore diameters. This information is useful if the molecular size range of the adsorbate is known, because adsorption is strongest in the instances where the molecular size of the adsorbate is just slightly smaller than the pore diameter of the adsorbent.

The molecular size of the adsorbate is a factor which can affect the adsorption process. This is due to the fact that the adsorbate must be able to enter the pores of the adsorbent in order to be removed from the solvent. If the pore size range of the adsorbents is known, then it is possible that a successful screening of the adsorbents could be made for a particular adsorbate upon inspection of the

pore volume distribution curves of the adsorbents.

An adsorbate that has a high solubility in a particular solvent would generally not be easily adsorbed from it. For adsorption to occur readily, the adsorbate should be present in a solvent in which it is relatively insoluble. There have been exceptions to this observation, so the factor of solubility can not always be relied upon for making the best judgement.

The most favorable situation for adsorption with regard to the factor of polarity is for the adsorbate and the adsorbent to be similar in nature. Granular activated carbon in an adsorbent which is slightly polar, and those adsorbates which have a low polarity or are non-polar are most successfully adsorbed onto it. This situation can be more favorable by using water as the solvent because of its polar nature (Weber, 1972).

The pH of the solvent can affect adsorption by altering the surface charge of the adsorbent as well as the charge on the adsorbate. Ionization of the functional groups on granular activated charcoal can increase or decrease the slightly negative charge that it typically carries. Ionization of the functional groups of the adsorbate can determine whether or not the species has a charge and also the magnitude of the charge. Adsorption would not be favored under the conditions where the adsorbate and adsorbent had a similar charge.

Temperature can affect both the rate at which adsorption

occurs and the extent to which adsorption occurs in a system. For instance, at elevated temperatures the rate of adsorption would increase while the extent of adsorption would decrease due to the exothermic nature of the process.

Further studies are needed to optimize conditions for removing patulin with charcoal and to evaluate the effect of charcoal treatment on the quality of resulting juices.

EXPERIMENTAL

MATERIALS

Apple Juice. Apple juice was made from McIntosh, Spy, and Golden Delicious apples. These fruits were washed and passed through a hammermill. The chopped apples were placed in nylon press cloths which were supported by wood press racks and pressed on a pilot scale batch hydraulic fruit press. The pressure was alternatively applied and disengaged to assure maximal expulsion of juice.

Pectic enzyme (Miles Laboratories, Inc., Naperville, IL) was added to the juice. The treated juice was allowed to stay at refrigeration temperature overnight. Bentonite at a level of 0.05% was added on the following day and the juice was shaken thoroughly. The juice was stored in the cooler overnight again and was filtered the next day through a Whatman No. 4 filter paper using a vacuum. The filtered juice was heated to 83°C for 5 min in steam kettles, bottled in glass jars, and heat sealed. The bottled juice was cooled as soon as possible under running tap water and subsequently stored at 4°C. The pasteurized juice was checked for pH, titratable acidity, soluble solids and patulin content.

Activated Charcoal. Several types of commercial

activated charcoal (granular and powdered) from different sources were investigated in this study. A brief summary of the basic properties of these carbons is given in Table 1. Carbons were washed with distilled water to remove leachable materials and carbon fines. The carbons were then dried at 105°C for 24 hr and immediately desiccated after removing from the drying oven. The carbons were stored in airtight glass bottles when reaching room temperature.

Standard Patulin. Patulin standard was purchased from Sigma Chemical Co. (St. Louis, MO).

Standard Apple Juice Flavor. The following eight compounds were used in identifying apple juice aroma. Standard propyl butyrate, trans-2-hexen-1-ol, cis-2-hexen-1-ol and methyl acetate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Trans-2-hexenal, heptanol and hexanal were purchased from Sigma Chemical Co. (St. Louis, MO). Ethyl-2-methyl butyrate was purchased from Fluka Chemical Corp. (Ronkonkoma, NY).

Reagents. All reagents and solvents utilized in this study were analytical grade and/or HPLC grade. The cyclohexane was distilled before being used for patulin analysis.

METHODS

I. Analysis of Patulin

Safety Procedures. Precautions were taken in the handling

Table 1. General properties* of activated charcoal used in the research.

Commercial name	Raw material	Surface area (m^2/g)	Total pore volume (cc^3/g)	pH	Density (lb/ft^3)
Granular**					
WVG	bituminous coal	1100	0.81	-	-
WVB	wood-based	1400	1.86	-	-
F-300	bituminous coal	950-1050	0.85	6.5	28
OL	bituminous coal	1000-1050	0.88	-	28.1
Powdered**					
S-51	lignite	650	1.0	-	32
ADPP	bituminous coal	1525	-	-	-
SA	bituminous coal	1400-1800	2.2-2.5	4-6	21-23
WPHP	bituminous coal	1000-1150	-	-	46
SN	-	1400-1800	2.2-2.5	6-8	21-23

* Sources: manufacture brochures, Faust and Aly (1987), and Lee et al., (1981).

** Manufacture:
WVG, WVB: Westvaco, Covington, Va.
F-300, OL: Calgon Corp., Pittsburgh, Pa.
S-51: American Norit Co., Jacksonville, Fl.
ADPP, WPHP: Calgon Corp., Pittsburgh, Pa.
SA, SN: Westvaco, Covington, Va.

of patulin. During manipulations with patulin, disposable gloves were worn and all work was performed under an approved hood. The surface of work areas was routinely sprayed with NaOCl bleach. Any glasswares and vials in contact with patulin were thoroughly rinsed with bleach. All TLC plates were soaked in bleach before discarding. The treated waste materials and discarded solvents derived from patulin analysis were removed by the MSU Chemical and Biological Safety Unit.

Patulin Analysis. Torres et al. (1986) developed a patulin analysis technique requiring less time and solvent as compared to what is needed by AOAC method (1984). A comparison between Torres and AOAC methods for the quantification of patulin in apple juice was performed. Apple juice was spiked with patulin standard to a final concentration of 1000 ppb for use in the comparison study.

Torres's method. A 2 mL toxin-spiked juice sample was injected into a silica Sep-Pack cartridge (Waters Associates, Inc.). It was then eluted with 6 mL of ether and 6 mL of ethyl ether-ethyl acetate (1:1) into a 4 dram vial. The vial was tightly closed with a Teflon lined screw cap and held at -20°C until quantitative analysis by high performance liquid chromatography.

Modified AOAC method. The official AOAC method uses benzene in the elution of patulin during the clean-up of the sample extracts. It was found that cyclohexane, which is less toxic, resulted in as good recovery as benzene. Cyclohexane

was, therefore, used instead of benzene in sample cleanup.

Extraction. Twenty five mL of apple juice were extracted four times with equal volumes of ethyl acetate in a 250 mL separatory funnel. The extracts were collected in a 500 mL round bottom flask and evaporated to approximately 10 mL using a rotary flash evaporator (Buchi Instruments Co., Switzerland) with a water bath temperature of 35±5°C. Overheating of the extracts was avoided to prevent sample decomposition. The concentrate was decanted into a 100 mL Erlenmeyer flask. The round bottom flask was washed with two 5 mL portions of ethyl acetate and the washings were combined with the concentrate. The combined sample was adjusted to 80 mL with cyclohexane before clean-up by column chromatography.

Cleanup. A sintered-glass filter disc was placed in the bottom of a 15 x 250 mm Fischer and Porter modular chromatographic column. The column was then filled with 10 mL glass-distilled cyclohexane and followed by the addition of approximately 5 g of anhydrous Na_2SO_4. A slurry of 10 g of silica gel 60 (70-230 mesh ASTM, E. Merk Reagents) in 50 mL cyclohexane was added. The gel was allowed to settle and the top of the adsorbent was evened with a mechanical vibrator. Ten gram of anhydrous Na_2SO_4 were then carefully layered on top of the gel.

Cyclohexane was drained to the top of the packing. The patulin extract was transferred to the column with a disposable glass pipet. The sides of the flask were washed

three more times using 5 mL of ethyl acetate-cyclohexane (1:3) each time, and the washings were added to the column. The column was drained to the top of the packing and the eluate was discarded.

The patulin was eluted from the silica gel column with 150 mL of ethyl acetate-cyclohexane (30:70). The eluate was collected in a 500 mL round bottom flask and evaporated to approximate 1 mL in a rotary evaporator as described earlier. The concentrated sample was transferred to a 1 dram vial with a disposable glass pipet. The acetate-cyclohexane solvent was evaporated to dryness under a stream of nitrogen (N-Evap Evaporator Model 106, Organomation Assoc Inc., South Berlin, MA). Two 1 mL portions of ethyl acetate were used to rinse the 500 mL round bottom flask and the washings were added to the dried patulin extract in the 1 dram vial. The vial was tightly closed and held at -20°C until analysis by HPLC.

High Performance Liquid Chromatography. A Waters HPLC instrument (Waters Associates, Inc., Milford, MA) equipped with a M-45 solvent delivery system, U6K septumless injector, and a reverse phase partisil 10 ODS column (Whatman Chemical Separation Inc., Clifton, NJ) was used for the quantification of patulin. Detection was made at 254 nm by a Spectrum Model 5480 UV monitor (Spectrum Medical Industries, Inc., Los Angeles, CA). Chromatograms were recorded on an HP 3390A integrator (Hewlett-Packard Co., Palo Alto, CA).

At the time of analysis, the patulin sample vials were withdrawn from the freezer and shaken well in a Vortex-Genie test tube shaker (Scientific Industries, Inc., Bohemia, NY). The solvent was evaporated to dryness under a gentle stream of nitrogen using a water bath temperature of 35±5°C. The residual was dissolved in 80 μL of ethyl acetate-methanol mixture (10:90) for samples prepared by Torres method and 1 mL for those prepared by AOAC method. The change of solvent from ethyl acetate to ethyl acetate-methanol (10:90) was suggested by Stray (1978) due to poor separation in HPLC analysis when patulin was present in ethyl acetate eluted by water (Appendix A-1). Ten μL of patulin sample in ethyl acetate-methanol mixture were injected and the toxin was eluted from the column with water at a flow rate of 0.8 mL/min.

Appendices A-2 and A-3 show the HPLC chromatograms of patulin samples prepared by Torres and AOAC methods, respectively. It can be seen that poor resolution, though good recovery (94.7%), was obtained for samples by the Torres method. Modified AOAC resulted in good recovery as well as good separation between the toxin and extraneous compounds in apple juice. In spite of the simplicity and time- and solvent-saving capacities of the Torres method, modified AOAC method was used for patulin analysis in apple juice.

A stock patulin standard solution was prepared by dissolving standard patulin in ethyl acetate to give a

concentration of 400 ppm. Various levels of patulin solutions were made from the concentrated standard to establish a standard curve for quantification. The patulin standard curve is presented in Appendix B.

To determine the percentage recovery of patulin from apple juice, proper volume of the stock patulin solution (400 ppm) was dried under N_2 and dissolved in juice to give final toxin concentrations of 100, 300, 600, and 1000 ppb. Duplicate spiked juice samples containing known levels of patulin were analyzed as described earlier and the percentage recovery was calculated. The recoveries ranged from 87-105% (Table 2).

Thin Layer Chromatography. A fraction of HPLC eluate from a juice sample with retention time corresponding to that of the patulin standard was collected. The collected eluate was dried under a gentle stream of nitrogen and immediately dissolved in ethyl acetate. Thin layer chromatography was carried out according to official AOAC TLC method (1984). The TLC techniques served as qualitative and confirmatory tests for patulin analysis.

Qualitative TLC. TLC analysis of patulin was carried out according to a modification of AOAC (1984) method. Precoated 20 x 20 cm silica gel plates (Sil-G-HR-25, Brinkmann Instruments Inc., Westbury, NY) were scored using Schoeffel Scoring Device SDA 303 (Kratos Analytical Instruments, Ramsey, NJ) to provide strips 10 mm wide, and were spotted using a 10 µL syringe (Hamilton Industries, Two

Table 2. Patulin recovery as a function of toxin concentration.

Patulin concentration (ppb)	Recovery ± S.D. (%)*
100	90.97 ± 7.31
300	87.34 ± 9.11
600	89.31 ± 4.03
1000	105.10 ± 6.68

* Average of triplicate samples.

Rivers, WI). Ten µl of patulin extracts and standards were spotted 4 cm from the bottom edge of TLC plates. Just before use, the plates were activated by drying in an oven at 100°C for 30 min, followed by cooling for approximately 10 min.

The spotted plate was developed with toluene-ethyl acetate-90% formic acid (5:4:1, v/v/v) in a sealed equilibrated tank. When the solvent front was 4 cm from the top, the plate was removed from the tank and air-dried in a hood. The dried plate was sprayed with 0.5% 3-methyl-2-benzothiazolinone hydrazone hydrochloride solution (MBTH) until the layer appeared wet. The MBTH solution was stored at refrigeration temperature and prepared fresh every 3 days. The sprayed plate was dried in an 130°C oven for approximately 15 min.

After drying, the plate was examined visually under visible light. Patulin was seen as a yellow spot. The sample chromatogram was compared with that of a standard. The spot in a sample was considered as patulin when R_f value and color were identical to the patulin standard spot.

Confirmatory TLC. Precoated 20 x 20 cm Sil-G-HR-25 silica gel plates (Brinkmann Instrument Inc., Westbury, NY) were scored, activated, and spotted with patulin standard and samples as described before. The plates were developed in each of the following three solvent systems using unequilibrated developing tanks: hexane-anhydrous ether (1:3), $CHCl_3$-methanol (95:5) and $CHCl_3$-acetone (90:10). The

R_f values of the standard and samples were compared and were used as confirmatory tests for identifying patulin.

II. Removal of Patulin by Complete Mixed Batch System

The experimental system selected for evaluating the kinetics of adsorption of patulin to activated charcoal from apple juice was a batch system. The batch technique was selected because of its simplicity and ease of evaluating the parameters which influence the adsorption process. Actual applications of activated charcoal will be more likely via column-type operations. However, the evaluation of the fundamentals of adsorption is much simpler in a batch system and the basic relationship developed herein can, with due care, be applied to the behavior of continuous adsorption systems.

A. Rate Study

Weighed samples (2.4 of granular activated charcoal and 0.24 g of powdered activated charcoal) of each charcoal were mixed by stirring on a magnetic stirrer with 100 mL of 600 ppb patulin-spiked apple juice. The mixing time was 0.5, 1.25, 2.5, 5, 10 and 20 min for granular activated charcoal (GAC) slurries and 0.5, 1.25, 3, 5, 10, and 15 min for powdered activated charcoal (PAC) slurries. After mixing, the slurries were filtered through Whatman #5 filter paper with water vacuum. Two 25 mL aliquots of filtrate were analyzed for patulin by procedures described earlier. The

experiment was repeated twice. The percentage of patulin adsorbed was calculated by the following equation.

$$\% \text{ patulin removed} = \frac{(600 - \text{patulin remaining}),\ \mu g/L}{600\ \mu g/L} \times 100$$

Adsorption profile was examined by plotting contact time (min) vs. % patulin adsorbed. Rate of adsorption was analyzed with first order reaction by plotting contact time (min) vs. log residual patulin concentration (μg/L). Rate parameters due to intraparticle diffusion were calculated by determining the slopes of the plots of square root of contact time vs. cumulative adsorption of patulin (μg/g charcoal).

B. Adsorption Capacity

Adsorption capacity was evaluated by equilibrium isotherms. To establish equilibrium isotherms, it is necessary to determine the time required to achieve equilibrium for the adsorption of patulin. Time to equilibrium was defined as the time at which there was a constant percentage of patulin adsorbed for two consecutive time periods. Results of previous rate studies showed that, in all cases, the percentage of patulin adsorbed after 10 min did not significantly add to the efficiency of patulin removal. Contact time was therefore set at 10 min for the adsorption capacity study.

Carefully weighed 0.4, 1.2, 2.0, or 2.8 g of GAC and

0.04, 0.12, 0.2, or 0.28 g of PAC were added to 100 mL of 600 ppb patulin spiked apple juice. The slurries were mixed by stirring for 10 min. At the end of mixing, slurries were filtered and the resulting juice was analyzed for residual patulin concentration (μg/L, C_e). Patulin adsorbed by unit gram of charcoal was calculated by the following equation.

$$\text{Adsorbed Patulin } (\mu g/g,\ q_e) = \frac{60\ \mu g - \text{Residual patulin } (\mu g)}{\text{Charcoal Wt. } (g)}$$

Isotherm for each charcoal was determined using the Freundlich equation.

$$\log q_e = \log K_f + 1/n \log C_e$$

Freundlich capacity parameters (K_f and 1/n) were obtained as the intercept and slope of log q_e vs. log C_e plot.

C. Effect of Initial Toxin Concentration on Patulin Removal

Apple juice was spiked with patulin to concentration of 100, 300, 600 or 1000 ppb. Carefully weighed granular charcoal OL (0.3, 0.9, 1.5, 1.8 or 2.4 g) was mixed with 75 mL of patulin-spiked juice for 10 min. Upon the completion of mixing, the slurries were filtered and analyzed for residual patulin. Patulin removed (μg) was calculated by substracting remaining patulin from initial amount of toxin.

D. Effect of Juice pH on Patulin Removal

The effect of apple juice pH on patulin removal was studied by adjusting juice to pH 2.8, 3.2, 3.5 or 3.9 with 0.1 N HCl or 0.1 N NaOH. Weighed samples (2.4 g of GAC and 0.24 g of PAC) of each charcoal were mixed with 100 mL of pH adjusted spiked apple juice (600 ppb) for 10 min by stirring. The percentage of patulin removal was determined.

E. Effect of Soluble Solids in Juice on Patulin Removal

The effect of soluble solids in apple juice on patulin removal was determined by adjusting juice to 10, 12 or 18° Brix with sucrose. As in the previous experiment, 2.4 g of each GAC or 0.24 g of each PAC was mixed with 100 mL of Brix adjusted, patulin spiked (600 ppb) juice for 10 min. The percentage of patulin adsorbed was calculated.

F. Effect of Juice Temperature on Patulin Removal

The effect of apple juice temperature on patulin removal was determined by heating 600 ppb patulin-spiked juice to 90°C in water bath. Upon reaching 90°C, 2.4 g of each GAC or 0.24 g of each PAC was added and mixed for 10 min. The temperature of the water bath was maintained at 90°C during mixing. Patulin degraded by heat was measured by keeping juice samples at 90°C for another 10 min without the addition of activated charcoal upon reaching 90°C. Percent patulin removal was calculated.

G. Effects of Charcoal Treatment on Juice pH, Soluble Solids and Titratable Acidity.

The effects of charcoal treatment on juice pH, soluble solids and titratable acidity were determined. A total of 2.4 g of each GAC or 0.24 g of each PAC was mixed with 100 mL of toxin free apple juice. The slurries were mixed for 10 min and filtered at the end of mixing. The resulting juice was checked for pH, soluble solids and titratable acidity.

pH. pH was measured using a Beckman Model 3560 Digital pH meter (Fullerton, CA).

Titratable acidity. Ten mL of juice and 10 mL freshly boiled and subsequently cooled distilled water were added to a 100 mL Erlenmeyer flask. To this, 4 drops of phenolphthalein solution were added. Using a magnetic stirrer, the diluted juice was titrated with 0.1 N NaOH to reach an end point of pink color. The volume of NaOH used was recorded and the percentage of acid (w/v) in apple juice expressed as malic acid was calculated using the following equation.

$$\% \text{ Malic} = \frac{(\text{mL NaOH}) \times (0.1\text{N}) \times (0.001 \text{ eq/meq}) \times (67.03 \text{ g/eq}) \times 100}{5 \text{ mL}}$$

Soluble solids. Soluble solids were determined using a refractometer (Bausch & Lomb, Rochester, NY) and expressed as Brix (°B).

H. Effect of Charcoal Treatment on Juice Color

The color of juice samples resulting from Section G were determined by measuring the absorbance spectrum and Hunter b value.

Absorbance spectrum. The absorbance spectrum of apple juice was measured using a Perkin-Elmer Lamda 4B Spectral Photometer (Norwalk, CT) equipped with a scan spectral processing cartridge. The scanning was carried out between 190 and 600 nm.

Hunter b value. Hunter Color Difference D25-2 Meter (Hunter Associates Laboratory, Inc., Reston, VA) was used to measure color differences in the treated apple juice samples. A standard white tile (L=92.3, a=-1.2, b=0.5) was utilized to calibrate the instrument. One hundred mL of juice samples were placed in an optical glass cylinder cup (7.4 x 1.9 cm). Color measurements were taken, aided by covering the samples with an inverted white-lined can for standard optical background.

I. Effect of Charcoal Treatment on Juice Flavor

Gas stripping-trapping, vacuum stripping-trapping and Likens-Nikerson extraction methods were compared for their recoveries of selected apple juice aroma compounds. Appendix C (1-3) shows the gas chromatograms obtained for juice samples prepared by these three methods. It can be seen that gas stripping-trapping method resulted in higher recoveries for most of the selected aroma compounds and was,

therefore, used in this study. Apple juice was treated with charcoal by procedures described in Section G. The aroma components of resulting juice as well as no charcoal-treated juice were collected using gas stripping-trapping method.

Gas Stripping and Trapping. A gas stripping with subsequent trapping procedure described by Poll and Flink (1984) was modified to collect apple juice aroma. A 250 mL round bottom flask containing 100 mL of sample and 40 μL of methyl acetate (internal standard) was placed in a 50°C oil bath. The flask was tightly fitted with a Teflon-wrapped rubber stopper containing two glass tubes. A cylinder of nitrogen was connected to one of the tubes and nitrogen was bubbled through the flask. The exiting vapor was collected in a trap attached to the flask outlet. The trap consisted of a glass tube (12 x 0.4 cm) containing 1 g of anhydrous Na_2SO_4, followed by 50 mg of Tenax-GC (60/80 mesh, Alltech Associates, Inc., Deerfield, IL) and another 0.5 g of anhydrous Na_2SO_4. After 1 hr, the Tenax-GC trap was removed from the round bottom flask. The retained volatiles were eluted with approximately 10 mL of isopentane with the aid of centrifugation at approximately 650 rpm (IEC Clinical Centrifuge Model CL, International Equipment Co., Needham Hts., MASS). The sample was stored at -20°C until analysis.

The Tenax-GC tube used in this experiment was chemically pre-conditioned by washing the packed tube first with 10 mL of methanol and then 10 mL of isopentane. Following chemical preconditioning, the tube was thermally pre-treated

by heating the tube to 150°C for at least 2 hr in a stainless steel jacket while purging with nitrogen. The tube was allowed to cool before stopping gas flow and was stored in a tightly sealed glass container.

Vacuum stripping-trapping method. The setup of vacuum stripping-trapping method was similar to that of gas-stripping trapping method. Juice sample was poured into a two-exits 250 mL round bottom flask and closed tightly at one end. Vacuum was pulled from the other end for 1 hr and the aroma were collected in a Tenax GC trap. Components trapped in the GC trap was eluted by isopentane by procedures described before.

Likens-Nikerson Extraction. One hundred mL of juice and 40 μL of internal standard methyl acetate, and boiling chips were place in a 500 mL flat bottom flask. The flask was placed in a Likens-Nikerson extraction apparatus. The system was allowed to reflux for 1 hr and the volatiles were collected in 10 mL of isopentane in a 25 mL flask. Upon completion of reflux, the flask containing isopentane was removed from the apparatus and isopentane was dried by passing through anhydrous Na_2SO_4. The dried isopentane was collected in a graduated test tube. The volatile-collecting flask was rinsed with two 3 mL portions of isopentane and the washings were combined with dried volatile extracts. The combined volatile extracts were stored at -20°C until analysis.

Gas Chromatography. At the time of analysis, isopentane was concentrated to 0.2 mL by gently blowing nitrogen gas over the isopentane surface. To this concentrate, 3-5 grains of anhydrous Na_2SO_4 were added and 2 µL were injected into the gas chromatograph (Hewlett-Packard Model 5840A Chromatograph equipped with flame ionization detector). The split ratio was adjusted to 1:25. The GC separation was conducted using a 60 m x 0.25 mm fused silica capillary column packed with non-bonded 0.25 µm thickness of Carbowax 20M. Head pressure for helium carrier gas was regulated at 140 psi. The injection port and detector temperatures were 210 and 275°C, respectively. The column oven was programmed as follows: 15 min at 30°C, 30-180°C at 2.5°C/min, isothermal at 180°C for 30 min. The retention time and peak area were measured with a Hewlett-Packard 3390A Electronic Digital Integrator. Each sample was analyzed in duplicate.

III. Removal of Patulin by Fixed Bed Mini-Columns

A. Effect on Patulin Removal

Mini-columns were used to investigate the effects of flow rate, column size, and carbon loading on patulin removal from apple juice. The concentration of toxin in juice was 600 ppb. Granular activated charcoal WVG, WVB, and OL were tested. The amount of carbon used to adsorb patulin was 2.4, 3.2, 4.0 or 4.8 g of GAC per 100 mL of juice. The column diameter (inner) was 0.7, 1.9 or 2.9 cm. The flow rate was controlled as close to 12.5, 25, or 37.5

mL/min as possible using a metering pump. The eluate was collected per 25 mL fraction and analyzed for unadsorbed patulin. Patulin remaining as functions of flow rate, column diameter, and carbon loading was determined by multiple linear regression analysis (PlotIt, Module MLRA).

The feasibility of using column-type operation to remove patulin from apple juice was evaluated by constructing the breakthrough curve and usage-rate curve. The equations and calculation are presented in Appendix D.

Juice was unable to flow in column packed with powdered activated charcoal with the available laboratory instrument. Mini-column experiments were, therefore, not carried out for powdered activated charcoal.

B. Effect on Juice Quality

The pH, titratable acidity, Brix, and color of the juice eluted from mini-columns were determined as described before for analysis of juice quality in complete mixed batch systems.

The pH, titratable acidity, Brix, and color were also determined for an 8-liter juice passed through a column containing 192 g of carbon WVB and of inner diameter of 2.9 cm. The flow rate was 25 mL/min.

RESULTS AND DISCUSSION

(I) Removal of Patulin by Complete Mixed Batch System

A. Rate Study of Patulin Adsorption

The rate profile of patulin removal by granular activated charcoal is presented in Figure 2. Results showed a rapid initial rate of adsorption for all 4 tested carbons. This rate decreased markedly after 2.5 min of contact and was followed by a gradual approach to equilibrium. The equilibrium was reached in approximately 10-20 min. At equilibrium the removal efficiency was 90-97%, which corresponded to final patulin concentrations of 20-60 ppb depending on charcoal variety (Table 3).

Figure 3 shows a first-order plot of the experimental data from rate profile. It reveals that patulin adsorption by GAC is characterized by two processes, a quick step followed by a much slower one. The slow-down of patulin adsorption after 2.5 min contact with granular charcoal is not clear. However, three possible explanations can be proposed to explain the observed decrease.

First, it was suspected that after contacting with the juice for 2.5 min, the charcoal was saturated with the toxin under the experimental conditions. But if that were the case, then at equilibrium there should be no net change in

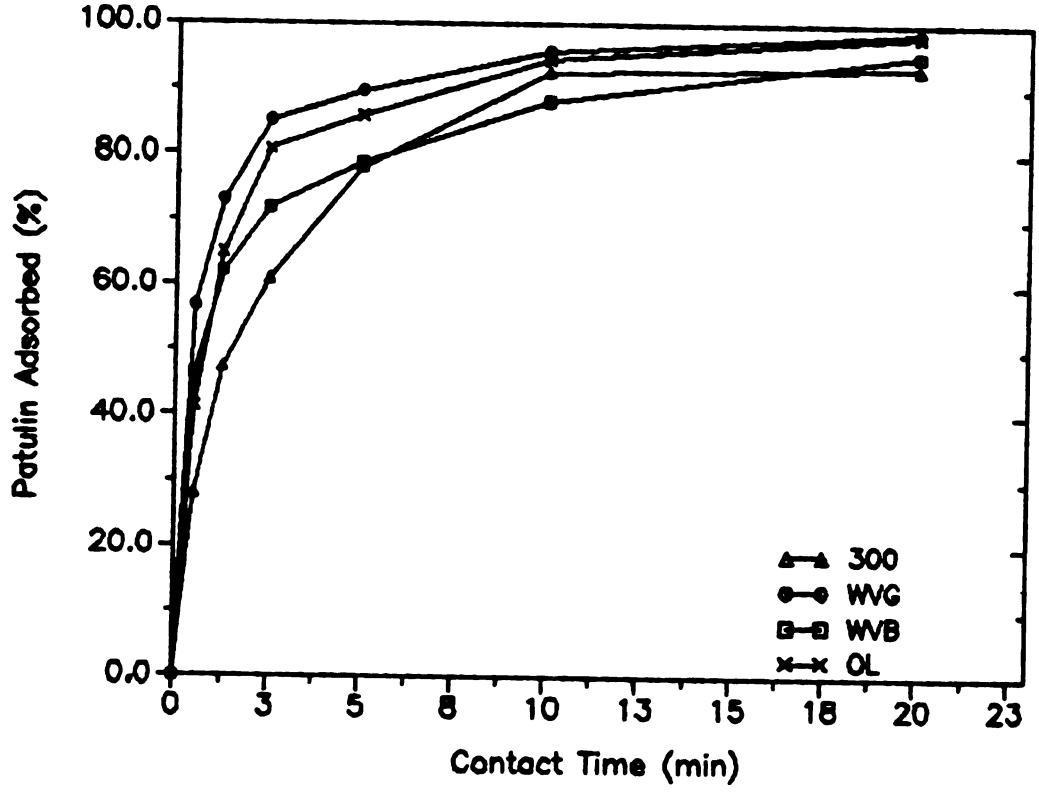

Figure 2. Rate profile of patulin removal by four different types of granular activated charcoal from apple juice.

Table 3. Residual patulin concentration (ppb) in apple juice* treated by granular activated charcoal.

Contact time (min)	Charcoal			
	OL	WVG	WVB	300
0.5	351.78** (18.06)	259.25 (10.62)	322.44 (8.36)	431.98 (15.43)
1.25	210.23 (29.79)	162.44 (13.67)	227.82 (13.13)	351.95 (6.81)
2.5	115.28 (8.83)	89.46 (12.43)	169.12 (18.87)	234.18 (13.64)
5.0	85.46 (11.56)	62.24 (10.65)	128.11 (11.72)	132.48 (9.98)
10.0	37.29 (8.23)	25.05 (6.50)	59.88 (5.03)	54.16 (12.89)
20.0	17.20 (8.97)	14.28 (5.61)	29.28 (6.44)	40.81 (12.24)

* Initial patulin concentration = 600 ppb.

** Mean ± (standard deviation).

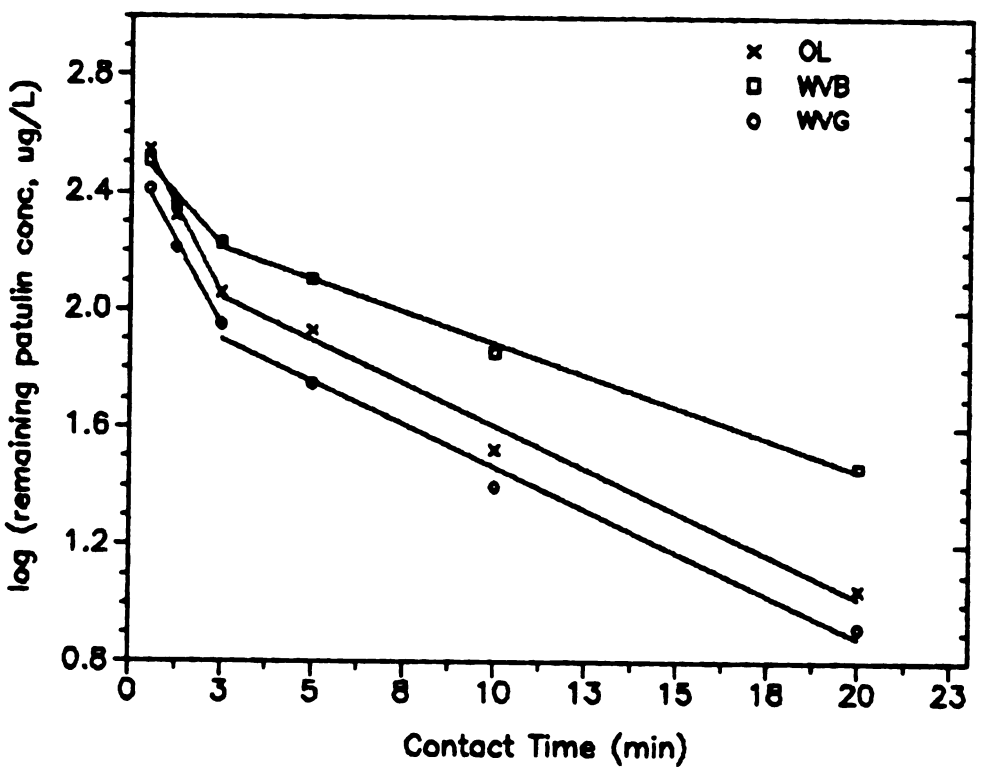

Figure 3. First-order rate analysis of patulin adsorbed by three types of granular activated charcoal from apple juice.

toxin adsorption. A 10-15% increase in toxin removal efficiency, however, was observed. The slow-down, therefore, is unlikely to be caused by the saturation of charcoal.

A second explanation that could justify the decreased adsorption at lowered toxin concentration may be related to the rate-limiting factor of external diffusion. Helfferlich (1962) reported that external diffusion can become rate-limiting when the concentration of adsorbate in the system is low. In this study it appears that when patulin concentration was lowered to a certain level, the transfer of toxin from bulk juice phase to charcoal surface was lowered because of the lowered driving force. As a consequence, an overall decrease in removal rate was observed at the lowered
toxin concentration.

The assumption of concentration gradient-caused dependence is supported by Zogorski et al. (1976). These researchers observed that the removal of 2,4-dichlorophenol by granular activated charcoal was dependent on the initial concentration of adsorbate. They also noted that the rate of adsorption of 2,4-dichlorophenol was limited by external diffusion at low phenol concentration and by intraparticle diffusion at high phenol concentration. They concluded that concentration gradient caused the removal dependence.

Third, the decreased adsorption at low patulin concentration could be caused by the existence of competing

components in apple juice. It is believed that at the beginning of the adsorption process, patulin was the predominant adsorbent in apple juice. Thus, rapid adsorption occurred at this period. Since apple juice is a complex system, substances which are also adsorbed by activated charcoal may become so competitive that they hindered the adsorption of patulin when the toxin concentration decreased to certain levels.

To test the second and third hypotheses, patulin was added to double deionized distilled water (DDDW) to the same level as in spiked apple juice. Charcoal OL was introduced to the patulin-containing DDDW at a level of 24 mg/mL water and rate experiment was proceeded as in juice. It is theorized that if the decreased adsorption was caused by hypothesis two, the break should still be observed in DDDW system. However, one can not exclude the influence of competing components in juice since these substances may compound the decreasing effect in juice. On the other hand, if the break point is not observed in DDDW system, then the decrease in adsorption rate is more likely caused by the existence of competing components than the rate-limiting effect of external diffusion.

The rate curve plotted by first order reaction for patulin removal from DDDW is shown in Figure 4. It can be seen that the break-point occurring in juice was also observed in the DDDW study. The two systems, however, differed in the concentration at which the break took place.

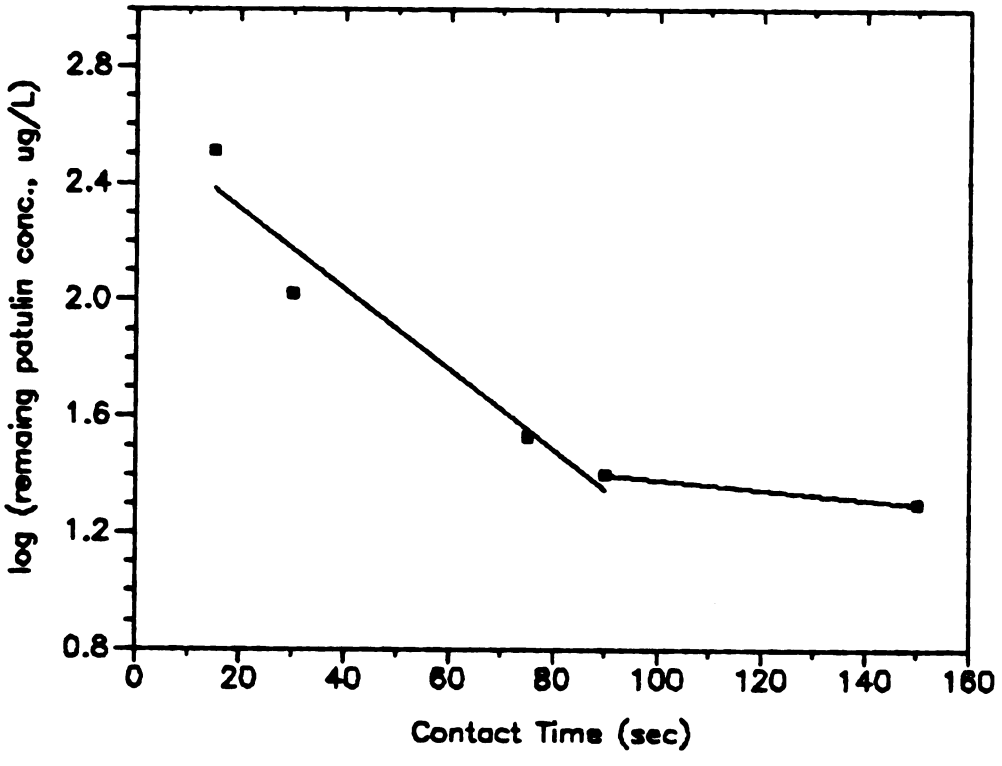

Figure 4. First-order rate analysis of patulin adsorbed by granular activated charcoal OL from double deionized distilled water.

In juice, the break-point occurred at toxin concentrations of approximately 90-130 ppb, whereas it occurred at 30 ppb in DDDW. Due to the occurrence of the break-point in DDDW system, the rate-limiting factor of external diffusion is believed to cause the decreased adsorption at lowered patulin concentration.

The patulin removal efficiency showed that adsorption of the toxin from DDDW occurred at a much faster rate than in the multi-component juice system (Table 4). Nearly 95 % of the spiked toxin was adsorbed from DDDW in 75 seconds, whereas, it took at least 10 minutes for the same amount of granular charcoal to reach the 95% removal efficiency in juice. The overall decreased adsorption rate of juice can be caused by competing components in juice as the adsorption sites on/in charcoal could be occupied by these substances. The existence of competing components can also attribute to the increase in toxin concentration where the break occurred. Results of the DDDW study also suggested that it is not a good approach to study patulin removal in a pure water model system since it can lead to over-estimation of the rate of patulin removal in apple juice.

Helfferlich (1962) reported that in a batch system with good mixing and high adsorbate concentration, intraparticle diffusion is the rate-limiting step. In the juice study, intraparticle diffusion appears to be rate-limiting before patulin concentration decreased to 90-130 ppb.

According to Crank (1956), for systems in which

Table 4. Patulin removal by charcoal OL from double deionized distilled water spiked to 600 ppb toxin.

Contact time (min)	Residual conc. (ppb)	Removal efficiency (%)
0.25	322.63±15.81	46.22
0.5	105.75± 9.22	82.37
1.25	34.12± 8.76	94.31
1.5	20.26± 4.83	96.64
2.5	trace	100.00
5	0	100.00
10	0	100.00

intraparticle transport is the rate limiting step, data for uptake of solutes from solution by the adsorbent generally follows a linear relationship when plotted as a function of the square root of time. The slope of the least-squares regression line from such a plot is indicative of the relative rate of uptake. It can function as an empirical rate parameter for comparison of the carbons.

The rate data were plotted in this manner. Good linearity of the data for the rate study was observed (Figure 5). Values for rate parameters and correlation coefficients for the least-square regression fits to the rate data are tabulated in Table 5. Charcoal 300 exhibited the smallest intraparticle rate parameter among the four granular charcoal. This could be caused by its larger pore size distribution. One aspect worth pointing out is that charcoal 300 achieved similar removal efficiency at the end of 20 min reaction time, even though it had the lowest intraparticle rate parameter. This can be due to the fact that charcoal 300 retained the same removal rate between 2.5-5 min reacting time, while the rates of adsorption of the other three carbons had slowed down after 2.5 min.

The adsorption of patulin by powdered activated charcoal was tested. When used at the same level (24 mg/mL) as granular charcoal, all five carbons exhibited removal efficiencies of 100% after contacting the juice for less than 1 min. It is apparent that powdered charcoal had much higher patulin removal efficiencies than those of granular

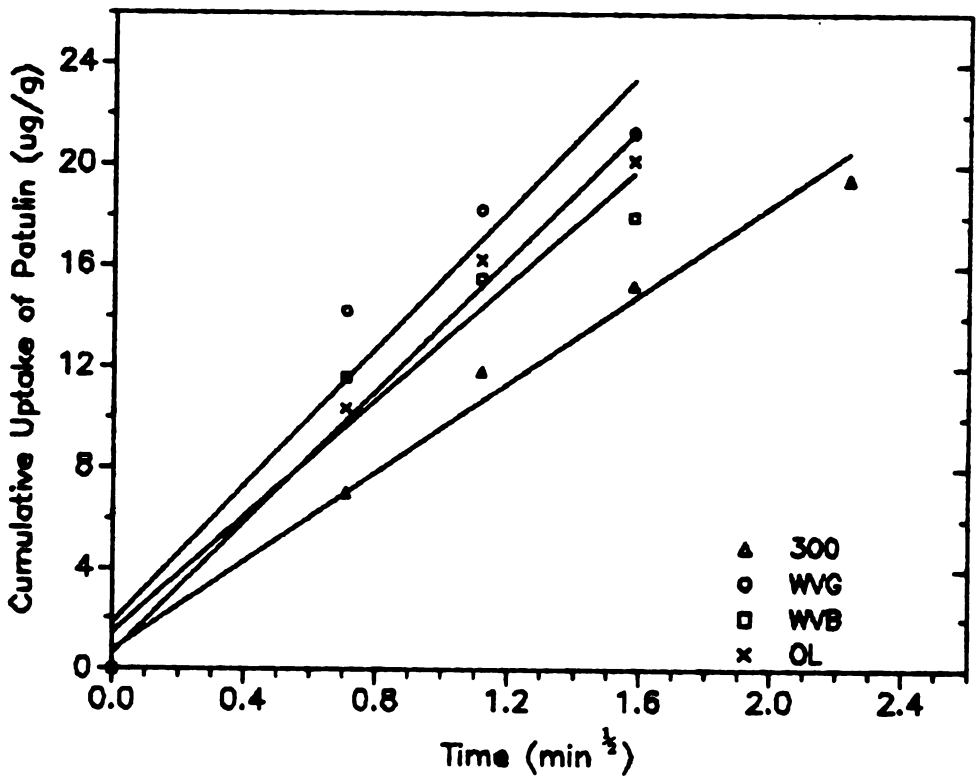

Figure 5. Plot of cumulative removal of patulin by four different types of granular activated charcoal from apple juice vs. square root of contact time.

Table 5. Intraparticle rate parameters for adsorption of patulin from 600 ppb toxin spiked apple juice on activated charcoal.

Charcoal	Rate parameter*	R^2**
Granular (24mg/mL)		
WVG	14.58	0.945
WVB	11.53	0.937
300	9.83	0.994
OL	13.02	0.988
OL	22.06***	0.937
Powdered (2.4mg/mL)		
S-51	49.96	0.931
ADPP	62.77	0.943
SA	53.15	0.966
WPHP	53.08	0.981
SN	58.92	0.943

* Intraparticle rate parameter = $(\mu g/gm)/(min)^{0.5}$

** R^2 = correlation coefficient for fit of the rate rate parameter to the experimental data.

*** Rate parameter for adsorption of patulin from 600 ppb toxin spiked double deionized distilled water.

charcoal. The resulting juice, however, was colorless. The task was then to determine the concentration of powdered charcoal that could be used without interfering with juice color. Apple juice was treated with charcoal ranging from 0.1-10 mg/mL of juice. Results showed that levels above 2.8 mg/mL significantly decolorized juice as examined visually. A rate study for removal of patulin by powdered activated charcoal was carried out at the 2.4 mg/mL of juice level.

The rate profile for patulin adsorption by five powdered activated charcoal is shown in Figure 6. In general, adsorption reached equilibrium in 5 min. Removal efficiency at plateau ranged from 67 to 89%, with ADPP exhibiting the highest efficiency and S-51 the lowest. S-51 had a significantly smaller total surface area than the other 4 powdered charcoals tested (Table 1). The smaller surface area could lead to less adsorption of patulin. Adsorption data plotted as a function of the square root of time is presented in Figure 7. The slope of the plot is shown in Table 5. Charcoal ADPP had the highest empirical rate parameter and S-51 had the lowest.

Powdered charcoal differed from granular charcoal in toxin adsorption in two respects. First, adsorption of patulin by powdered charcoal was a lot faster than granular charcoal. This is demonstrated by the significant higher empirical rate parameters than those of granular charcoal. This may be due to the fact that the powder is more accessible for patulin adsorption. Second, the level-off

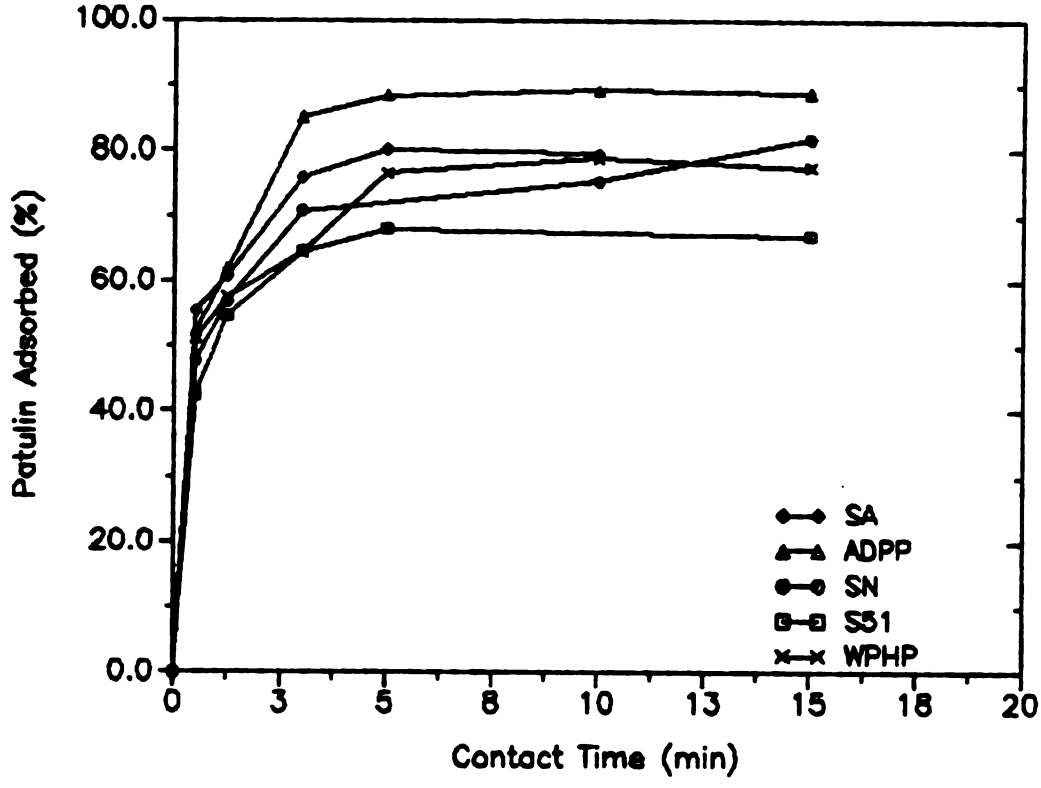

Figure 6. Rate profile of patulin removal by five different types of powdered activated charcoal.

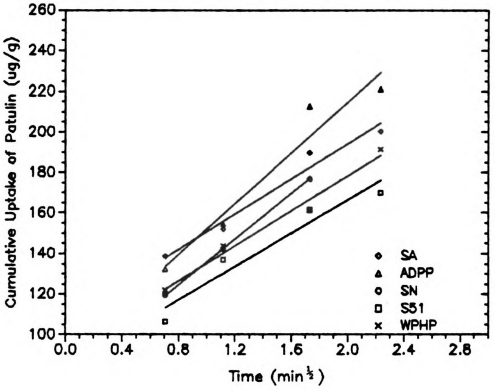

Figure 7. Plot of cumulative removal of patulin by five fifferent types of powdered activated charcoal from apple juice vs. square root of contact time.

of the toxin adsorption by powdered charcoal at 2.4 mg/mL usage level is more likely caused by saturation of powdered charcoal than the shift of rate-limiting step from intraparticle to external diffusion. This is because powdered charcoal showed very little additional toxin adsorption after reaching its plateau.

B. Adsorption Capacity of Patulin by Activated Charcoal

Figures 8 and 9 illustrate Freundlich adsorption isotherms for patulin adsorbed from 600 ppb toxin spiked apple juice by granular and powdered activated charcoal, respectively. The isotherms showed initial linearity, then curved at the latter part of the plots. The curvature was sharp for granular carbons WVB and F-300 as well as for powdered charcoals SN, SA and S-51. The occurrence of the curvature corresponds to samples treated with lower levels of charcoal.

The reason for experimental values higher than those obtained from the Freundlich equation is not clear. However, it can be caused by the additional adsorption of the toxin to the less available sites present in charcoal. These adsorption sites may not be utilized by the toxin when the amount of added charcoal is providing sufficiently the readily available sites. But when the readily available sites are inadequate, as in the case where significantly less charcoal is added, the concentration gradient of the

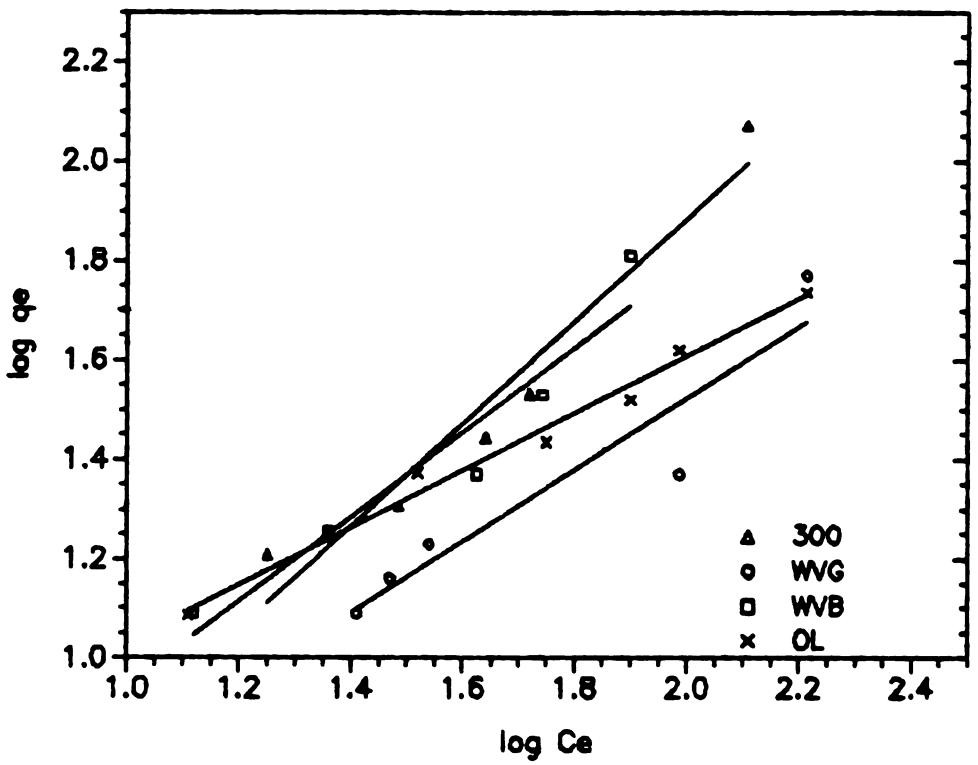

Figure 8. Freunlich adsorption isotherms for patulin removal from apple juice spiked with 600 ppb toxin by granular activated charcoal.

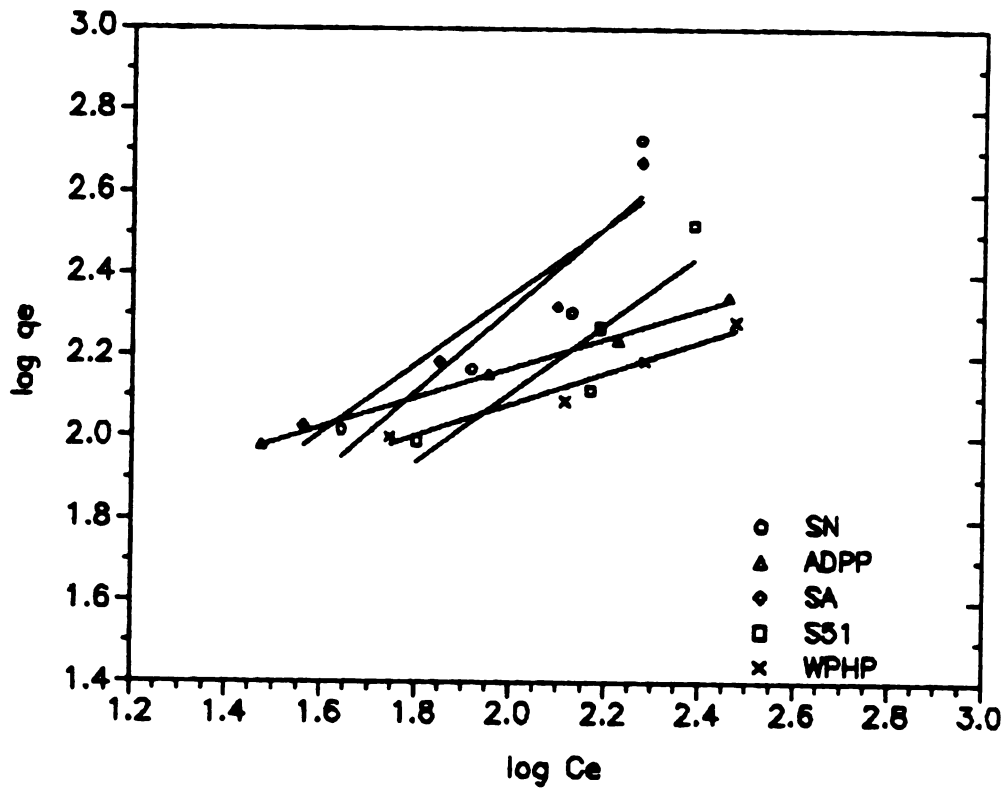

Figure 9. Freunlich adsorption isotherms for patulin removal from apple juice spiked with 600 ppb toxin by five different types of powdered activated charcoal.

toxin between the juice and carbon may force the toxin to seek the structurally or energetically less available sites. This explanation is supported by the observation of surface heterogenicities of activated charcoal (Parkash, 1974; Mattson and Mark, 1971). Similar phenomena have also been reported in isotherms described by the Freundlich equation in the removal of organic compounds from water (Ronstke and Snoeyink, 1983).

Points corresponding to the sharp increase in Freundlich isotherms were eliminated in the calculation of the Freundlich parameters. The parameters are presented in Table 6. The capacity factors for powdered carbon are approximately an order of a magnitude higher than those obtained for granular charcoal. The result confirmed earlier findings that powdered charcoal is a better patulin adsorber than the granular. Results of capacity factor indicate that in order to achieve similar removal efficiency only one tenth of charcoal used for the granular is required for the powdered carbons. This is exactly what was used.

From Table 6, powdered charcoal S-51 had the lowest capacity factor among the five tested powdered carbons. This can be due to its significantly lower total surface area. The extent of toxin adsorption can not be exclusively considered as affected by total surface area only since powdered charcoal S-51 has a total surface area smaller than granular carbons but has significantly higher capacity. The four granular charcoals tested showed similar K_f values.

Table 6. Freundlich isotherm* parameters of patulin adsorption by activated charcoal from 600 ppb toxin spiked apple juice.

Charcoal	K_f **	1/n **	R^2
Granular			
WVG	2.88	0.57	0.964
WVB	2.26	0.65	0.984
300	2.17	0.51	0.956
OL	2.51	0.72	0.952
OL	8.98***	0.65***	0.811
Powdered			
S-51	11.00	0.59	0.996
ADPP	29.90	0.34	0.996
SA	24.67	0.55	0.998
WPHP	26.26	0.32	0.949
SN	23.49	0.43	0.964

* Freundlich isotherm equation $q_e = K_f C_e^{1/n}$

** Calculated based on C_e in µg/l and q_e in µg/gm.

*** Parameters for adsorption of patulin from 600 ppb toxin spiked double deionized distilled water.

It is known that the capacity factor is characteristic of the entire adsorption system. In order to examine the influence of apple juice substances on adsorption capacity, Freundlich capacity of 600 ppb patulin spiked DDDW was determined using granular charcoal OL at a level of 24 mg/mL as the adsorbent. The Freundlich isotherm of 600 ppb DDDW is shown in Figure 10. The capacity factor calculated from the slope is 8.98, which is approximately 3.58 times higher than that obtained from apple juice (Table 6). It appears that substances present in apple juice had occupied some of the adsorption sites and significantly decreased the capacity of charcoal for patulin.

C. Effect of Initial Toxin Concentration on Patulin Removal

The influence of initial toxin concentration on the removal of patulin by granular charcoal OL is presented in Table 7. Results showed that the extent of adsorption is dependent on patulin concentration. Increasing the initial toxin concentration increased the amount of patulin being adsorbed by the same level of charcoal. The increase could be caused by the increase in driving force due to increased concentration gradient between charcoal and bulk juice solution. In addition, less competitiveness of native juice components for adsorption sites for juice with higher toxin concentration may also play a role in the increase.

Weber et al. (1964) reported the concentration dependence

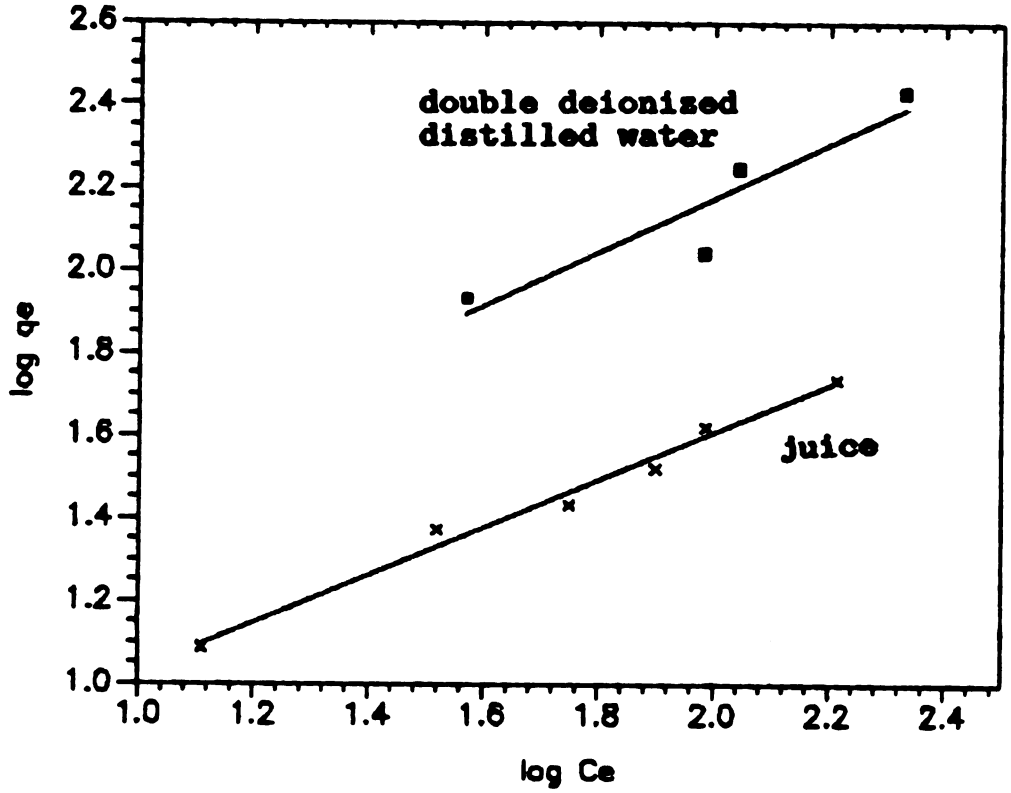

Figure 10. Freunlich adsorption isotherms for patulin removal by charcoal OL from double deionized distilled water spiked with 600 ppb toxin.

Table 7. Effect of initial patulin concentration on patulin adsorption (μg) from juice spiked with various levels of toxin by charcoal OL.

Charcoal level (mg/mL juice)	Initial patulin concentration			
	100	300	600	1000
4	4.96 (5.31)	15.58 (8.97)	34.08 (7.76)	59.56 (10.45)
12	6.41 (3.07)	19.90 (6.28)	40.79 (11.63)	67.51 (6.82)
20	6.90 (4.75)	20.54 (7.30)	42.26 (5.24)	69.72 (8.69)
24	6.73 (2.84)	20.77 (5.16)	43.42 (9.78)	71.84 (3.94)
28	7.20 (5.17)	21.68 (3.54)	43.60 (15.23)	72.62 (8.47)
32	7.29 (2.02)	21.43 (2.53)	43.83 (6.40)	73.46 (5.29)

for phenol. They proposed the terms "ultimate capacity" and "effective capacities" to describe the observed phenomenon. For a particular adsorbate, there is only one ultimate capacity. The ultimate capacity is independent of equilibrium concentration in solution and is measured in the adsorbate's nearly saturated solution. An effective capacity, on the other hand, is a fraction of the ultimate capacity and is encountered when adsorption on carbon occurred in solutions containing trace amounts of the adsorbate. How much of the ultimate capacity used is dependent on the adsorbate's concentration in dilute solution. It appears that the observed dependence of patulin removal on initial toxin concentration is very likely caused by the use of effective capacities of charcoal.

D. Effect of Juice pH on Patulin Removal

The effect of juice pH on the removal of patulin is shown in Table 8. It can be seen that patulin adsorption was not affected by juice pH regardless of carbon types. The effect was not studied over a wider pH range due to the unlikelihood of apple juice exhibiting a pH outside the values of this study.

The lack of influence of juice pH on toxin removal can be attributed to the fact that patulin is a neutral mycotoxin. For electroneutral substances, the adsorption by activated charcoal from aqueous systems is generally not affected by

Table 8. Effect of juice* pH on patulin removal (%) by activated charcoal.

Charcoal	pH			
	2.8	3.2	3.5	3.9
Granular** (24mg/mL)				
WVG	91.79*** (1.64)	92.76 (3.73)	89.02 (0.84)	92.84 (2.80)
WVB	94.12 (2.27)	94.48 (4.52)	95.83 (1.08)	93.49 (1.46)
300	91.43 (3.28)	90.44 (3.17)	90.97 (2.14)	92.88 (2.48)
OL	93.74 (2.91)	94.50 (1.13)	93.82 (3.61)	92.83 (3.79)
Powdered** (2.4mg/mL)				
S-51	69.93 (2.58)	69.15 (1.70)	68.00 (2.94)	67.84 (5.25)
ADPP	85.65 (4.49)	86.91 (2.63)	89.24 (3.16)	88.83 (2.57)
SA	78.35 (1.79)	75.41 (2.31)	79.51 (1.82)	79.55 (1.55)
WPHP	74.74 (2.83)	76.62 (4.12)	78.87 (1.60)	76.39 (3.29)
SN	78.12 (4.53)	72.96 (2.40)	75.32 (0.75)	77.83 (2.68)

* Initial patulin concentration = 600 ppb.

** Mixing time = 10 mins.

*** Mean ± (standard deviation).

the solution pH (Hauge and Willaman, 1927: Anderson, 1947, Faust and Aly, 1983).

Mechanisms proposed to explain the dependency of activated carbon's adsorptive capacity for weak acids and bases on solution pH can provide, to some extent, the information leading to the present observation. Snoeyink et al. (1969) attributed the decrease in capacity for a weak acid at alkaline conditions to the development of repulsive forces between the anions (dissociated from the weak acid) and the carbon surface. They further suggested that the increase in the negative surface charge of the carbon by adsorbing the hydroxyl ions or by ionizing the very weak acidic functional groups on the carbon surface could also contribute to the observed decrease.

Being a neutral compound, the characteristics of patulin are unlikely to be affected by the solution pH. As a consequence, adsorption was not affected by solution pH.

E. Effect of Juice Soluble Solids on Patulin Removal

Table 9 illustrates that the removal efficiency of patulin from apple juice was decreased by 18° Brix juice soluble solids at levels of 24 and 2.4 mg/mL of juice for granular and powdered charcoal, respectively. Sugar represents the major soluble solid in apple juice. The adsorption of sugar onto activated charcoal and its adverse effect on the removal of certain drugs have been reported

Table 9. Effect of soluble solids in juice* on patulin removal (%) by activated charcoal.

Charcoal	Soluble solids (Brix)		
	10	12	18
Granular** (24mg/mL)			
WVG	93.14 (1.79)	89.02 (0.84)	86.21 (2.03)
WVB	94.68 (1.54)	93.49 (1.08)	87.56 (1.91)
300	88.82 (1.46)	90.97 (2.14)	85.46 (1.73)
OL	96.47 (2.39)	93.14 (1.46)	89.78 (2.57)
Powdered** (2.4mg/mL)			
S-51	69.28 (1.13)	68.00 (2.94)	59.31 (0.99)
ADPP	88.82 (2.75)	89.24 (3.16)	83.81 (1.63)
SA	80.94 (2.44)	79.51 (1.82)	74.23 (2.34)
WPHP	83.67 (1.23)	78.87 (1.60)	73.46 (2.41)
SN	75.09 (2.19)	75.32 (0.75)	73.68 (1.55)

* Initial patulin concentration = 600 ppb.

** Mixing time = 10 mins.

*** Mean ± (standard deviation).

(Cooney and Roach, 1979; Neve, 1976; Hauge and Willaman, 1927). Sugar may well act in a similar manner as in drug systems where it competes for adsorption sites.

Table 10 shows patulin removal efficiency affected by various levels of charcoal OL and ADPP. The removal efficiency of 12° Brix juice was lowered at charcoal levels of 4 for OL and 0.4 mg/mL for ADPP, respectively. Juice of 18° Brix exhibited decreased toxin adsorption at levels less than 24 mg/mL for OL and all levels for ADPP.

The difference in the available adsorption sites between low and high levels of charcoal may cause the rather marked decrease in the removal efficiency when substantially less amount of carbon was used. The total adsorption sites on charcoal was less at lower carbon levels. When some of the sites were occupied by sugar, the available sites for patulin might decrease significantly in a relative sense. On the other hand, when charcoal was added at higher levels, there might still be plenty of space for patulin adsorption even though some sites were taken by sugar molecules. Consequently, the removal efficiencies were unaffected by sugar adsorption.

F. Effect of Juice Temperature on Patulin Removal

Results of the effect of juice temperature on patulin removal by granular charcoal OL are shown in Table 11. Due to the adsorption of patulin to charcoal and to the heat

Table 10. Effect of soluble solids on patulin removal (%) by various levels of charcoal OL and ADPP.

Charcoal level (mg/mL)	Temperature (°C)		
	10	12	18
OL			
4	79.36 (3.86)	74.52 (1.17)	69.28 (1.25)
12	86.91 (4.63)	85.73 (1.09)	80.67 (2.41)
20	92.59 (1.83)	93.91 (2.65)	88.24 (2.74)
24	96.47 (2.39)	95.14 (2.46)	89.78 (2.57)
28	97.23 (1.75)	96.88 (3.79)	95.28 (4.08)
32	97.84 (3.26)	97.40 (1.42)	97.15 (2.30)
ADPP			
0.4	69.38 (2.77)	65.04 (2.23)	58.47 (1.45)
1.2	76.73 (3.06)	77.91 (2.15)	71.36 (2.58)
2.4	88.82 (2.78)	89.24 (4.30)	83.81 (1.63)
2.8	92.16 (1.32)	91.73 (1.89)	86.25 (2.74)

* Initial patulin concentration = 600 ppb.

** Mixing time = 10 mins.

*** Mean ± (standard deviation).

Table 11. Effect of juice* temperature on patulin removal (%) by various levels of activated charcoal OL.

Charcoal level (mg/mL)	Temperature (°C)	
	22	93
4	75.53 (4.32)	77.58 (6.73)
12	90.64 (3.87)	92.24 (1.95)
20	93.91 (2.26)	93.76 (3.43)
24	93.48 (1.09)	92.25 (3.91)
28	96.88 (3.65)	97.27 (1.72)
32	97.42 (2.44)	97.73 (3.68)

* Initial patulin concentration = 600 ppb.

** Mixing time = 10 mins.

*** Mean ± (standard deviation).

destruction (approximately 5%) under current experimental conditions, it was expected to observe a higher percentage of patulin disappearance from juice heated to 93°C. Data, however, revealed that similar % of disappearance was obtained within each charcoal level.

A possible explanation for this phenomenon is that temperature can not only affect the rate at which adsorption occurs but also the extent to which adsorption occurs in a system (Weber, 1972). Weber (1972) reported that the rate of adsorption increased, while the extent of adsorption decreased in an exothermic adsorption process at elevated temperatures.

Adsorption of patulin by charcoal is exothermic. This was evidenced by the release of heat during the span of adsorption process. Thus, raising the reaction temperature favors the equilibrium toward desorption. It was likely that at elevated temperature of 93±5°C the disappearance of patulin from juice due to heat and the increase in rate of patulin adsorption were counteracted by the increase of patulin desorption due to higher temperature. As a consequence, the % of total patulin removal by granular activated charcoal OL was unaffected by the increased temperature.

The temperature effect on patulin removal of the other three granular charcoal and powdered charcoal is shown in Table 12. Temperature had little effect for all tested charcoal on % of removal except powdered carbons ADPP, SA

Table 12. Effect of juice* temperature on patulin removal (%) by activated charcoal.

Charcoal	Temperature (°C)	
	22	93
Granular** (24mg/mL)		
WVG	89.02 (0.84)	87.64 (2.53)
WVB	93.49 (1.08)	94.25 (3.22)
300	90.97 (2.14)	93.26 (3.35)
OL	93.48 (1.09)	92.25 (4.97)
Powdered** (2.4mg/mL)		
S-51	68.00 (2.94)	64.89 (1.75)
ADPP	89.24 (3.16)	93.46 (4.83)
SA	79.51 (1.82)	85.72 (3.15)
WPHP	78.87 (1.60)	77.39 (4.46)
SN	75.32 (0.75)	78.63 (2.07)

* Initial patulin concentration = 600 ppb.

** Mixing time = 10 mins.

*** Mean ± (standard deviation).

and S-51. ADPP and SA exhibited significant increases in patulin removal while S-51 showed a significant decrease when temperature increased to 93°C. It is not clear why temperature increased patulin adsorption for some charcoals while showing little or an opposite effect on the others. The balance among toxin destruction by heat, increased adsorption by the increase in rate and decreased adsorption by the increase in desorption may determine the gross effect of temperature.

G. Effects of Charcoal Treatment on Juice Quality

The effects of various levels of activated carbon OL on pH, total acidity, soluble solids, and color of juice are presented in Table 13. Results showed a general trend with increasing carbon dose, of increased pH and decreased acidity in the juice. Activated carbons are known to carry a net negative surface charge (Weber, 1972). The negative charge can be significant for the adsorption of hydrogen ions present in juice and results in the increase in pH and decrease in total acidity.

The total soluble solids also decreased with the increase in the amount of added carbon. The decrease was significant when OL was used at 28 and 32 mg/mL levels ($p<0.05$). The decrease of Brix could be due to the adsorption of fruit sugar by the charcoal. As discussed earlier, sucrose has been demonstrated to be adsorbed by activated charcoal

Table 13. Effects of various levels of charcoal OL on pH, titratable acidity, soluble solids and Hunter b values of juice in complete mixed batch system.

Charcoal level (mg/mL)	pH	TA*	Brix	Hunter b
0	3.48[a]	0.46[a]	11.4[a]	3.5[a]
4	3.48[a]	0.45[a]	11.4[a]	3.5[a]
12	3.49[a]	0.43[ab]	11.3[a]	3.5[a]
20	3.52[a]	0.41[b]	11.3[a]	3.3[a]
24	3.57[b]	0.39[bc]	11.1[ab]	3.2[a]
28	3.60[b]	0.38[c]	10.9[b]	3.0[ab]
32	3.62[b]	0.37[c]	10.7[b]	2.9[b]

* Expressed as % malic acid.

** Mixing time = 10 mins.

*** Values in the same column bearing similar superscripts are not siginificantly different at 5% level.

(Cooney and Roach, 1979). A decrease in juice yellowness, expressed in Hunter b values, was also significant at the 28 and 32 mg/mL usage levels.

Table 14 shows the effects of charcoal treatment on juice quality when used at levels of 24 and 2.4 mg/mL for granular and powdered carbons, respectively. It can be seen that charcoal treatment resulted in decreases in pH, titratable acidity, total soluble solids and Hunter yellowness under the experimental conditions. There was not much difference between granular and powdered charcoal and among the same types of charcoal.

Figure 11 is the spectrum of apple juice and Figure 12 shows a typical scan spectrum of juice treated with charcoal. A decreased adsorption in the visible and UV wave length range was observed for juice treated with charcoal.

H. Effect of Charcoal Treatment on Juice Flavor

Ethyl-2-methyl butyrate, propyl butyrate, hexanal, trans-2-hexanal, cis-2-hexen-1-ol, trans-2-hexen-1-ol, and heptanol have been reported as important contributors to apple juice aromas (Flath et al., 1967; Poll and Flink, 1984; Schobinger, 1981). The effect of charcoal treatment on apple juice flavor was, therefore, studied with these selected components (Figures 13, 14, and 15). Results show that both granular and powdered activated charcoal significantly reduced these important apple juice aromas.

Table 14. Effects of charcoal treatment on pH, titratable acidity, soluble solids and Hunter b values of juice in complete mixed batch system.

Charcoal	pH	TA*	Brix	Hunter b
None	3.48	0.46	11.4	3.5
Granular** (24mg/mL)				
WVG	3.51	0.43	11.0	3.1
WVB	3.55	0.39	11.0	3.4
300	3.53	0.40	11.1	3.3
OL	3.57	0.39	11.1	3.2
Powdered** (2.4mg/mL)				
S-51	3.50	0.41	11.2	3.0
ADPP	3.51	0.42	11.2	3.1
SA	3.50	0.41	11.2	3.0
WPHP	3.50	0.41	11.2	3.3
SN	3.49	0.41	11.2	3.0

* Expressed as % malic acid.

** Mixing time = 10 mins.

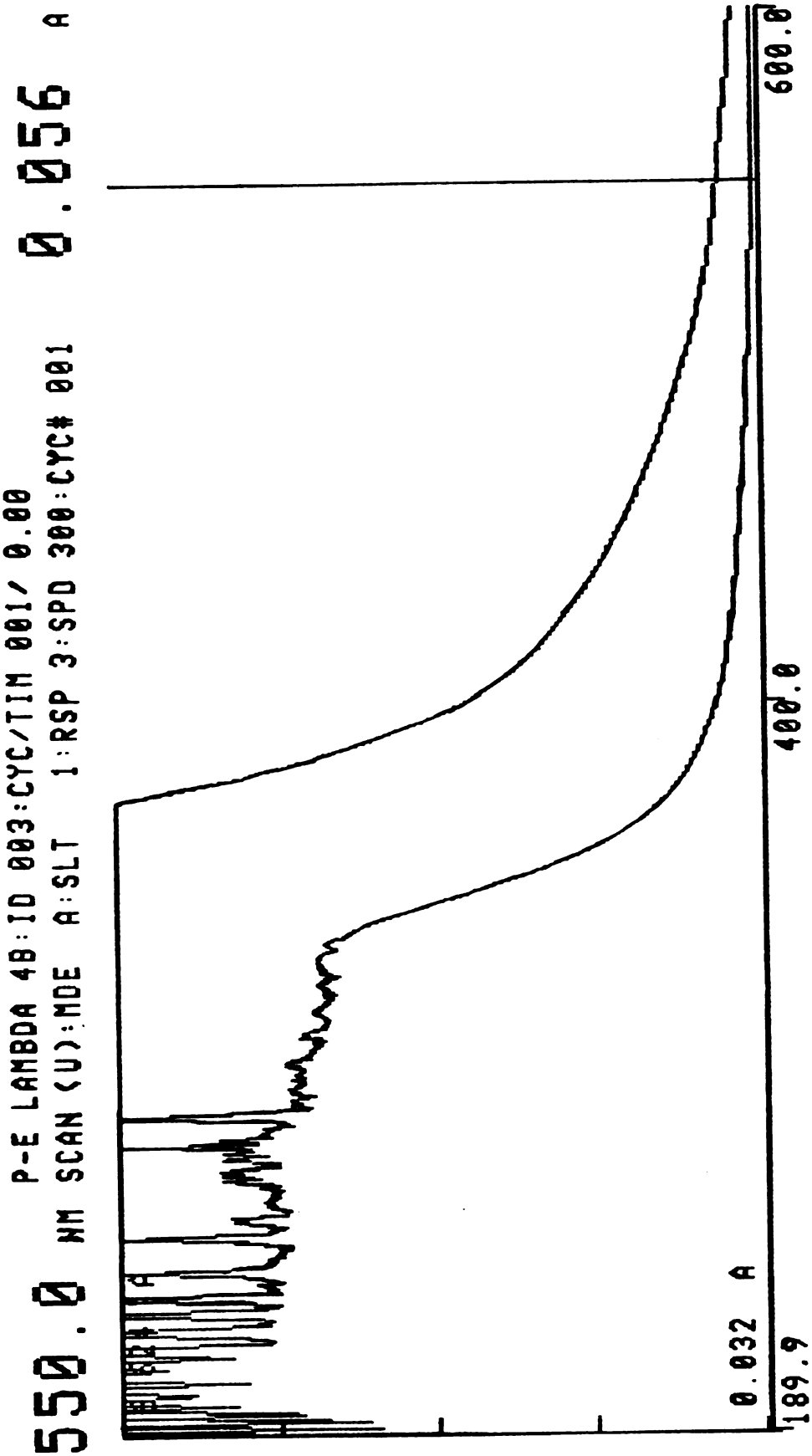

Figure 11. Spectrum absorption (190-600 nm) of apple juice.

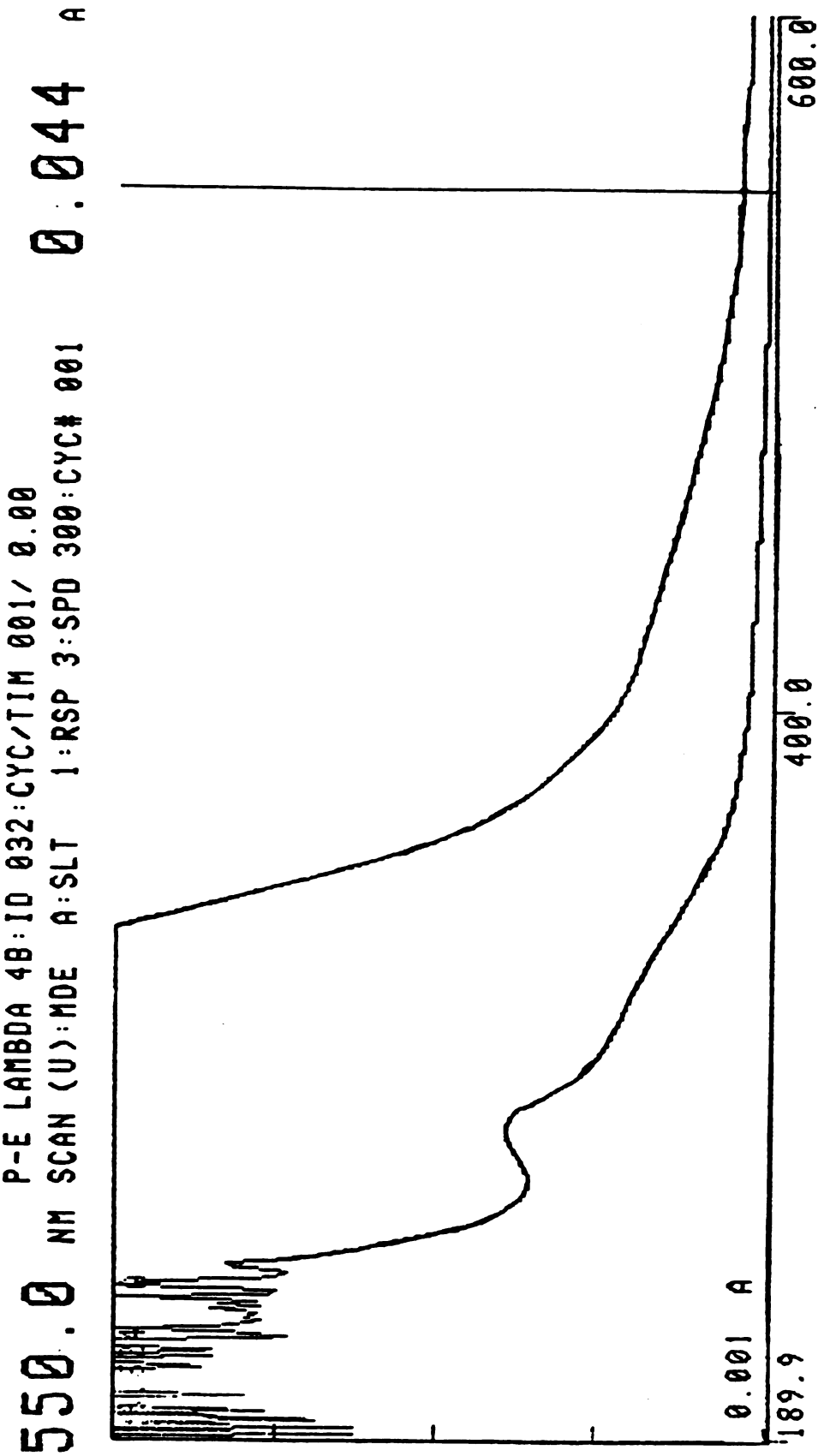

Figure 12. Spectrum absorption (190-600 nm) of apple juice treated with charcoal.

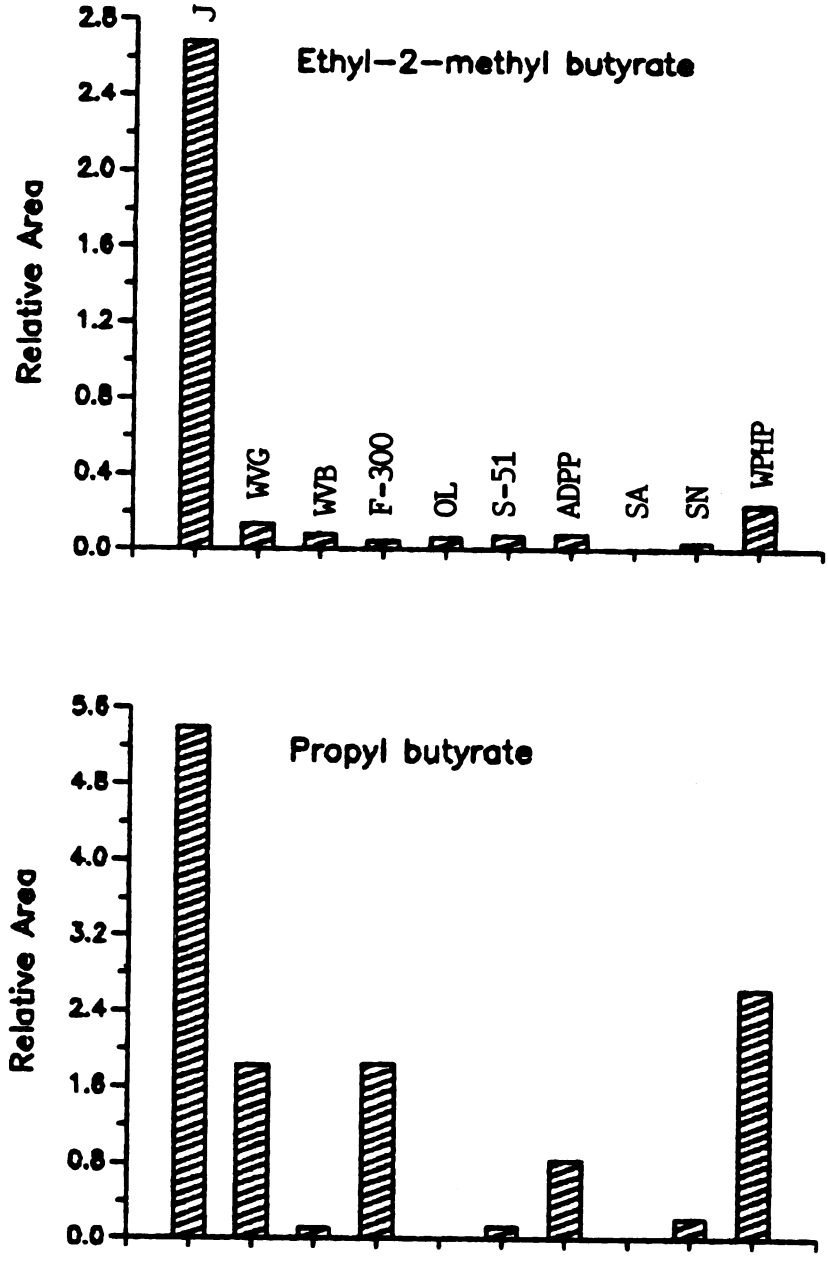

Figure 13 – Effect of charcoal treatment on selected esters of apple juice flavor

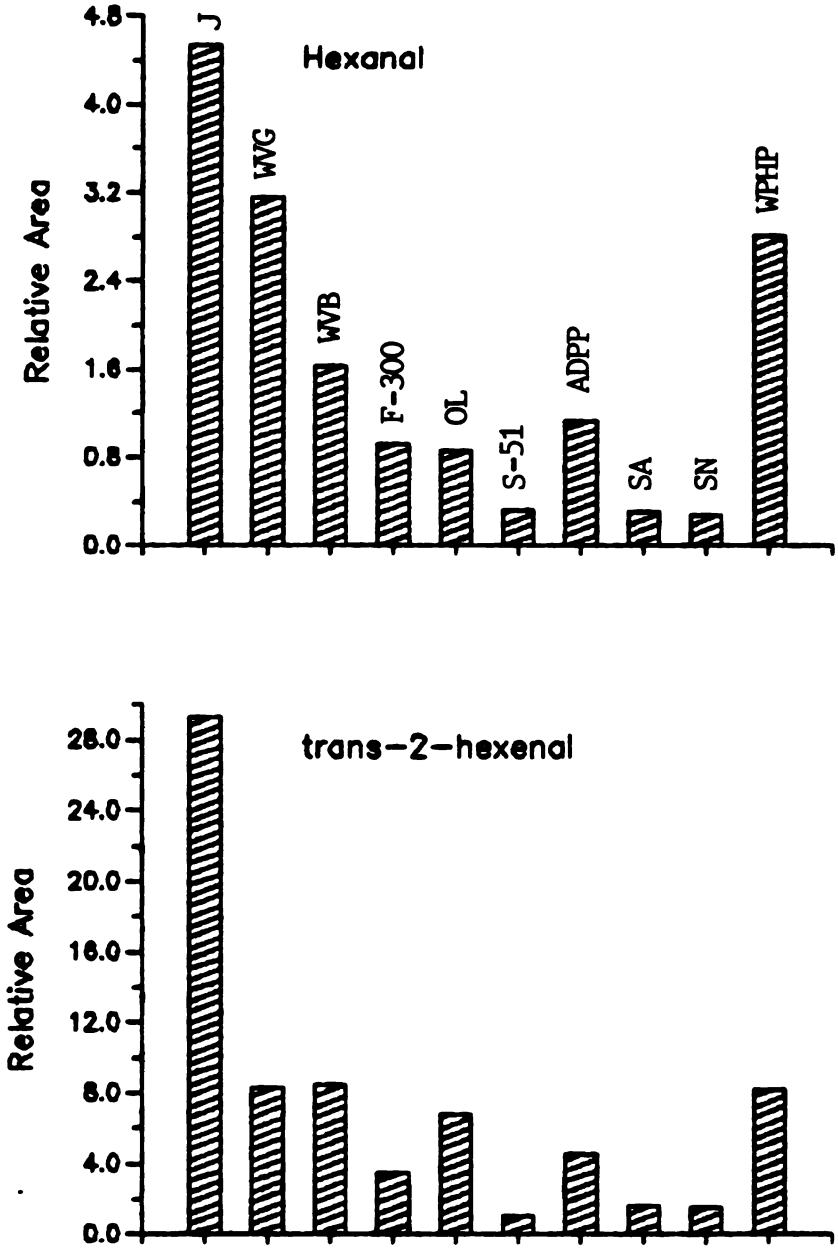

Figure 14 – Effect of charcoal treatment on selected aldehydes of apple juice flavor

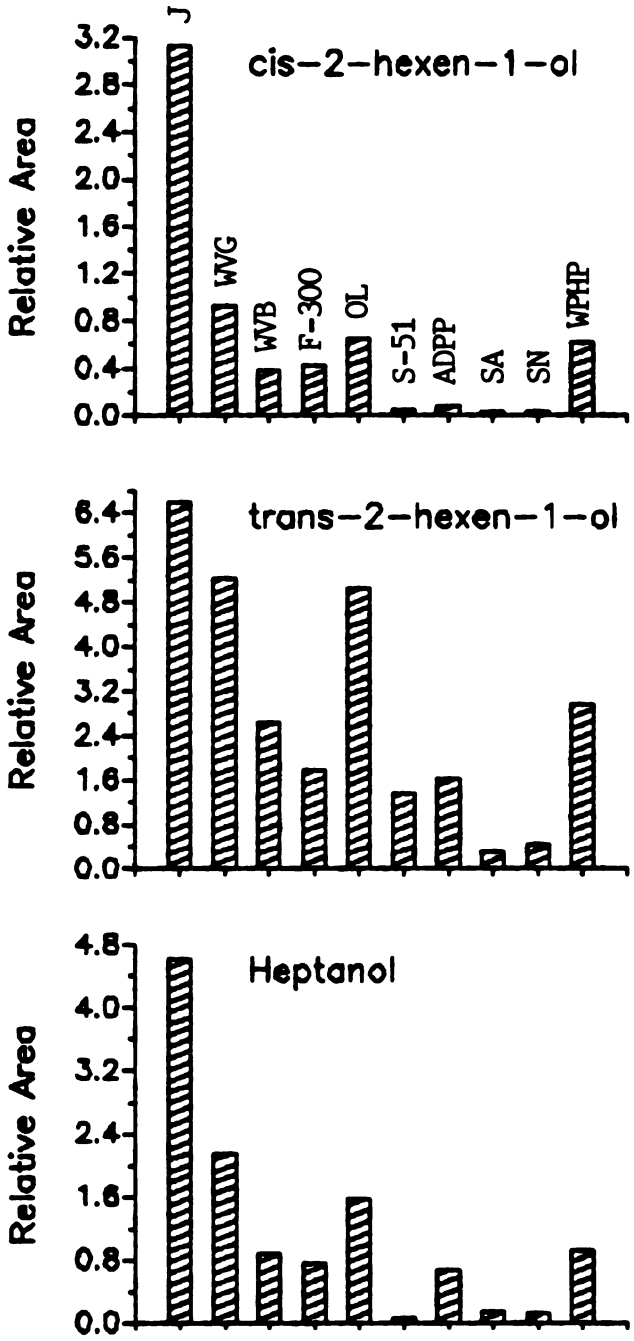

Figure 15– Effect of charcoal treatment on selected alcohols of apple juice flavor

Although powdered carbons were used at much lower level than granular charcoal, their adverse effect was greater than that of granular activated charcoal. Charcoal has been recognized and used as an odor absorber. Results of this study further demonstrated its capacity to absorb flavor compounds.

(II) Removal of Patulin by Fixed Bed Mini-Column

A. Effect on Patulin Removal

Tables 15, 16 and 17 present patulin remaining in 600 ppb spiked juice after passing through columns of various carbon loading, column diameter, and flow rate. It can be seen that increased carbon loading, decreased flow rate and column diameter decreased residual patulin. Results were analyzed by multiple linear regression procedures and the resulting regression equations are listed in Table 18. The low R^2 values indicated that equations derived from design parameters of carbon loading, column diameter and flow rate cannot satisfactorily predict residual patulin in the effluent.

Mathews and Weber (1975) used PRAXIS model to estimate external and intraparticle diffusion coefficients and these coefficients along with design parameters were used to simulate results from mini-columns by a second model "MADAM" (Michigan Adsorption Design and Application Model). Good agreement between experimental and predicted profile was

Table 15. Residual patulin concentration in apple juice* eluted from mini-columns packed with charcoal WVG of various loading, column diameter and flow rate.

Loading (gm)	Flow rate (mL/min)	Diameter (cm)	Eluate (mL)	Res. patulin (ppb)
12	12.5	0.7	100-500	N.D.
12	25	0.7	0-300	N.D.
			375-400	2.94
			475-500	8.54
12	37.5	0.7	0-100	N.D.
			175-200	2.77
			275-300	9.44
			375-400	15.04
			475-500	24.91
12	25	0.7	0-300	N.D.
			375-400	2.94
			475-500	8.54
12	25	1.9	0-100	N.D.
			175-200	3.8
			275-300	14.16
			375-400	28.28
			475-500	39.97
12	25	2.9	75-100	4.37
			175-200	18.89
			275-300	27.30
			375-400	43.73
			475-500	55.84
24	25	2.9	0-400	N.D.
			475-500	2.77
12	25	0.7	0-300	N.D.
			375-400	2.94
			475-500	8.54
16	25	0.7	100-400	N.D.
			475-500	1.3
20	25	0.7	100-500	N.D.
24	25	0.7	100-500	N.D.
24	25	1.9	100-500	N.D.

* Initial patulin concentration = 600 ppb.

Table 16. Residual patulin concentration in apple juice* eluted from mini-columns packed with charcoal WVB of various loading, column diameter and flow rate

Loading (gm)	Flow rate (mL/min)	Diameter (cm)	Eluate (mL)	Res. Patulin (ppb)
12	12.5	0.7	0-500	N.D.
12	25	0.7	0-300	N.D.
			375-400	5.54
			475-500	9.46
12	37.5	0.7	0-200	N.D.
			275-300	6.67
			375-400	10.22
			475-500	16.87
12	25	0.7	0-300	N.D.
			375-400	5.54
			475-500	9.46
12	25	1.9	0-100	N.D.
			175-200	6.51
			275-300	14.87
			375-400	22.31
			475-500	30.16
12	25	2.9	0-100	N.D.
			175-200	13.80
			275-300	23.22
			375-400	38.03
			475-500	51.63
16	25	2.9	0-200	N.D.
			275-300	9.74
			375-400	18.89
			475-500	33.12
20	25	2.9	0-400	N.D.
			400-500	5.36
			575-600	10.47
			675-700	17.71
			775-800	32.29
24	25	2.9	0-600	N.D.
			675-700	1.58
			775-800	11.64
			975-1000	28.97
12	25	0.7	0-300	N.D.
			375-400	5.54
			475-500	9.46
16	25	0.7	0-400	N.D.
			475-500	1.85
20	25	0.7	0-500	N.D.
24	25	0.7	0-500	N.D.

* Initial patulin concentration = 600 ppb.

Table 17. Residual patulin concentration in apple juice* eluted from mini-columns packed with charcoal OL of various loading, column diameter and flow rate.

Loading (gm)	Flow rate (mL/min)	Diameter (cm)	Eluate (mL)	Res. Patulin (ppb)
12	12.5	0.7	0-500	N.D.
12	25	0.7	0-300	N.D.
			375-400	1.18
			475-500	5.32
12	37.5	0.7	0-300	N.D.
		0.7	375-400	5.54
			475-500	13.79
12	25	0.7	0-300	N.D.
			375-400	1.18
			475-500	5.32
12	25	1.9	0-100	N.D.
			175-200	2.68
			275-300	9.45
			375-400	25.39
12	25	2.9	75-100	3.09
			175-200	15.75
			275-300	24.19
			375-400	33.85
			475-500	49.51
24	25	2.9	0-500	N.D.
12	25	0.7	0-300	N.D.
			375-400	1.18
			475-500	5.32
16	25	0.7	0-400	N.D.
			475-500	2.45
20	25	0.7	0-500	N.D.
24	25	0.7	0-500	N.D.
24	25	1.9	0-500	N.D.
24	25	2.9	0-500	N.D.

* Initial patulin concentration = 600 ppb.

Table 18. Multiple linear regression equations for the prediction of residual patulin in juice eluted from mini-columns.

Carbon	P*	Equation**	R^2
WVG	0.05	$Y=0.029X_4-1.152X_1+4.417X_3+8.821$	0.536
WVB	0.05	$Y=-1.216X_1+7.119X_3+0.032X_4+7.018$	0.557
WVB	0.10	$Y=-1.216X_1+7.119X_3+0.032X_4+0.417X_2-3.412$	0.591
OL	0.05	$Y=2.171X_5-0.856X_1+0.021X_4+7.566$	0.511
Pooled***	0.05	$Y=1.922X_5-1.079X_1+0.026X_4+0.281X_2+3.795$	0.527

* α level.

** X_1: carbon loading (gm); X_2: flow rate (mL/min); X_3: column diameter (cm); X_4: eluate volume (mL); X_5: column area (cm^2).

*** Pooled: data generated from 3 carbons were pooled and analyzed.

reported. External and intraparticle diffusion coefficients have been demonstrated as important factors in determining the rate of patulin adsorption by activated charcoal. Future prediction of remaining patulin in effluent from mini-columns should proceed in the direction of searching for these two mass transfer coefficients and use them along with design parameters to see if better prediction can be resulted in.

The practical feasibility of removing patulin from apple juice using column-type operation was evaluated using charcoal WVG as an example. This is performed by constructing the breakthrough curve of data obtained from columns of 2.9 cm in diameter and packed with 12, 16, 20, or 24 g of charcoal WVG (Appendix D) The flow rate was 25 mL/min. Thirty ppb was chosen as the maximal allowed residual patulin concentration in the effluent. The breakthrough points occurred at 12, 20, 32, and 40 min for columns loaded with 12, 16, 20, and 24 g of charcoal, respectively.

The equations used in calculation were adapted from Faust and Aly (1987). Equations and calculation are listed in the appendix D. Based on a 5,000 gallons/day processing volume, an adsorber of 6 ft in height, 2.18 ft in diameter and a loading of 416 lbs of charcoal are required to decrease toxin concentration from the initial 600 to 30 ppb. The calculated column diameter and height make it seems feasible from a physical construction point of view. Charcoal values

at approximately $3/lb, depending on its source and variety. A charcoal loading of 416 lbs costs $1248. A 5,000 gal/day juice processing corresponds to a production of 10014 bottles of 1.89 Liters/bottle. Thus, a bottle of 1.89 liters costs approximately 13 cents more after patulin treatment. Economically, it also seems feasible. Of course, in-depth calculation of process design parameters, capital investment and cost analysis are required.

B. Effects on Juice Quality

The titratable acidity, Brix, and Hunter b values of juice eluted from mini-columns as affected by column diameter and flow rate are presented in Tables 19 and 20, respectively. Table 18 shows that regardless of column diameter, total acidity, soluble solids and the yellowness of juice were significantly decreased in the first couple hundred mLs of effluent. The adsorption was lessened at the latter stage of inflow. Soluble solids and Hunter b values of the effluent at the end of the flow were nearly the same as those of the untreated juice. These two juice quality characters were less affected by the variation in column diameter. Titratable acidity was lowered not only at the beginning but also at the end of the flow and the decrease was the most for effluent from the smallest column diameter. Carbons OL and WVG resulted in juice similar in quality.

Effluent eluted from mini-columns differed in flow rate

Table 19. Effect of column diameter on titratable acidity, soluble solids and Hunter b value of juice eluted from mini-columns.

Column Diameter (cm)	Elute (mL)	TA*	Soluble Solids (B°)	Hunter b
Juice	-	0.49	11.2	3.6
OL**				
0.7	100	0.14	8.0	2.7
	200	0.27	10.2	2.9
	300	0.31	11.0	3.0
	400	0.34	11.2	3.0
	500	0.36	11.2	3.3
1.9	100	0.19	8.4	2.9
	200	0.32	10.4	2.9
	300	0.38	10.8	3.1
	400	0.39	11.0	3.1
	500	0.40	11.2	3.6
2.9	100	0.19	9.8	2.9
	200	0.33	10.6	3.0
	300	0.39	11.0	3.2
	400	0.40	11.2	3.3
	500	0.42	11.2	3.6
WVG**				
1.9	100	0.23	9.2	2.4
	200	0.33	10.7	2.8
	300	0.38	11.0	3.0
	400	0.40	11.2	3.4
	500	0.42	11.2	3.4
2.9	100	0.29	9.8	2.6
	200	0.40	10.8	2.8
	300	0.41	10.0	3.2
	400	0.43	11.2	3.5
	500	0.43	11.2	3.6

* Titratable acidity is expressed as % malic acid.

** Carbon loading = 24 mg/mL; flow rate = 25 mL/min.

Table 20. Effect of flow rate on titratable acidity, soluble solids and Hunter b value of juice eluted from mini-columns.

Flow Rate (mL/min)	Elute (mL)	TA*	Soluble Solids (B°)	Hunter b
Juice	-	0.49	11.2	3.6
OL**				
12.5	100	0.21	8.4	2.7
	200	0.24	9.8	2.8
	300	0.31	10.6	2.9
	400	0.33	11.2	3.1
	500	0.36	11.2	3.4
25	100	0.19	8.8	2.9
	200	0.33	10.4	3.0
	300	0.39	10.8	3.2
	400	0.40	11.0	3.3
	500	0.42	11.2	3.6
37.5	100	0.21	9.8	3.1
	200	0.33	10.6	3.4
	300	0.38	11.0	3.6
	400	0.39	11.2	3.6
	500	0.42	11.2	3.6

* Titratable acidity is expressed as % malic acid.

** Carbon loading = 24 mg/mL; column diameter = 2.9 cm.

also showed significant decrease in titratable acidity, soluble solids and Hunter b values for the first couple hundred mLs of influent (Table 20). The adverse effect of charcoal treatment was less at the end of inflow. Quality of juice from columns with a flow rate of 12.5 mL/min appeared to be most affected by charcoal treatment.

Table 21 shows the juice quality of effluent from an 8-liter inflow. It can be seen that yellowness of juice as determined by Hunter b value as well as the total soluble solids were the same as the influent juice and the titratable acidity was only 0.02% lower than the control value. These results indicated that in column-type operation, the adverse effects of charcoal treatment on juice quality appear only at the beginning of the effluent and should not pose a problem when large volumes of juice are passed through the column. The influence of charcoal on juice flavor is yet to be determined.

Table 21. Effect of charcoal treatment on titratable acidity, soluble solids and Hunter b value of 8-liter juice eluted from mini-column packed with charcoal OL.

Elute (mL/min)	TA* (%)	Soluble Solids (B°)	Hunter b
Juice			
-	0.50	11.2	3.6
Treated			
500	0.08	8.0	0.5
1000	0.25	11.0	1.1
1500	0.35	11.0	1.4
2000	0.40	11.2	2.0
2500	0.40	11.2	2.5
3000	0.43	11.2	2.7
3500	0.46	11.2	3.0
4000	0.46	11.2	3.1
4500	0.46	11.2	3.2
5000	0.46	11.2	3.3
5500	0.46	11.2	3.3
6000	0.46	11.2	3.3
6500	0.46	11.2	3.6
7000	0.47	11.2	3.6
7500	0.47	11.2	3.6
8000	0.47	11.2	3.6

* Titratable acidity is expressed as % malic acid.

** Carbon loading = 24 mg/mL; column diameter = 2.9 cm; flow rate = 25 mL/min.

CONCLUSIONS

In this study, the significance of patulin contamination in apple juice and the need of developing an effective and acceptable approach for patulin removal were discussed. The basic chemical properties and biological activities of patulin as well as the characters of activated charcoal were reviewed.

Attention was directed to four aspects: 1) the rate and adsorption capacity of patulin by activated charcoals in batch model systems, 2) the effect of apple juice pH, soluble solids, temperature, and toxin concentration on the efficiency of patulin removal, 3) the effect of charcoal treatment on juice flavor and overall quality, and 4) the feasibility of patulin removal by mini-column models.

The adsorption of patulin was a very fast process for both types of charcoals. It reached equilibrium in 10 and 5 min for granular and powdered carbons, respectively. Two adsorption rates, a fast adsorption followed by a slower one, were noted for granular charcoal. The slow-down of toxin adsorption could be caused by the shift of rate-limiting step from intraparticle to external diffusion, which in turns was related to toxin concentration in bulk juice phase.

The slow-down observed for the granular charcoal was not

noted for powdered charcoals. The level-off of the adsorption process by powdered carbons was, therefore, believed to be caused by the saturation of powdered charcoals. The empirical rate parameters of the powdered charcoals were 2-3 times that of the granular charcoal. Rate data from double deionized distilled water indicated that it is not a good approach to study patulin removal in pure water system since it can lead to over-estimation of the rate of adsorption.

Adsorption capacity, determined by Freundlich isotherms, was affected by total surface area of charcoal and the components in apple juice. In general, powdered charcoals had adsorption capacity approximately ten times larger than that of the granular charcoals. Capacity data of the double deionized distilled water further suggested that study of patulin removal in pure water system can over-estimate the performance of charcoal.

The extent of patulin adsorption depended on the initial toxin concentration. Increasing the initial toxin concentration increased the amount of patulin being adsorbed. The magnitude of concentration gradient could affect the rate-limiting step and result in differences in the effective adsorption capacity.

Patulin adsorption was not affected by juice pH regardless of charcoal types; whereas the adsorption efficiency decreased as the soluble solids of juice increased. High temperature fastened the rate of adsorption, however,

temperature had little effect on the percentage of total patulin removed. This could be due to the fact that high temperature favored the desorption of toxin from charcoal.

The acidity and total soluble solids in the juice decreased as the carbon dosage increased. Although a decreasd absorption in the visible and U.V. range for juice treated with charcoal was observed, the yellowness of juice as determined by Hunter b value was not significantly affected. Both granular and powdered charcoal significantly reduced the amount of major flavor components in apple juice.

Mini-column model indicated that patulin could be effectively removed through continuous flow. The adverse effects of charcoal treatment on juice quality appeared only at the initial stage of the effluent and should not be considered as a problem when large volume of juice are passed through the column and pooled.

RECOMMENDATIONS FOR FUTURE RESEARCH

1. Identify the compound(s) that competes with patulin for adsorption to activated charcoal and study its adsorption relation with patulin in juice samples. Such information can be used to better monitor the removal of patulin from apple juice.

2. Examine the use of superactivated charcoal on patulin removal and its effect on resulting juice quality. Superactivated charcoal has been used successfully in the treatment of drug overdose due to the lower requirement in dose. The advantage of using less charcoal can be beneficial to the adverse effect of charcoal on juice color and flavor.

3. Determine the growth of microbials on activated charcoal. The growth of microbials has been reported to affect the life span of charcoal. It will be important to know how charcoal is affected and how it affects juice quality.

4. Develop procedures for the regeneration of used charcoal in order to economically reutilize charcoal.

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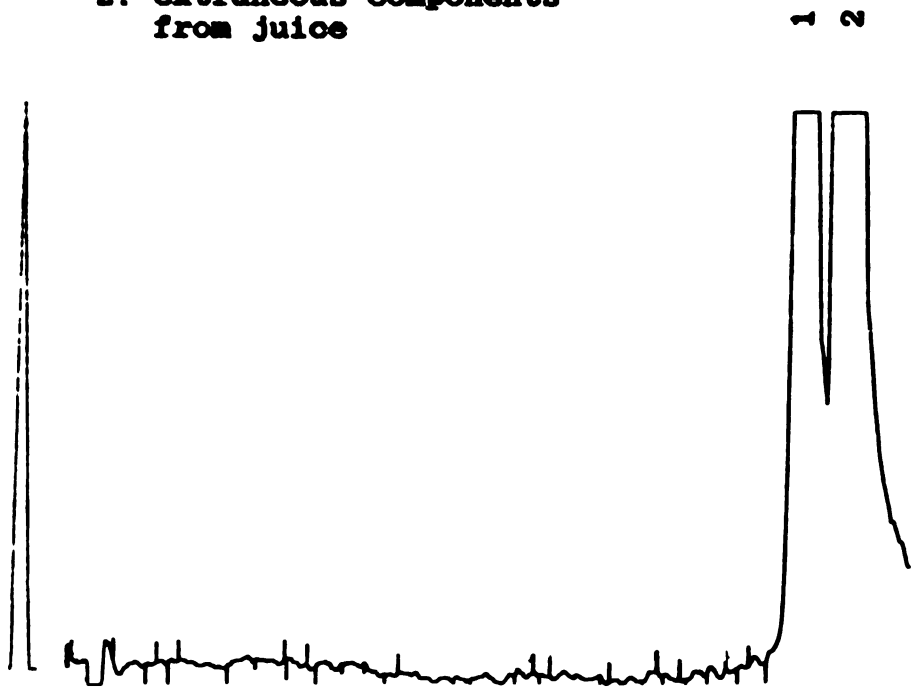
APPENDICES

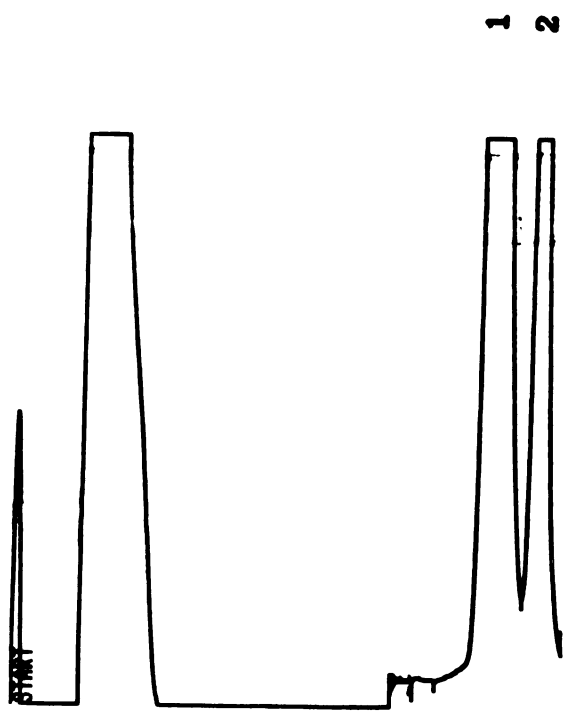
Appendix A. HPLC chromatograms of patulin extracted from apple juice.

1. patulin
2. extraneous components from juice

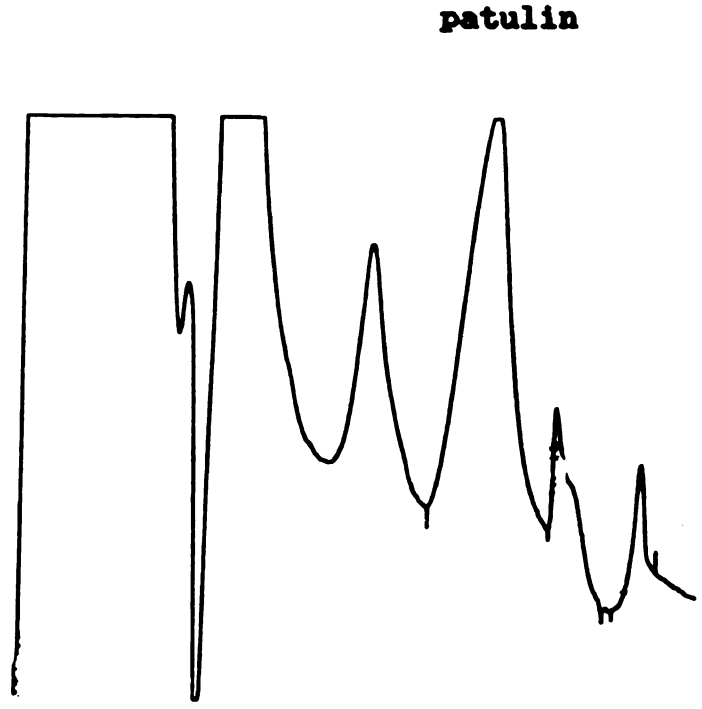


A.1. HPLC chromatogram of patulin in ethyl acetate extracted by AOAC method from apple juice.

1. patulin
2. extraneous components from juice

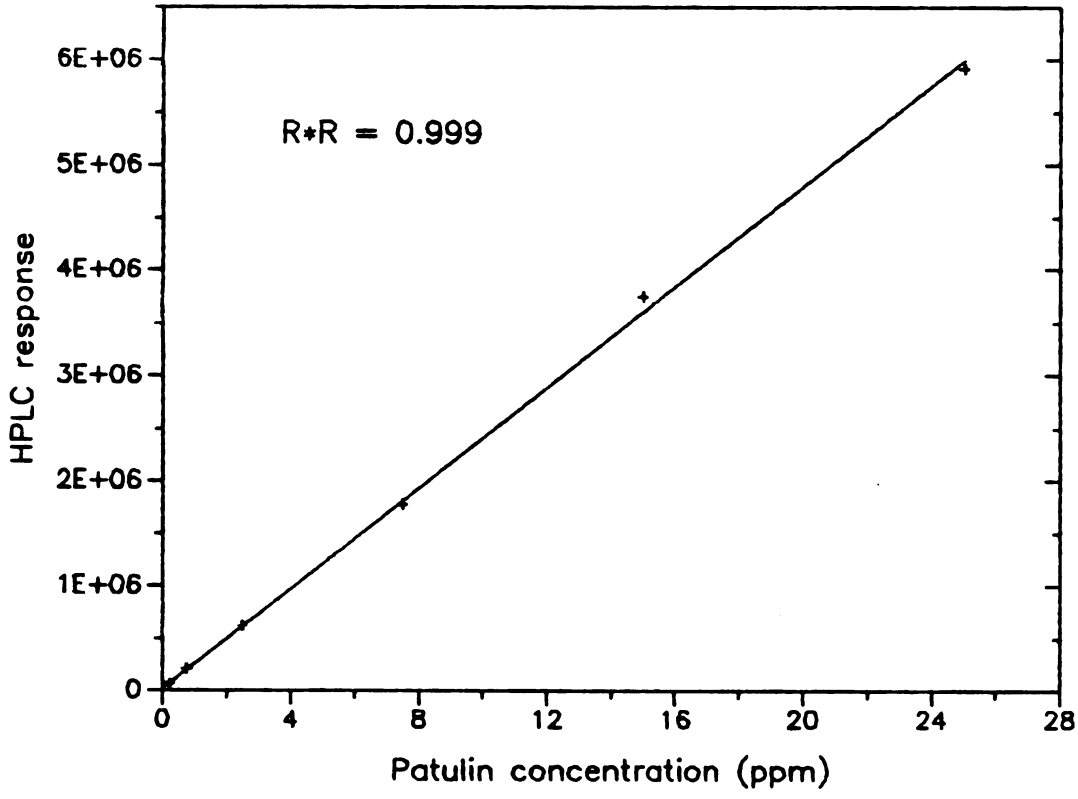


A.2. HPLC chromatogram of patulin in ethyl acetate-methanol (10:90) extracted by AOAC method from apple juice.



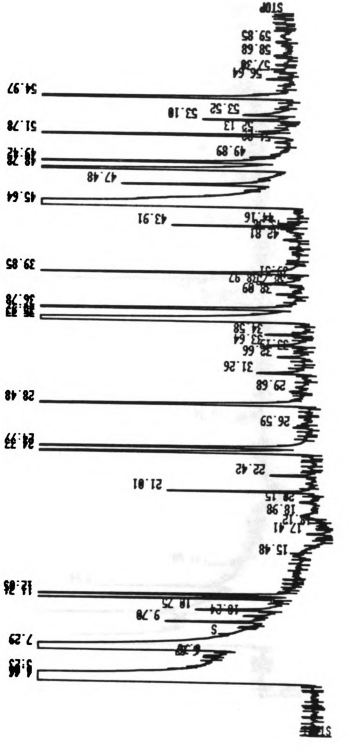
A.3. HPLC chromatigram of patulin in ethyl acetate-methanol (10:90) extracted by Torres method from apple juice.

Appendix B. Standard curve of patulin quantitation by HPLC.

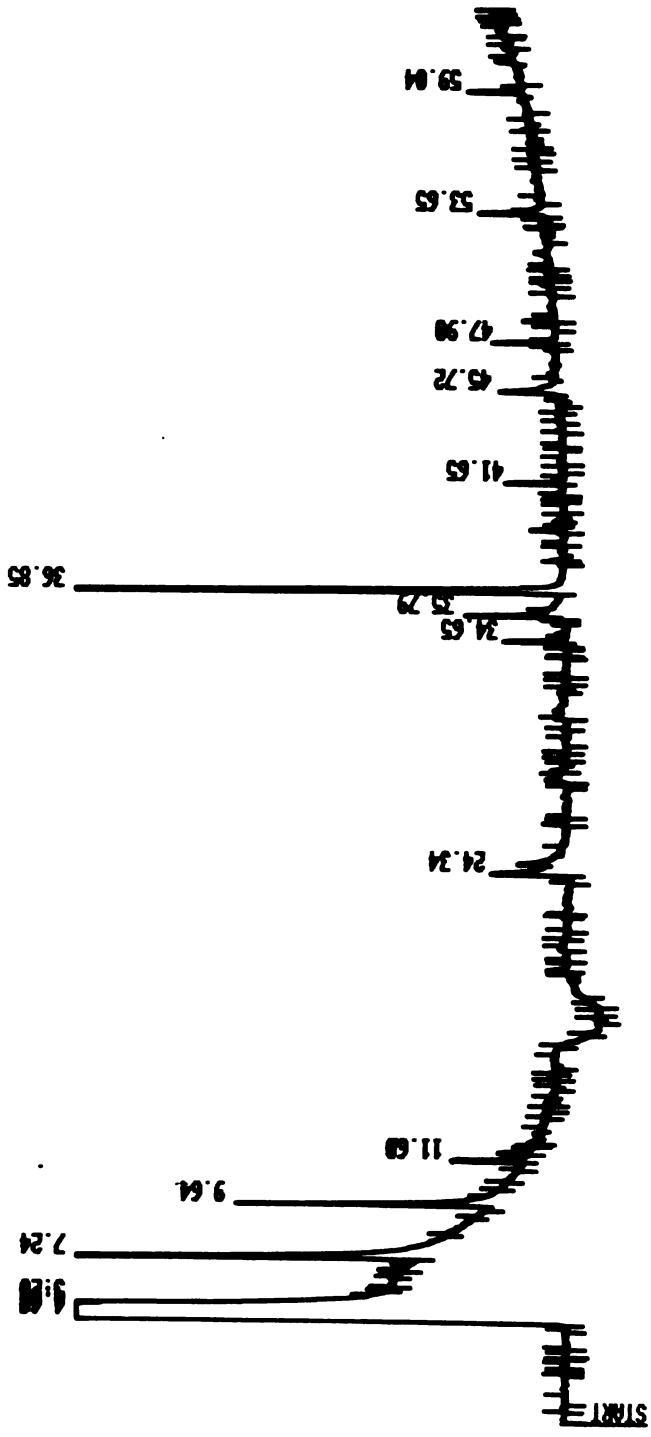


Appendix B. Standard curve of patulin quantitation by HPLC.

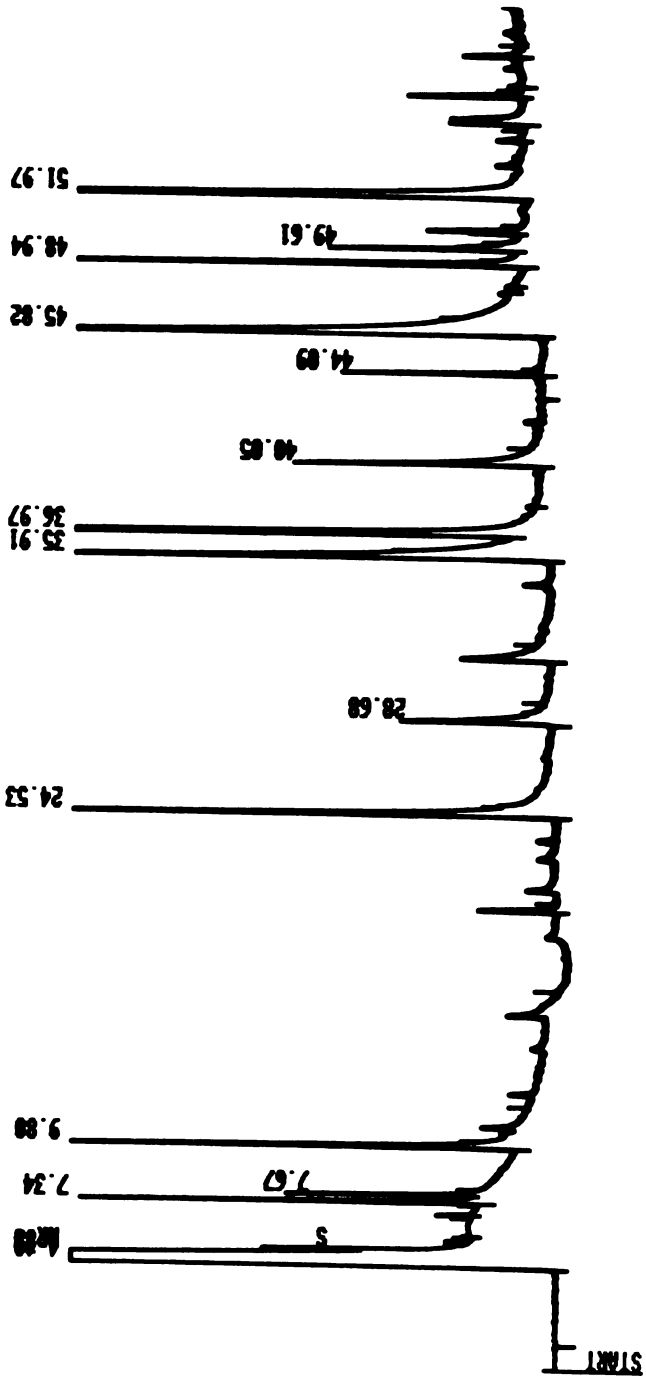
Appendix C. GC chromatograms of selected aroma compounds in apple juice collected by three different methods.



C.1. GC chromatogram of apple juice flavor extracted by gas stripping-trapping method.



C.2. GC chromatogram of apple juice flavor extracted by vacuum stripping-trapping method.



C.3. GC chromatogram of apple juice flavor extracted by Likens-Nikerson method.

Retentition time	Compound
7.67	methyl acetate
22.40	ethyl-2-methyl butyrate
24.72	hexanal
28.51	propyl butyrate
35.83	trans-2-hexenal
48.90	trans-2-hexen-1-ol
49.55	cis-2-hexen-1-ol
51.91	heptanol

Appendix D. Equations and calculation of charcoal loading and adsorber diameter and height of patulin removal by column-type operation.

EQUATIONS

$$\text{contact time} = \frac{\text{bed volume } (ft^3) \times 7.48\ (gal/ft^3)}{\text{flow rate (gal/min)}}$$

$$\text{usage rate} = \frac{\text{weight of carbon in column (lb)}}{\text{volume at breakthrough (gal)}} \times 1000$$

$$\text{hydraulic loading } (gal/min/ft^2) = \frac{\text{flow rate (gal/min)}}{\text{column area } (ft^2)}$$

$$\text{adsorber area} = \frac{\text{flow (gal/day)}}{\text{hydraulic load.}(gal/min/ft^2) \times 1440(min/day)}$$

CALCULATION

Production volume: 5,000 gal/day

$$\text{bottle} = \frac{5{,}000\ (gal/day) \times 3.785\ (L/gal)}{1.89\ (L/Bot)}$$

$$= 10014 \text{ bottles}$$

$$\text{flow } (ft^3/min) = \frac{5{,}000\ (gal/day)}{1440\ (min/day) \times 7.48\ (gal/ft^3)}$$

$$= 0.464\ ft^3/min$$

contact time = 32 mins (from exhaustion curve)

$$\text{volume of carbon } (ft^3) = 32\ (min) \times 0.464\ (ft^3/min)$$

$$= 14.854\ ft^3$$

flow rate = 25 (mL/min) x 0.000264 (gal/mL)

= 0.0066 gal/min

$$\text{hydraulic loading} = \frac{0.0066\ (\text{gal/min})}{[2.9/2\ (\text{cm}) \times 0.0328\ (\text{ft/cm})]^2 \times \pi}$$

$$= 0.928\ \text{gal/min/ft}^2$$

$$\text{adsorbing area } (\text{ft}^2) = \frac{5{,}000\ (\text{gal/day})}{1440\ (\text{min/day}) \times 0.928\ (\text{gal/min/ft}^2)}$$

$$= 3.74\ \text{ft}^2$$

adsorber diameter = $2 \times (3.74\ \text{ft}^2/\pi)^{0.5}$ = 2.18 ft

carbon bed depth = $14.854\ \text{ft}^3 / 3.74\ \text{ft}^2$ = 3.97 ft

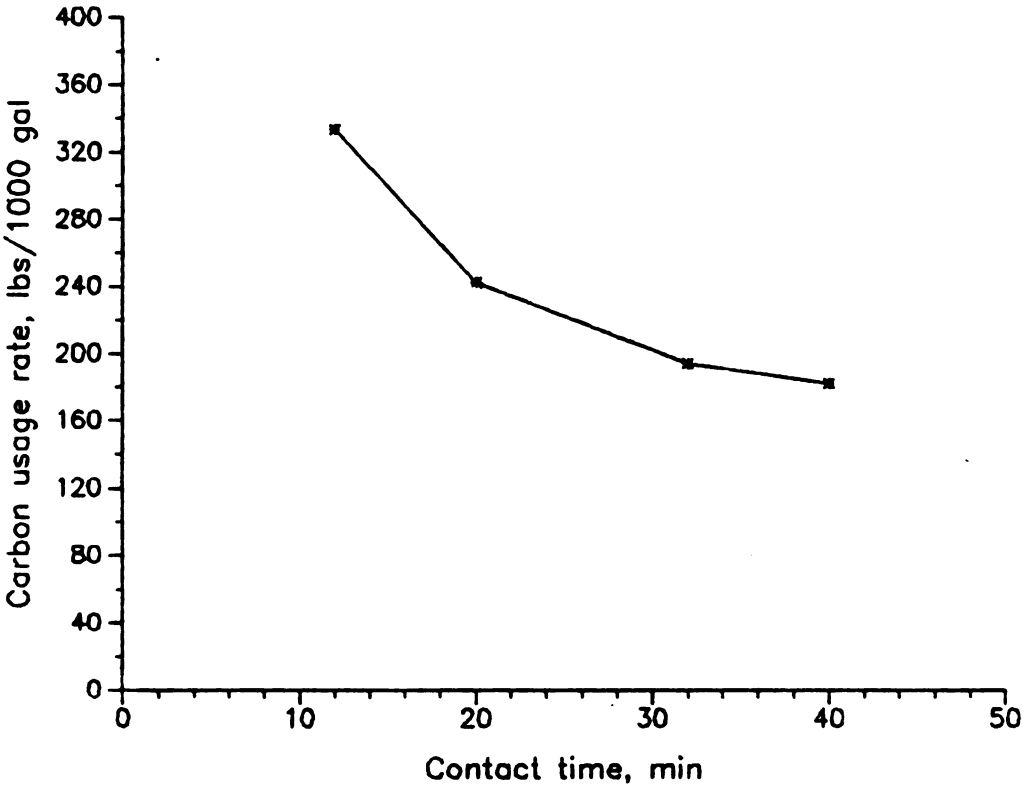
height of adsorber = 3.97 ft x 1.5

= 6 ft (based on 50% bed expansion)

weight of carbon = $14.854\ (\text{ft}^3) \times 28\ (\text{lb/ft}^3)$

= 415.92 lb

$$\text{cost/bot} = \frac{3\ (\$/\text{lb}) \times 415.92\ (\text{lb})}{10{,}014\ (\text{bot})} = 0.13\ (\$/\text{bot})$$



Carbon usage rate vs. system resistance time.