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**MATRIZES PARA LIBERAÇÃO CONTROLADA DO FEROMÔNIO DE  
AGREGAÇÃO RINCOFOROL**

**SALVADOR – BAHIA**

**2018**

**ARÃO CARDOSO VIANA**

**MATRIZES PARA LIBERAÇÃO CONTROLADA DO FEROMÔNIO DE  
AGREGAÇÃO RINCOFOROL**

Tese apresentada ao Curso de Doutorado em Biotecnologia do Programa de Pós-graduação em Biotecnologia – RENORBIO do instituto multidisciplinar em saúde da Universidade Federal da Bahia, como requisito parcial à obtenção do título de doutor em Biotecnologia. Área de concentração: Recursos Naturais.

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Elaborada por:

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*(Marthin Luther King)*



## RESUMO

VIANA, A. C. **Matrizes para liberação controlada do feromônio de agregação rincoforol. 2018. 133f. Tese (Doutorado)** - Programa de Pós-Graduação em Biotecnologia – RENORBIO, Universidade Federal da Bahia, Salvador-BA.

O uso de feromônios tem sido uma alternativa eficiente para monitoramento e controle de pragas, mantendo a população em níveis economicamente aceitáveis. Para o controle do *Rhynchophorus palmarum* (broca do coqueiro), o uso de agrotóxicos não é viável, sendo usado o feromônio de agregação rincoforol como isca para captura em conjunto com armadilhas. Em virtude de condições ambientais, a cinética de evaporação dessa substância pode ser prejudicada ocasionando taxas de liberação excessivas ou baixas para detecção pelo *R. palmarum*. O presente estudo teve como objetivo avaliar o uso de matrizes inorgânicas e uma matriz orgânica como dispositivo para liberação controlada do rincoforol. Foi realizado a validação de método analítico para identificação e quantificação do rincoforol por cromatografia gasosa acoplada a um detector de espectrometria de massas, tanto na sua forma pura como também do recuperado após adsorção nas matrizes. Foram sintetizadas ou adquiridas as estruturas inorgânicas: Zeólitos (ZSM-5 SAR 30/50/80; MCM-22 SAR 30/50/80; Zeólito L; Zeólito Y amoniacal e Sódico; Silicalita-1), Argilas (Montmorilonita K10 e KSF; caulim) e Na-Magadiíta, como também a estrutura orgânica amilose de batata. Caracterização das matrizes foram realizadas, mostrando similaridade com matrizes obtidas através de padrões fornecidos pela literatura. Avaliações da estabilidade do rincoforol adsorvido nas matrizes e posterior recuperação com solução de n-hexano, foram analisadas através do método analítico desenvolvido, mostrando no t = 24h resultados positivos para recuperação nas estruturas: Silicalita-1 (93,17%); Zeólito L (93,62%); ZSM-5 SAR30 (91,26%); Na-Magadiíta (89,05%), caulim (88,3%) e amilose de batata (95,23%). As demais estruturas avaliadas apresentaram degradação total ou parcial do rincoforol, obtendo produtos de degradação através de reações de desidratação e eterificação. Para avaliação da estabilidade do feromônio durante seu armazenamento já adsorvido nas matrizes, avaliação do recuperado foi realizada durante 180 dias com intervalos de 30 dias. Foi demonstrando que a estrutura Caulim não foi adequada, por apresentar degradação parcial e volatilização do rincoforol no tempo de até 60 dias. As demais estruturas apresentaram apenas perda na intensidade do sinal, demonstrando perda da massa adsorvida por evaporação sem ocorrer conjuntamente formação de novos produtos de degradação. As estruturas que apresentaram maior resultado para uso como dispositivo de liberação controlada do rincoforol foram: Na-Magadiíta; Zeólito-Y e Silicalita-1. Com os resultados obtidos, foi possível concluir que as matrizes inorgânicas silicalita-1, zeólito L e Na-Magadiíta, como também matriz orgânica amilose de batata são as matrizes mais promissora para uso como dispositivo de liberação controlada do feromônio de agregação rincoforol.

**Palavras-Chave:** Zeólito; Argila; Amilose; Cromatografia Gasosa; *Rhynchophorus palmarum*.

## ABSTRACT

VIANA, A. C. **Matrizes para liberação controlada do feromônio de agregação rincoforol. 2018. 133f. Tese (Doutorado)** - Programa de Pós-Graduação em Biotecnologia – RENORBIO, Universidade Federal da Bahia, Salvador-BA.

The use of pheromones has been an efficient alternative for the monitoring and control of pests, keeping the population at levels that are economically acceptable. For the control of *Rhynchophorus palmarum* (South American palm weevil), the use of pesticides is not viable, and the aggregation pheromone rhynchophorol is used as bait to capture it along with traps. Due to environmental conditions, this substance's evaporation kinetics can be damaged, causing excessive or low release rates for detection by *R. Palmarum*. The objective of this study was to evaluate the use of inorganic matrices and one organic matrix as a device for controlled release of rhynchophorol. Validation of analytical method to identify and quantify rhynchophorol was carried out by gas chromatography coupled to a mass spectrometry detector, for both its pure form and the amount recovered after adsorption on the matrices. The following inorganic structures were synthesized or purchased: Zeolites (ZSM-5 SAR 30/50/80; MCM-22 SAR 30/50/80; zeolite-L; ammonium and sodium zeolite-Y; Silicalite-1), Clays (K10 and KSF Montmorillonit; Kaolin) and Na-Magadiite, as well as the organic structure potato starch. The matrices were characterized and showed similarities to matrices obtained through standards provided by the literature. Stability of the rhynchophorol adsorbed on matrices and subsequently recovered with n-hexane solution was analyzed using the analytical method developed, showing positive results at t = 24h for recovery in the structures: Silicalite-1 (93.17%); zeolite-L (93.62%); Zsm-5 SAR30 (91.26%); Na-Magadiite (89.05%), kaolin (88.3%) and potato starch (95.23%). The other structures evaluated showed total or partial degradation of rhynchophorol, obtaining degradation products by dehydration and etherification reactions. To assess the stability of the pheromone during its storage already adsorbed on the matrices, the recovered amount was evaluated for 180 days, at 30-day intervals. It was demonstrated that the Kaolin structure was not adequate, since it led to partial degradation and volatilization of rhynchophorol in the time of up to 60 days. The other structures showed only loss of signal intensity, demonstrating loss of the adsorbed mass by evaporation without simultaneous formation of new degradation products. The structures that showed the best results for use as rhynchophorol controlled-release device were: Na-Magadiite; zeolite-Y and Silicalite-1. With the obtained results, it was possible to conclude that the inorganic matrices silicalite-1, zeolite-L and Na-Magadiite, as well as the organic matrix potato starch, are the most promising ones to be used as a device for controlled release of the aggregation pheromone rhynchophorol.

**Key Words:** Zeolite; Clay; Amylose; Gas Chromatography; *Rhynchophorus palmarum*.

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## LISTA DE ABREVIATURAS E SIGLAS

PIB	Produto Interno Bruto
MIP	Manejo integrado de pragas
NHV	Voláteis de plantas não hospedeiras
DRX	Difratometria de Raios X
EDX	Espectrometria de Energia Dispersiva de Raios-X
FTIR	Espectroscopia na Região do Infravermelho
TG/DTG	Termogravimetria e Termogravimetria Derivada
TPD-NH3	Dessorção Termoprogramada de Amônia
MEV	Microscopia Eletrônica de Varredura
mg	Miligrama
mL	Mililitro
µL	Microlitro
Kv	Quilovolt
mA	Miliampère
µm	Micrometro
min	Minuto
mm	Milímetro
α	Coeficiente Lagmuir
2θ	Ângulo Bragg
Å	Angstrom
°C	Graus Celsius
h	Hora
HPLC	Cromatografia Líquida de Alta Eficiência
CG-EM	Cromatografia à gás acoplada à espectrometria de Massas
CG-DIC	Cromatografia à gás acoplada a detector de ionização de chamas
INPI	Instituto Nacional de Propriedade Intelectual

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## 1. INTRODUÇÃO GERAL

Com o aumento da população mundial, possuindo expectativa de valores acima de 10 bilhões já no final deste século, a busca pela produção de alimentos esta cada vez mais otimizada e eficiente onde busca cada vez mais alinhar a produtividade com a qualidade o máximo possível [1]. Buscando evitar perdas já no plantio, o controle de pragas agrícolas continua sendo um dos principais parâmetros para evitar perdas substanciais no manejo de uma cultura agrícola. Estima-se que a perda causada por artrópodes chegue a valores acima de \$ 470 bilhões, correspondendo a valores de 18 – 26% dos plantios agrícolas em todo o mundo [2].

No controle de pragas agrícolas a técnica mais utilizada ainda é a eliminação pelo uso de agrotóxicos, método eficiente que promove rápida eliminação da praga alvo. Entretanto, o uso de agrotóxicos é causador de diversos problemas, como contaminação de mananciais hídricos; problemas à saúde dos manipuladores; contaminação de alimentos e destruição de inimigos naturais de pragas, provocando, a longo prazo, danos irreparáveis ao ecossistema [3,4].

Substâncias utilizadas por insetos para promover a comunicação entre indivíduos da mesma espécie são chamadas de feromônios. Estas estão sendo identificadas e produzidas de forma sintética, viabilizando sua utilização para captura dos insetos em armadilhas, seguida de eliminação destas no campo. Na metodologia do Manejo Integrado de Pragas (MIP), o uso de feromônio vem aumentando a possibilidade de controlar a população das pragas, possibilitando a diminuição, ou mesmo a eliminação do uso de agrotóxicos [3-5].

Técnicas que utilizam feromônios estão sendo pesquisadas para o controle de diversas pragas. Para facilitar a identificação pelo inseto, sua cinética de evaporação não poderá ser prejudicada por condições ambientais, tais como: temperatura, velocidade do vento e umidade. Para evitar esta liberação não controlada, pesquisas buscam selecionar matrizes que consigam reter o feromônio por vias de adsorção, e promove-la em taxas viáveis para detecção pelo inseto o mais longo período.

O inseto *Rhyncophorus palmarum* (Broca do coqueiro), praga presente principalmente nas culturas do coqueiro, dendezeiro e cana-de-açúcar, promove danos às plantas prejudicando a produção, por danificar o caule destas culturas na busca por alimento e também as fêmeas no momento da postura dos ovos para

reprodução. Esse besouro também é o principal vetor do nematóide *Bursaphelenchus cocophilus*, agente causador da doença do anel-vermelho, que provoca a morte das plantas. Para controle da população dessa praga o uso de agrotóxicos não é indicado. O controle é alcançado eficientemente através de técnicas de manejo com feromônio rincoforol (feromônio de agregação deste besouro) como isca em armadilhas para captura, e posterior eliminação dos insetos [6-8].

O uso de materiais microporosos, que possuem elevada área superficial com grande capacidade de adsorção está cada vez mais sendo estudado para uso como suporte para liberação prolongada e controlando o feromônios. O uso destas matrizes permite uma liberação em concentrações adequadas do feromônio para identificação pelo inseto, além de promover sua proteção contra degradação por intempéries, oxigênio, umidade, luz ultravioleta entre outras [9].

Para melhorar o controle da taxa de evaporação do feromônio, estudos com matrizes microporosas a base de aluminossilicatos, como os zeólitos, são pesquisadas. Diferentes tipos de zeólitos com variações nas matrizes relacionadas com a disposição e conformação da rede de tubos; tamanho dos poros; área superficial de adsorção; acidez; dentre outros parâmetros vem sendo testados. A escolha do suporte zeolítico com as respectivas especificações deve levar em consideração a afinidade, a degradação e liberação do feromônio, visando aumentar a sua eficiência quando utilizado no campo [10-13].

Outro grupo de matrizes inorgânicos que possuem elevada área superficial são as argilas. Estes materiais são largamente utilizados como suporte para diversas reações químicas. Pesquisas onde utilizam as argilas como matriz para adsorção de herbicidas vem mostrando resultados positivos quando utilizado como dispositivo de liberação controlada e remediadores de áreas contaminadas com herbicidas [14-16].

Conjuntamente novas matrizes são capazes de promover liberação controlada para feromônios, agora com características orgânicas. Exemplo destas são a amilose que consegue formar um complexo cristalino com número elevado de compostos polares e não polares [17]. Estudos prévios comprovam que a amilose consegue formar complexos com diferentes polimorfismos, através de complexos de inclusão como exemplos: conjugados de ácidos linoleico [18]; álcoois de cadeia ramificada como terc-butil [17], drogas como ibuprofeno [19], iodo [20], álcoois pequenos e lineares como 1-butanol [21,22].

Através da identificação e quantificação do feromônio recuperado, este pode ser utilizado para adsorção em matrizes com posterior liberação prolongada, possibilitará avaliar sua utilização como suporte do feromônio. Através desta técnica, será possível ocorrer a seleção de matrizes de forma racional, visando sua aplicação no campo para controle de pragas [9].

## 2. OBJETIVOS:

### 2.1. GERAL:

Avaliar a estabilidade e cinética de liberação do feromônio sintético de agregação *6-metil-2-E-hepten-4-ol* (Rincoforol), adsorvidas em diferentes matrizes inorgânicas e uma orgânica.

### 2.2. ESPECÍFICOS:

- Validar o método para a identificação e quantificação de rincoforol por CG-EM;
- Avaliar a estabilidade a longo prazo do rincoforol adsorvido nas matrizes inorgânicas;
- Avaliar a cinética de liberação da forma ativa do rincoforol adsorvido pelos zeólitos Silicalita-1, Zeólito L, Zeólito Y, MCM-22;
- Avaliar a cinética de liberação da forma ativa do Rincoforol adsorvidos pelas argilas Caulim, montmorilonita K10, montmorilonita KSF e Na-magadiíta;
- Avaliar a capacidade de adsorção e liberação controlada da matriz amilose de batata;

### 3. REVISÃO DA LITERATURA

#### 3.1 SEMIOQUÍMICOS

No reino animal, é necessária a utilização de distintas formas de comunicação para garantir a interação entre os diferentes indivíduos de uma espécie ou entre espécies. A comunicação de forma rápida e eficiente garante a sobrevivência da comunidade de uma espécie, pois permite como exemplo: o alerta contra o ataque por predadores, auxilia na demarcação de território e localização de alimento, entre outros [3].

Para a comunicação no mundo dos insetos, os mesmos utilizam ferramentas importantes, tais como: Sinais visuais, acústicos, táteis e/ou substâncias químicas. O uso de substâncias químicas excretadas pelos membros da comunidade pode provocar diferentes comportamentos e consequente ação nos insetos. Assim, podem ocorrer respostas distintas, tanto para o inseto que emite quanto para o que recebe a informação. Estas substâncias de comunicação são denominadas semioquímicos, que por definição são: substâncias químicas que fornecem informações relativas à uma interação entre dois indivíduos, e quando liberada por um determinado indivíduo, provoca uma mudança fisiológica e/ou comportamental no indivíduo receptor [3].

Os semioquímicos são classificados em dois grandes grupos, sendo: o primeiro 1 - Aleloquímicos: Semioquímicos que mediam interações entre indivíduos pertencentes a espécies distintas; e o segundo 2 - Feromônios: “Substâncias que mediam interações entre organismos da mesma espécie, conferindo vantagens adaptativas para o organismo emissor, para o organismo receptor, ou para ambos” [4].

Quando promovida uma comunicação entre diferentes espécies, os aleloquímicos podem prover ganhos diferentes entre as espécies envolvidas, podendo favorecer apenas ao receptor do sinal (caiomônio) ou somente ao emissor do sinal (alomônio) ou tanto ao emissor quanto ao receptor (sinomônio). Estes tipos de semioquímicos participam de tarefas fundamentais dentro de um ecossistema. Por exemplo, a emissão de voláteis pelo milho *Zea mays* quando atacado por herbívoros (lagartas da mariposa *Spodoptera exigua*), atrai fêmeas da vespa *Cortesia*

*marginiventris*, a qual é seu parasitóide natural [3-5]. A classificação dos semioquímicos está esquematizada na Figura 1.

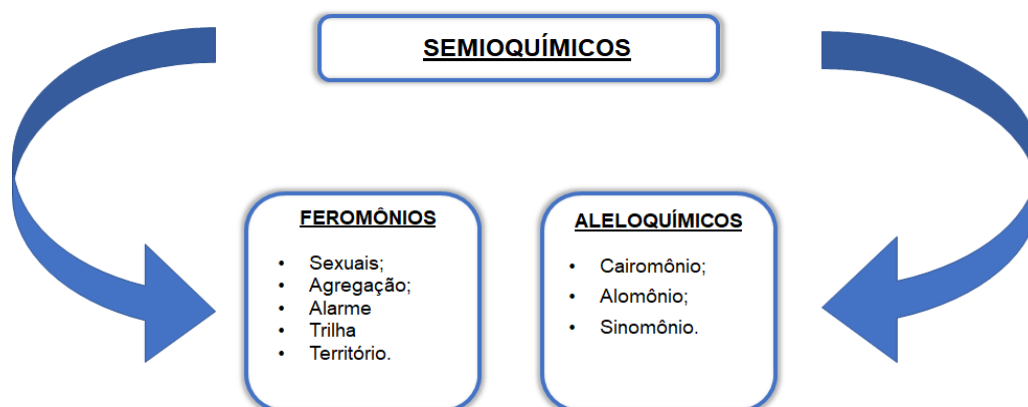


Figura 1: Classificação dos Semioquímicos [5]

Os feromônios transmitem informações para benefício da própria comunidade, garantindo sua sobrevivência [3,4]. Diferentes tipos de feromônios são reconhecidos através do comportamento que esses compostos produzem no organismo da mensagem: Feromônios sexuais (atração de indivíduos do sexo oposto para acasalamento), feromônios de agregação (atração de indivíduos de ambos os sexos para um local específico para alimentação e/ou acasalamento), feromônio de trilha (formação de trilhas), feromônio de marcação (demarcação de espaço), entre outros [23].

### 3.2 FEROMÔNIO DE AGREGAÇÃO RINCOFOROL

O besouro *Rhynchophorus palmarum* (Figura 02), importante praga de controle nas culturas do coqueiro (*Cocos nucifera*), dendezeiro (*Elaeis guineenses*) e abacaxizeiro (*Ananas comosus*), é responsável por provocar prejuízos econômicos nessas lavouras. Isso decorre não somente da utilização do caule destas culturas pelos besouros como fonte de alimentação e local de postura para seus ovos; mas principalmente, pela transmissão do nematódeo *Bursaphelenchus cocophilus*, que causa a doença do anel vermelho, mortal para plantas da família Arecaceae [24,25].



Figura 2: *Rhynchophorus palmarum* adulto.  
Fonte: Próprio autor.

Ao encontrar alimento, o *R. palmarum* libera um feromônio de agregação, tornando possível o encontro de vários indivíduos para se alimentar e reproduzir no mesmo local. Essa substância liberada é conhecida como rinfoforol (*6-metil-2-E-hepten-4-ol*), um álcool alifático de 8 carbonos, com 1 insaturação [2]. Sua forma estrutural está representada na Figura 3 [13].

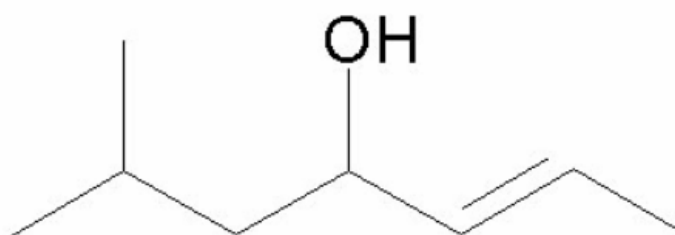


Figura 3: Ilustração estrutural do rinfoforol [13].

A identificação do rinfoforol ocorreu em 1991, por ROCHAT et al (1991) [5], através do uso de técnicas de cromatografia gasosa (CG) acoplado a detectores de espectrometria de massas (EM) e Espectroscopia na região do infravermelho por Transformada de Fourier (FTIR). Nesse trabalho, foi possível identificar a produção de dois principais voláteis liberados pelo inseto *R. palmarum*. Um deles nem sempre detectado; e o rinfoforol, que provou ser o componente essencial do feromônio de agregação, através das técnicas de CG e bioensaios comportamentais.

No trabalho citado, os autores sintetizaram o rincoforol a partir de reações crotonaldeído à  $-10^{\circ}\text{C}$  em tetrahidrofurano - THF, com reagentes de Grignard de brometo de isobutila, 2-bromobutano e 1-bromobutano. A análise por CG-MS permitiu registrar a abundância iônica em função de  $m/z$ : 113(2), 110(3), 95(8), 85(6), 71(100), 69(8), 67(11), 57(10), 53(8), 43(20) e 41(21). Esta informação tornou-se base para identificação do rincoforol em trabalhos que buscam desenvolver rotas de síntese alternativa para esse feromônio, tornando viável sua síntese e utilização para controle do *R. palmarum* [26].

O feromônio sintético tem sido utilizado como isca em armadilhas para capturar o inseto *R. palmarum*, que é em seguida eliminado. Essa técnica, denominada “atrai-e-mata”, propicia a redução de sua população em níveis aceitáveis ocorrendo a diminuição dos prejuízos causados à produção agrícola nas culturas em que esta praga ocorre [7]. Entretanto, técnicas que utilizam iscas baseadas em feromônio, sofrem influência das condições ambientais, tais como temperatura e velocidade do ar; e muitas vezes não permanecem em concentrações constantes e adequadas durante o período em que os insetos adultos estão presentes [27].

Para tanto, cuidados devem ser tomados na utilização do rincoforol como isca. Sua integridade deve ser garantida durante todo o período de aplicação, além de estar disponível em concentração perceptível pelo inseto, de forma constante.

### 3.3 CONTROLE DE PRAGAS

#### 3.3.1. **Agrotóxicos**

A busca por alimentos saudáveis tem sido apontada como essencial para garantir o bem-estar e longevidade da população. Contudo, para elevar a produção com elevada quantidade e qualidade dos produtos oriundos das lavouras, muitas vezes são utilizados agrotóxicos, definidos pela lei federal 7.802 de 11 de julho de 1989, como sendo:

Os produtos e os agentes de processos físicos, químicos ou biológicos, destinados ao uso nos setores de produção, no armazenamento e beneficiamento de produtos agrícolas, nas pastagens, na proteção de florestas, nativas ou implantadas, e de outros ecossistemas e de ambientes



urbanos, hídricos e industriais, cuja finalidade seja alterar a composição da flora ou da fauna, a fim de preservá-las da ação danosa de seres vivos considerados nocivos [28].

O uso destas substâncias nas produções agrícolas fornece um controle rápido e eficiente da praga alvo, sendo a justificativa primordial para sua utilização. Entretanto, a utilização desta técnica vem sendo substituída ao longo dos anos por causar diversos prejuízos ao meio ambiente e à saúde do ser humano, seja para as pessoas que aplicam o agrotóxico, como também para as que consomem esses produtos contaminados [2].

Estudos demonstram que o uso indiscriminado dos agrotóxicos tem prejudicado a bacia hidrográfica de regiões rurais, contaminando não só a água utilizada para a lavoura, como também aquela destinada para o consumo humano e animal. Essa situação tem sido relacionada, à longo prazo, ao risco de desenvolvimento de doenças cancerígenas [4, 29,30].

A contaminação também pode ser evidenciada nos alimentos quando o agrotóxico não é aplicado corretamente, ou até mesmo quando o período de quarentena após o seu uso não é respeitado. Essa situação corrobora para que substâncias nocivas à saúde humana estejam ativas em frutas e hortaliças no momento da comercialização e consumo [21-35]. Na Tabela 01 é disponibilizado o resultado de um estudo realizado pela Agência Nacional de Vigilância Sanitária (ANVISA), no ano de 2012 em relação aos alimentos que possuem maior contaminação por agrotóxicos.

Tabela 1: Alimentos com maior contaminação por agrotóxicos no Brasil

Posição	Alimento	Contaminação (%)	Posição	Alimento	Contaminação (%)
1°	Pimentão	91,8	10°	Tomate	16,3
2°	Morango	63,4	11°	Laranja	12,2
3°	Pepino	57,4	12°	Maçã	8,9
4°	Alface	54,2	13°	Arroz	7,4
5°	Cenoura	49,6	14°	Feijão	6,5
6°	Abacaxi	32,8	15°	Repolho	6,3
7°	Beterraba	32,6	16°	Manga	4,0
8°	Couve	31,9	17°	Cebola	3,1
9°	Mamão	30,4	18°	Batata	<1,0

Fonte: Resíduos de agrotóxicos em alimentos – ANVISA [35].


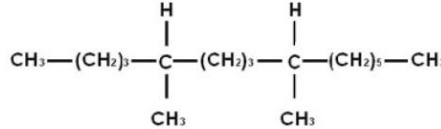
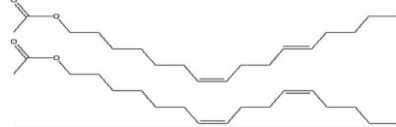
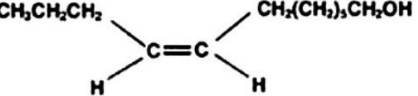

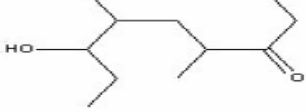
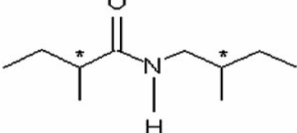
### 3.3.2. Feromônio para Manejo Integrado de Pragas

Visando garantir uma maior eficiência, é fundamental a observação de diversos parâmetros os quais irão determinar qual a melhor técnica para controle do inseto. Neste intuito, o uso da técnica de manejo integrado de pragas propõe monitoramento visando manter a população da praga causadora do prejuízo econômico visando seu controle à níveis aceitáveis. Por definição o conceito de MIP é: “um sistema de controle de pragas que procura preservar e aumentar os fatores de mortalidade natural das pragas pelo uso integrado dos métodos de controle selecionados com base em parâmetros técnicos, econômicos, ecológicos e sociológicos” [5].

Neste conceito de trabalho, diversas culturas tais como: macieiras, pereiras, pessegueiro e citros já utilizam técnicas baseados na utilização do feromônio para o controle de suas pragas. O uso desta técnica proporciona o monitoramento e ajuda à evitar o aparecimento de pragas recorrentes antes mesmo do seu surgimento [38]. Na Tabela 02 é possível observar exemplos de culturas com o respectivo feromônio usado para o controle da praga alvo. Considerando os pressupostos do MIP, o uso de feromônio vem sendo objeto de estudo com o objetivo de promover a redução populacional de pragas de diversas culturas. Como consequência, o uso de agrotóxicos também é reduzido, evitando prejuízos ao meio ambiente e ao ser humano, tanto durante a aplicação, quanto no consumo dos alimentos.

Uma prospecção realizada por Bergmann et al (2009) [38], verificou o quantitativo de artigos relacionados aos estudos com feromônios na América do sul, durante o período de 1998 até 2008. Os autores relataram 261 artigos sobre o tema abordado. Uma evolução crescente para pesquisas no tema foi observada, subindo de 2 artigos publicados anualmente para números de 20 até 30 artigos publicados nos anos de 2006 até 2008. O Brasil destacou-se como maior pesquisador, com 187 artigos publicados.

Tabela 2: Principais feromônios comercializados no Brasil.

Feromônio	Formula estrutural	Inseto-praga	Cultura
Acetato de (E,Z)-4,7-tridecadienila		<i>Phthorimaea operculella</i> (Traça-da-batatinha)	Batata, tomate, fumo, berinjela e pimentão
5,9-dimetilpentadecano		<i>Leucoptera coffeella</i> (Bicho-mineiro-do-café)	Café
Gosiplure (Z,E)-7,11-hexadecadienil acetato; (Z,Z)-7,11-hexadecadienil acetato.		<i>Pectinophora gossypiella</i> (Lagarta-rosada)	Algodão
Álcool láurico		<i>Grapholita molesta</i> (mariposa-oriental)	Maçã e pêssego
Acetato de (Z)-8-dodecenila + acetato de (E)-8-dodecenila		<i>Grapholita molesta</i> (Mariposa-oriental)	Maçã e pêssego
Serricornin (4,6-dimetil-7-hidroxi-nonan-3-ona)		<i>Lasioderma serricorne</i> (Bicho-do-fumo, carruncho-do-fumo)	Fumo
N-2'S-metilbutil-2-metilbutilamida		<i>Migdolus fryanus</i> (Broca-da-cana, migdolus)	Cana-de-açúcar

Fonte: RAMOS (2012) com modificações [7]; BRASIL (2003) [82].

Silva et al (2014) [39], na busca por uma técnica alternativa para o monitoramento do percevejo marrom (*Euschistus heros*), praga recorrente na cultura da soja, avaliou os métodos de liberação do feromônio sexual deste inseto por diferentes formulações liberadas em iscas (pastilhas celulósicas revestidas com plástico nanoporoso). Os autores destacam que as formulações baseadas em feromônio testadas foram eficientes para o monitoramento da praga. A formulação na forma de isca apresentou maior eficiência quando comparada com outras técnicas. Comparando com a técnica convencional de pano batido, a técnica proposta conseguiu detectar o inseto em período anterior ao método convencional em aproximadamente 45 dias. Assim, o controle da praga pôde ser realizado com antecedência, o que preveniu a elevação da população, onde evitou danos à lavoura e permitiu o aumento da produtividade.

Não há agrotóxico disponível para o gerenciamento do *R. palmarum*. O controle químico do besouro mediante pulverizações não é permitido, pois as larvas encontram-se profundamente no interior da planta e não são atingidas. Medidas fitossanitárias direcionadas à redução das populações do besouro vetor e do número das fontes de inóculo são os métodos mais usados nas tentativas de controle da doença. Para isso é comum o uso de armadilhas contendo como isca roletes de cana-de-açúcar e melação de cana diluído, feitas em um balde plástico (100 L) coberto com uma tampa furada, onde são adaptados funis que permitem a entrada, porém não permitem a saída dos besouros. No entanto, devido à sua pouca eficiência, essas iscas foram sendo aplicadas em conjunto com o feromônio rincoforol [5]

As iscas baseadas no feromônio rincoforol têm sido comercializadas em tubos do tipo Eppendorf safe-lock®, contendo um orifício de 1mm de diâmetro na tampa. Navarro et al (2002) [5] determinaram uma taxa de evaporação de 4,3 mg/dia (equivalente a 5µL) do rincoforol a partir desse dispositivo.

A utilização de pedaços de abacaxi e voláteis mais abundantes do extrato hexânico obtido da aeração do abacaxi também foram testados como compostos sinérgicos. Não houve melhora na captura dos insetos quando comparada às iscas com rincoforol associada à cana-de-açúcar [2].

### 3.4 MATRIZES PARA LIBERAÇÃO CONTROLADA DE FEROMÔNIOS

Para utilização dos feromônios como controle da população de pragas na agricultura, o mesmo precisa ser liberado no ambiente em concentrações adequadas. Tal concentração deve ser o suficiente para ser detectada pelo inseto-praga, mas não excessiva, de forma que torne sua aplicação economicamente elevada. Além disso, sua eficiência não deve ser influenciada, ou mesmo minimizada, por condições ambientais [8, 39, 40].

No intuito de manter constante a cinética de evaporação/liberação do feromônio, pesquisas estão sendo desenvolvidas para buscar uma matriz onde esse feromônio seja adsorvido e liberado de forma eficiente. Em sistemas matriciais, diferentes velocidades de liberação podem ser conseguidas através de modificações estruturais. Assim, a velocidade de liberação desejada pode ser alcançada por modulação dos parâmetros geométricos da matriz [8, 11, 13, 39, 41].

Realizando busca no intuito de visualizar o perfil de pesquisas relacionados nas últimas duas décadas onde foram utilizadas uma ou mais matrizes, tanto orgânicas ou inorgânicas. Estas matrizes foram usadas visando formação de um complexo objetivando a liberação controlada de feromônios. Utilizando uma base de dados para busca como a Science Citation Index Expanded (SCI Expanded), foi escolhida a plataforma de busca Web of Science. Conjuntamente para aumentar o espectro de busca, também foi utilizada na mesma avaliação a plataforma de artigos científicos ScienceDirect (Elsevier).

Durante as buscas, as bases de dados foram consultadas utilizando o período iniciando em 1997 até agosto de 2017, utilizando as seguintes palavras como critérios para busca: os termos “feromônio, liberação e armadilha” foram usadas como tópicos de buscas para estarem presentes nos títulos, keywords ou abstract. O operador Bolleano “AND” foi utilizado para busca entre os termos utilizados. Para identificação quanto ao assunto abordado durante a consulta nos artigos encontrados, o título e o abstract foram consultados. Em casos de dúvidas quanto ao assunto relacionado à busca, todo o artigo foi lido.

As bases consultadas apresentaram resultados de buscas distintos, sendo verificado um total de 657 artigos relacionados para a plataforma Web of Science e um total de 1684 artigos para a plataforma ScienceDirect. Após avaliação de ambos

os bancos de dados fornecidos, foram selecionados os artigos correlacionados com o objeto da busca, sendo classificados como “relevantes” obtendo um novo total de 159 e 129 respectivamente para as plataformas citadas, sendo analisados em seguida em diferentes aspectos (Figura 4).

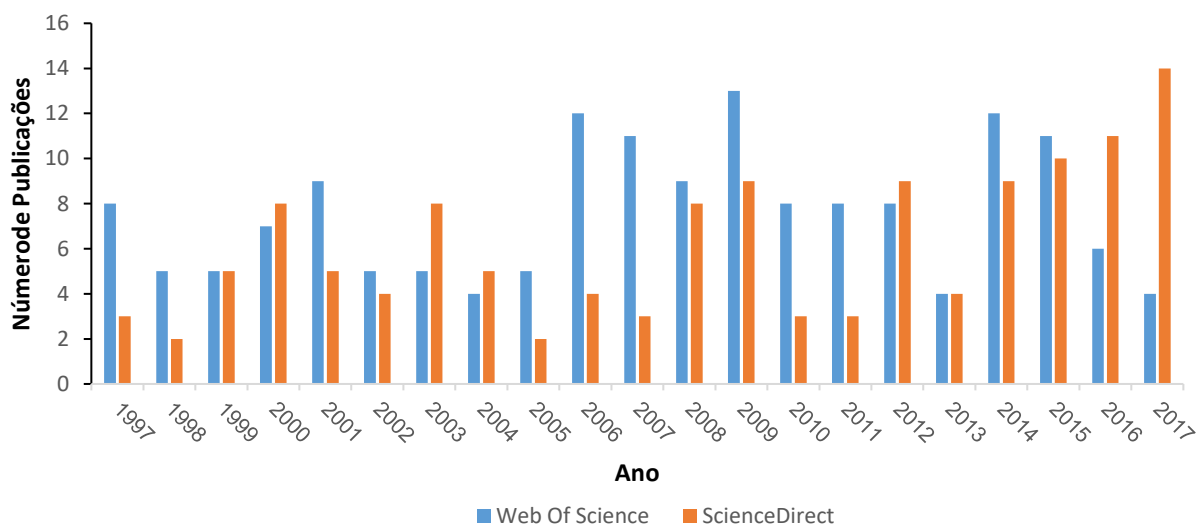


Figura 4: Evolução de Artigos científicos com liberação controlada de feromônios.

É possível visualizar que nas últimas duas décadas que vem ocorrendo o aumentando significativo no interesse para pesquisas no uso destas matrizes adsorventes em conjunto com feromônios. Nas bases consultadas, é observado ainda que este interesse elevando a partir do ano de 2006. Dentre os artigos observados, podemos ainda destacar os países com as maiores quantidades de pesquisas no assunto sendo os: Estados Unidos da América; Reino Unido; China e Canada, respectivamente (Tabela 3). Vale destacar que o país Coreia do Sul possui uma quantidade elevada de produção totalizando 13 publicações no referido período na base de dados ScienceDirect, entretanto com parceria com outros países que realizaram o depósito dos artigos.

Tabela 3: Número de publicações nas bases de dados por países.

País	Base de Dados		Média
	ScienceDirect	Web of Science	
Estados Unidos	32	68	50
Reino Unido	14	12	13
Canada	6	12	9
Espanha	6	9	7,5
China	7	7	7
Austrália	5	5	5
Alemanha	4	2	3
Brasil	1	1	1
Outros	54	43	48,5
<b>Total</b>	<b>129</b>	<b>159</b>	<b>144</b>

### 3.5. MATRIZES INORGÂNICAS PARA LIBERAÇÃO DE FEROMÔNIO

#### 3.5.1. Zeólitos

Apesar do primeiro zeólito ter sido descoberto em 1756, somente em 1926 as características de adsorção desses materiais foram explicadas pelo tamanho de poro. Por possuir diâmetros de poros de aproximadamente 5 Å, sua estrutura tridimensional permitem a inserção de pequenas moléculas, ocasionando também a exclusão de moléculas maiores, surgindo a denominação “peneira molecular” [42].

Os zeólitos também são conhecidos como materiais cristalinos microporosos com estruturas regulares compostas por canais e poros de tamanho molecular. Possuem definição baseada em aluminossilicatos interligados por ligações tridimensionais em rede, através de tetraedros de  $AlO_4$  e  $SiO_4$  ligados entre si, compartilhando átomos de oxigênio comum [43]. A modificação causada pela substituição do íon Si por Al, pode implicar em modificações na carga da matriz modificando sua característica funcional, como exemplo na elevação ou diminuição da sua acidez. Sua fórmula geral pode ser representada por:  $M_2O_n \cdot Al_2O_3 \cdot xSiO_2 \cdot yH_2O$  [44,45].

Os zeólitos obtidos por fontes naturais apresentam contaminação na matriz, dificultando sua aplicação para muitas finalidades industriais. Diante dessa dificuldade, os zeólitos começaram a ser produzidos sinteticamente, de forma a obter uma matriz homogênea que viesse a atender as demandas da indústria química [46].

Variações durante a síntese do zeólito pode promover alteração na conformação da rede, modificando o diâmetro dos canais que fazem parte da matriz microporosa do zeólito. O uso de substâncias pré-poliméricas como direcionadores e a substituição do íon formador na síntese, pode resultar na formação de novas matrizes com características elevada na área superficial e capacidade para liberação de compostos bioativos [10].

Devido a formação de poros e canais na rede conformacional, com diâmetros inferiores a 20Å, o zeólito permite não só abrigar pequenas moléculas, como também excluir moléculas maiores. Sua elevada área superficial constituída pela rede porosa torna o zeólito uma matriz adequada para atuar como matriz para liberação de compostos ativos.

Os zeólitos ZSM-5, faujasita e zeólito  $\beta$  foram estudados por Muñoz-Pallares et al (2001) [11] com o objetivo de avaliar a aplicabilidade desses materiais como “dispenser” para liberação controlada dos feromônios *n*-decanol e trimedlure. Parâmetros geométricos e químicos relativos à capacidade de adsorção, emissão e velocidade difusional foram estudados.

O aumento da razão Si/Al na matriz faujasita implicou em uma redução na velocidade de emissão do *n*-decanol. O aumento da força de adsorção do feromônio foi devido ao aumento da polarizabilidade da rede zeolítica e diminuição da polaridade da ligação de oxigênio. Como as interações de Van der Waals são proporcionais à polarizabilidade da rede, que aumenta com o aumento com o incremento de Si/Al, os autores concluíram que forças intermoleculares de Van der Waals representam 67% da interação *n*-decanol/zeólito.

Por apresentar o menor tamanho de poro, o zeólito ZSM-5 foi avaliado em relação à influência do tamanho dos poros na velocidade de difusão do *n*-decanol. Os autores verificaram que, independente da razão Si/Al, o nível de retenção é muito maior para esses zeólitos.



A influência do cátion de compensação foi avaliada através da troca dos cátions  $\text{Cs}^+$  por  $\text{Na}^+$ , resultando em uma faujasita X com quantidade de cátions fora da rede com 50% de  $\text{Na}^+$  e 50% de  $\text{Cs}^+$  (menor razão carga/raio). A cinética de emissão do n-decanol a partir da faujasita com 50% de  $\text{Cs}^+$  extra rede diminuiu, quando comparadas com o zeólito NaX. Foi verificado que a troca de  $\text{Na}^+$  por  $\text{Cs}^+$  implicou no aumento da polarizabilidade devido à estrutura eletrônica do  $\text{Cs}^+$  e um aumento do nível de interações de van der Waals com a molécula adsorvente. Além disso, a localização do  $\text{Cs}^+$  próxima à supercavidade favorece a interação e produz alto nível de adsorção do semioquímico.

A acidez também aumentou a retenção do feromônio trimedlure em zeólitos faujasita com diferentes concentrações de hidrogênio. Esse aumento foi devido à maior interação entre o oxigênio do éster e o grupo ácido. No entanto, o nível de retenção, em uma determinada acidez, foi menor com o trimedlure. Isso foi devido à menor capacidade do grupo éster em formar ligações de hidrogênio; e ainda, ao impedimento estérico do grupo éster de butila terciário do trimedlure. Além disso, uma hidrólise parcial do grupo éster ocorreu com o feromônio trimedlure para as amostras com quantidade de H de 80%, alterando a cinética de emissão e a eficácia do semioquímico [11].

Para avaliar a influência do diâmetro do poro e a cinética de dessorção do n-decanol, as matrizes zeólito ZSM-5 e zeólito  $\beta$  foram estudadas por possuírem razão Si/Al similares. Como esperado, o nível de retenção elevasse em função da diminuição do tamanho dos poros. Isso ocorre não somente devido às restrições geométricas, mas também porque o diâmetro menor possibilita a maior interação do adsorbato com as paredes do zeólito. Além disso, verificou-se que a retenção de zeólitos menos polares é mais forte. Finalmente, os autores verificaram a influência do preparo das pastilhas utilizadas como “dispenser”, sendo esta forma de aplicação viável para uso como forma para liberação do feromônio decanol [47].

A cinética de emissão do n-decanol foi avaliada para amostras de zeólito Y com concentração de hidrogênio de 80% (Y HNa 80%) a partir de pastilhas com diferentes forças de compressão. O nível de retenção aumentou em função do aumento da força de compressão. Isso ocorreu devido à diminuição do tamanho dos macroporos, diminuindo a velocidade de difusão da molécula adsorvida. Apesar de fácil, o controle desse parâmetro pode ser alterado somente até um determinado limite. Quando a

matriz é carregada com grande quantidade de adsorbato pode exsudar. Além disso, em alguns zeólitos, a pressão excessiva poderá alterar a matriz cristalina, resultando em mudanças indesejáveis nas propriedades químicas [47].

O zeólito A por possuir a característica de elevada capacidade de troca iônica foi utilizado por Seo et al (2016) [12] como dispenser do feromônio de agregação do *R. pedestris*. Tal feromônio consiste de três compostos: (E)-2-hexenil-(Z)-3-hexenoato (EZ), (E)-2-hexenil-(E)-2hexenoato (EE) e miristil isobutirato (MI). Para aumentar a sorção do feromônio, o zeólito A foi modificado com cetiltrimetilamônio e foi denominado SMZ-A. Aproximadamente metade do feromônio foi liberada em 10 semanas, indicado um bom potencial como para esse feromônio. Ainda avaliando a eficácia dos “dispensers” PSMZ-A (SMZ-A na forma de pó), CSMZ-A (SMZ-A na forma cilíndrica), titanossilicato ETS-10 e um produto comercial (CP) foi realizado através da captura de *R. pedestris* adulto em culturas de soja e ameixa. Em ambas as culturas o “dispenser” constituído por PSMZ-A teve desempenho superior, seguido dos seguintes materiais: CSMZ-A, ETS-10 e CP.

Jakus et al (2003) [48] avaliaram, em laboratório, a liberação da verbenona, um feromônio antiagregante emitido pelos besouros *Tomicus piniberda L.* e *Ips typographus*, a partir dos zeólitos: silicalita, mordenita e zeólito Y. Essas pragas atacam florestas de coníferas. O zeólito Y foi escolhido para teste em campo por ter apresentado maior liberação prolongada. Em campo, os experimentos foram parcialmente bem-sucedidos para ambas as espécies. Os autores também avaliaram a eficiência da verbenona a partir de um “dispenser” comercial baseado em zeólito natural (IT-REPELENT). Uma suspensão do “dispenser” em água, aplicado por pulverização aérea, é constituído pelo zeólito natural (Chepalit PM-05 Agropur) combinado com um polímero, em concentração de 10% de verbenona.

Contudo, verificou-se que semioquímicos podem ser adsorvidos em zeólitos e sua velocidade de dessorção (difusão) pode ser modulada por modificações nos parâmetros físico-químicos e estruturais. No entanto, estudos de adsorção devem ser conduzidos de forma que os fatores determinantes para a instabilidade do composto ativo sejam conhecidos.

### 3.5.2. Argilas

Argilas são compostos minerais que ocorrem naturalmente compostos por finos grãos (geralmente, com diâmetro em torno de  $2\mu\text{m}$ ), que adquire característica plástica quando adicionado de quantidade apropriada de água e que endurece com secagem [49]. São rochas constituídas por argilominerais, que são silicatos lamelares de Al, Fe e Mg hidratados, com matrizes cristalinas em camadas (filossilicatos), constituídos por lamelas contínuas de tetraedros  $\text{SiO}_4$ , ordenados de forma hexagonal, condensados com folhas octaédricas de hidróxidos de metais tri e divalentes [14]. As argilas também podem fazer parte de outros tipos de minerais, tais como: quartzo, feldspato, carbonatos, óxidos metálicos e matéria orgânica [50]. Na Figura 5 é apresentada a representação esquemática das conformações tetraédricas e octaédricas.

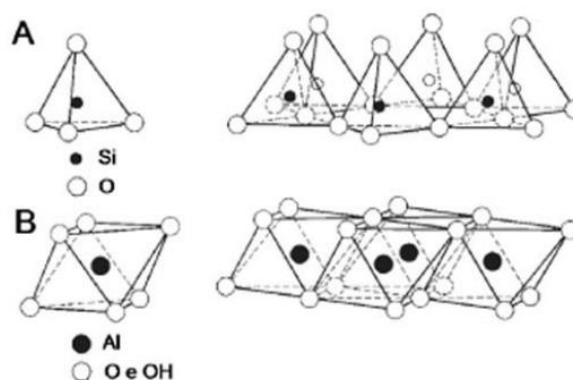


Figura 5: Diagrama esquemático de uma folha tetraédrica (a) e de uma folha octaédrica (b) [47]

As lamelas são formadas pelo compartilhamento de três dos quatro oxigênios do tetraedro de  $\text{SiO}_4$ ; da mesma forma, octaedros contendo um átomo de metal (Al, Mg, ou Fe) no centro e seis átomos de oxigênio nos vértices também formam matrizes bidimensionais devido ao compartilhamento de átomos de oxigênio [51].

A organização dessas lamelas dá origem à classificação das argilas. A estrutura 1:1 é formada por uma lamela de tetraedros de Si (T) condensada com uma lamela de octaedros (O), sendo denominada estrutura OT. A estrutura 2:1 é formada por duas lamelas de tetraedros intercaladas por uma lamela de octaedros (TOT) [51]. A Figura 6 ilustra estas duas conformações.

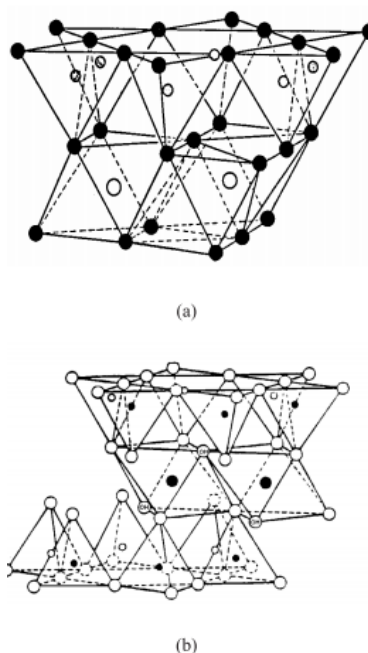


Figura 6: Modelo esquemático da camada estrutural básica de uma argila 1:1 (a) e 2:1 (b) [51].

As argilas, além de possuírem características de modelagem, usada na fabricação de cerâmicas desde tempos remotos pelo homem, ganharam elevada versatilidade no estudo das reações químicas, participando de diversas aplicações, tais como: (1) agente catalisador ativo em reações, geralmente ácidas; (2) utilização como suporte inerte ou bifuncional; (3) como agentes de preenchimento, quando são necessárias propriedades físicas, como exemplo resistência, aumento da densidade e atrito [34].

O tamanho e a falta de polaridade dificultam a entrada da molécula de semioquímicos nos canais de argilas, e por isso, a adsorção, na maioria dos casos, é superficial. CORMA et al (1998) [53] modificaram a argila natural sepiolita para melhorar as características de retenção do feromônio trimedlure.

A influência dos cátions superficiais foi avaliada para os seguintes materiais: sepiolita Mg natural, sepiolita H Mg 25% em  $H^+$  e sepiolita Na Mg 25% em  $Na^+$ . A retenção máxima foi observada para o material sepiolita Na Mg 25%, seguida da sepiolita Mg natural.

Os materiais sepiolita H e sepiolita H Mg 25% em  $H^+$  foram avaliados em relação à camada de adsorção. O aumento da área superficial de  $400 \text{ m}^2 \text{ g}^{-1}$  na sepiolita H Mg

25% para  $520 \text{ m}^2 \text{ g}^{-1}$  na sepiolita H promoveu uma diminuição na percentagem de trimedlure adsorvida na segunda camada de 33% para 13%, respectivamente. As argilas sepiolita também sofreram modificações na pressão de compactação. O aumento da retenção foi observado em função do aumento da pressão de compactação.

Finalmente, a eficácia, considerando a quantidade de insetos (mosca-das-frutas) capturados e vida útil do emissor, foi verificada para a sepiolita Na Mg 25% e o dispositivo usual (barco de plástico perfurado). O material sepiolita Na Mg 25% permitiu a captura de maior quantidade de moscas e manteve a atividade por mais tempo, com um período de efetividade de 185 dias. Enquanto que o dispositivo usual foi eficaz apenas durante um período de 132 dias.

INO et al (2003) [54] desenvolveram um “dispenser” baseado em argilas minerais para liberação prolongada de feromônios. Os autores impregnaram 5 partes em massa do feromônio (Z)-8-dodecenil acetato (Z-8-12AC) em 95 partes em massa das argilas paligorskita e sepiolita secas por 24 horas à  $105 \text{ }^\circ\text{C}$ . As mesmas argilas foram submetidas à temperatura de  $500 \text{ }^\circ\text{C}$  por 30 minutos e impregnadas com o feromônio. O período de liberação foi maior para a amostra submetida à alta temperatura.

A inclusão de fotoestabilizadores em complexos argila-feromônio de (Z)-7-tetradecenal e da mistura *E-11-hexadecenil acetato/E-11-hexadecenal* melhorou substancialmente a velocidade de liberação e manteve o nível de feromônio adequado [51]. Verificou-se, portanto, que poucas argilas minerais foram testadas para liberação de feromônios.

As argilas montmorilonitas já foram utilizadas como suporte para liberação controlada de fertilizantes, pesticidas, herbicidas, fármacos, entre outros. Na busca realizada até o presente estudo, nenhuma aplicação utilizando semioquímicos foi encontrada [55-59]. O mesmo fato foi observado para a caulim [60, 61].

A montmorilonita é a mais comum esmectita dos solos. Caracteriza-se pela considerável substituição isomórfica de Al por Mg nas camadas octaédricas. Os cristais são compostos de camadas do tipo 2:1 e permite variar reversivelmente a distância basal devido à penetração da água entre as camadas. A distância interplanar basal é da ordem de  $15,4 \text{ \AA}$ , e a espessura da camada 2:1 é da ordem de  $8,97 \text{ \AA}$ . A substituição isomórfica de certa quantidade de alumínio por magnésio na lâmina

dioctaédrica e de silício por alumínio na lâmina tetraédrica, deixa os cristais de esmectita com um saldo de carga negativa. Isso confere às esmectitas elevada capacidade de troca de cátions [62].

As argilas montmorillonita estão disponíveis comercialmente sob o nome K10 e KSF. Tais materiais modificados a partir da montmorillonita natural possuem características físico-químicas similares, mas área superficial muito diferente. A montmorillonita K10 tem área superficial em torno de  $250 \text{ m}^2 \text{ g}^{-1}$ , enquanto que a KSF tem em torno de  $10 \text{ m}^2 \text{ g}^{-1}$  [63].

A argila caulim é constituída principalmente pelo argilomineral caulinita. A caulinita é um silicato de alumínio hidratado, constituída por cristais triclinicos pseudo-hexagonais com diâmetro entre  $0,2\text{-}10 \mu\text{m}$ , espessura de  $0,7 \text{ nm}$  e densidade de  $2,6 \text{ g.cm}^{-3}$ . Possui uma estrutura lamelar 1:1, constituída pelo empilhamento de camadas de lamelas iguais. Estas camadas são formadas pelo empilhamento de folhas com disposição regular de unidades básicas. Em cada camada da caulinita existem uma folha tetraédrica e uma folha octaédrica sobrepostas. A folha tetraédrica é formada por tetraedros  $\text{SiO}_4^{-4}$  colocados em um plano, lado a lado, compartilhando seus vértices.

A folha octaédrica é formada pela disposição de octaedros  $(\text{Al}(\text{OH})_6)_3^-$  em um plano. A folha tetraédrica une-se à folha octaédrica através do compartilhamento de átomos oxigênio. Três dos átomos de oxigênio de cada tetraedro formam um plano, enquanto que o quarto oxigênio está ligado a um octaedro. Ao todo, dois de cada seis átomos de oxigênio do octaedro ligam-se aos tetraedros. Duas das seis hidroxilas de cada octaedro se desfazem, conservando a neutralidade elétrica. Através de forças de Van der Waals entre os átomos de oxigênio da folha tetraédrica e os átomos de hidrogênio da folha octaédrica uma camada pode se unir a outra [64].

A conformação geométrica das disposições entre as camadas 1:1 e 2:1 propiciam diferenças na capacidade de troca iônica entre as estruturas. A troca iônica realizada pela argila caulinita (1:1) é consideravelmente menor que as argilas do tipo montmorillonita (2:1), na ordem de  $1\text{-}10 \text{ meq}/100\text{g}$ , dependendo do tamanho da partícula. No entanto, a velocidade de troca é alta, quase instantânea [65].

A caulinita é única entre os argilominerais lamelares a possuir camadas assimétricas eletricamente neutras, tendo ânions  $\text{OH}^-$  de um lado e  $\text{O}_2^-$  do outro lado

das camadas 1:1. O caráter polar da camada e os grupos OH permitem a intercalação reversível de moléculas orgânicas polares pequenas, por interação dipolo/dipolo, ou por ligação de hidrogênio. Moléculas como: dimetilsulfóxido, hidrazina, uréia, cloreto de sódio, acetato de potássio e amidas (formamida e N-metilformamida) são consideradas apropriadas para intercalação [66].

Apenas uma aplicação de caulinita como carreador de semioquímicos foi encontrada na literatura. Paramasivan e Paul [66] desenvolveram oito formulações contendo diferentes proporções dos seguintes componentes: extrato das folhas da fase de floração do milho, girassol híbrido e lavagem do ovo de *Chilo partellus*. As formulações foram avaliadas em cultura de tomate e a fórmula com igual proporção dos componentes registrou o maior parasitismo.

As características químicas e estruturais descritas para as argilas montmorilonita e caulim e o baixo custo de aquisição desses materiais sugerem que são candidatos apropriados para uso como “dispenser” de semioquímicos.

### 3.5.3. Amilose

O uso de estruturas orgânicas já é conhecido e estudado para liberação controlada de pequenas moléculas que possam estar encapsuladas. A amilose é uma substância biodegradável e proveniente de diversas fontes renováveis, onde sua principal fonte é o amido. O teor de amilose geralmente está entre 15 a 25% dos amidos nativos, sendo uma fonte que necessita ser quimicamente trabalhada para melhorar sua eficiência [67]. Quimicamente, é um polissacarídeo composto de repetidas unidades de glicose. Estruturalmente, é composta por ligações glicosídicas do tipo  $\alpha$ -1,4, produzindo uma cadeia linear [68, 69].

O teor de amilose cristalina pode favorecer a formação de novos complexos (encapsulados) com um número elevado de compostos polares e não-polares. Estudos de difração de raios-x indicaram que o polissacarídeo assume uma conformação helicoidal, e que o diâmetro da hélice é controlado pelo tamanho do agente complexante [70]. Apesar do amido possuir unidades de amilopectina, apenas a fração de amilose é capaz de formar complexos de inclusão com as frações polares e não-polares devido a sua conformação estrutural.

Estudos anteriores já haviam provado que o teor de amilose tem diferentes polimorfos (-A, -B) quando cristalizado com moléculas de água dentro da cavidade e (-V) quando faz as inclusões complexos novos complexos. Alguns relatos como: ácidos linoléico conjugado [71], Como álcoois de cadeia ramificada -terc butil [71], Drogas como ibuprofeno [72], Iodo [70], A pequena e álcoois lineares como 1-butanol [73, 74], e de compostos bioativos como genisteína [75], suportam a evidência sobre a formação de complexos de inclusão com teor de amilose.

Ainda a capacidade complexante é favorecida por causa do V-polimorfo da amilose, onde apresenta um interior da hélice de característica hidrofóbica que desempenha um importante papel na estabilização dos complexos de inclusão com moléculas orgânicas [76]. Wokadala, Ray, & Emmambux (2012) [77], discutiram as características estruturais e alguns fatores que afetam o teor de amilose V, sendo a formação do complexo influenciado como o tipo de amido, grau de polimerização de amido, teor de umidade, efeito da estrutura e concentração do ligante, calor, pH e presença de aditivos.

### 3.6. MÉTODOS CINÉTICOS PARA IDENTIFICAÇÃO E QUANTIFICAÇÃO DE SEMIOQUÍMICOS

Para avaliar a velocidade de liberação do semioquímico a partir de “dispensers”, bem como sua estabilidade durante o período de liberação, um sistema *in vitro* é aconselhável antes dos testes em campo.

Na literatura destacam-se três metodologias de avaliação cinética: (1) método por gravimetria - baseado na quantificação da perda de massa através da volatilização do feromônio e expressão do resultado em porcentagem da perda de massa; (2) Extração total do feromônio através de solvente orgânico – utilização de solvente orgânico para recuperação do feromônio adsorvido em uma matriz, verificando sua perda através da diferença da concentração final com a concentração inicial e (3) coleta dinâmica da liberação dos voláteis em câmara de aeração – aprisionamento dos voláteis em cartuchos adsorventes que possuem afinidade com o feromônio, com posterior extração por solvente orgânico e quantificação. Nas técnicas (2) e (3), a cromatografia gasosa é utilizada em conjunto para identificação e quantificação do feromônio e seus produtos de degradação [9].



Takahashi et al (2008) [78] avaliaram a taxa de evaporação do feromônio adsorvido em peça polimérica na forma de iscas conjuntamente com peças vítreas sinterizadas, através do uso de balança analítica interligada a computador, realizando 90 leituras por minuto em intervalo de 210 minutos. Diferenças foram observadas nas taxas de liberação entre as duas matrizes. A matriz polimérica apresentou maior eficiência por promover maior interação com o feromônio. Apesar de a técnica citada fornecer dados substanciais ao longo do período avaliado, a mesma possui falhas por erros na precisão e exatidão obtidos para as taxas de liberação, influenciadas pela umidade do ambiente e sujidades que possam se depositar no sistema.

No intuito de viabilizar uma técnica rápida e eficiente para monitorar taxas de volatilização para feromônios, Stipanovic et al (2004) [46] propuseram a utilização da termogravimetria como ferramenta eficiente para esta finalidade. Realizando estudo com as substâncias 1-dodecanol e dispartlure, feromônios de atração sexual da traça egípcia, foi possível verificar que a técnica é viável quando a substância adsorvida não possui característica de solubilidade na matriz inorgânica onde poderia provocar o intumescimento da matriz e não sua adsorção. A técnica ainda fornece dados como a temperatura de volatilização do feromônio que com a informação da área de absorção e morfologia da matriz, é possível realizar uma correlação entre elas podendo prever a taxa de liberação no ambiente.

Cuthbert [80] e Meighen [81] e seus respectivos colaboradores, avaliaram a liberação do feromônio adsorvidos em matrizes na configuração de iscas. Para recuperação do feromônio, as iscas foram submetidas a um fluxo constante de gás inerte, sendo o material recolhido em um tubo no formato de “U” mantido sobre refrigeração. Esquema do sistema de extração é representado na Figura 7. Após período de extração, hexano foi utilizado para recuperação do feromônio condensado dentro do tubo. Em ambos os artigos, o eluído foi analisado por cromatografia à gás acoplada à detector de ionização de chama (CG-DIC), seguido de comparação com padrão do feromônio. Esta técnica permite a quantificação e identificação do feromônio como também de possíveis produtos de degradação voláteis. Porém, para garantir a eficiência da técnica, é necessário que ocorra a total extração do feromônio para a obtenção de resultados exatos.

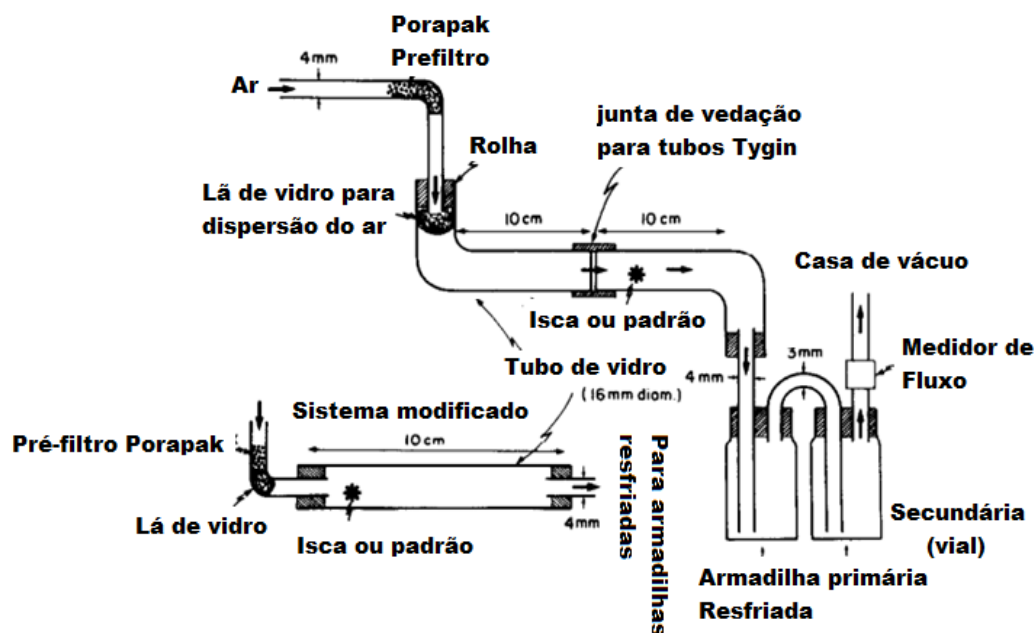


Figura 7: Modelo de câmara de aerção para avaliação da cinética de liberação de feromônios a partir de “dispensers” [70].

Muñoz-Pallares et al (2001) [11], para quantificar o feromônio liberado a partir de pastilhas de zeólitos, utilizaram câmara com fluxo constante de gás inerte. As pastilhas foram eluídas com etanol ou  $\text{CH}_2\text{Cl}_2$ , e sendo posteriormente avaliada por CG-DIC. O método mostrou-se adequado quantificar e mensurar as velocidades de liberação do feromônio nos respectivos zeólitos.

Tomaszewska et al (2005) [27] utilizaram cartuchos de substâncias adsorventes como C18, sílica, Super-Q, Tenax, Porapak-Q e carvão ativado para recuperar o feromônio codlemone volatilizado e carregado pelo gás de arraste oriundo do sistema. Para identificação e quantificação das substâncias volatilizadas, o equipamento de Cromatografia à gás acoplada à espectrometria de Massas (CG-EM) foi utilizado, propiciando não somente a identificação do feromônio, mas também os seus produtos de degradação e substâncias que possam provocar erros por ocorrência de contaminação. Apesar da técnica propiciar a identificação de produtos de degradação, no presente estudo não foi identificado produtos de degradação do codlemone.

A escolha da técnica para avaliação da cinética de evaporação de um feromônio, como também da sua estabilidade após adsorção deve considerar as características da matriz, nesse aspecto a característica de adsorção de umidade do ambiente é o fator determinante; e as características de interação do feromônio com a matriz.

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## 4. CAPÍTULOS

### 4.1. ARTIGO 01 - SYNTHESIS, CHARACTERIZATION AND EVALUATION OF MFI ZEOLITES AS MATRIXES FOR RHYNCHOPHOROL PROLONGED RELEASE

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ARTIGO 01 - SYNTHESIS, CHARACTERIZATION AND EVALUATION OF MFI ZEOLITES AS MATRIXES FOR RHYNCHOPHOROL PROLONGED RELEASE

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## ABSTRACT

Rhynchophorol has been used as lure for monitoring and controlling the beetle *Rhynchophorus palmarum* L. in palm tree culture. However, for the efficient use of techniques based on pheromone it is required a constant and adequate release of this compound for long period, while adult insects are present. In order to evaluate the potential use of MFI zeolites (ZSM-5 and silicalite-1) as matrixes to slow down the release of rhynchophorol, the silicalite-1 and ZSM-5 zeolites in SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> molar ratio (SAR) of 24, 41 and 52 were synthesized and characterized by XRD, ED XRF, <sup>27</sup>Al MAS NMR, FTIR, TG, SEM, textural analysis and NH<sub>3</sub>-TPD. Recovery studies showed that the rhynchophorol was completely degraded in ZSM-5, independently of the SAR. The degradation mechanism was proposed to occur via an intramolecular E1 elimination. Recovery above 90% was obtained only from silicalite-1 (SAR = ∞). This result indicated that not only acidity, but diffusional effects determine the stability of rhynchophorol in MFI zeolites.

Keywords: ZSM-5; Silicalite-1; Pheromone dispenser; *Rhynchophorus palmarum*; Rhynchophorol.

## INTRODUCTION

Rhynchophorol (2-methyl-5(E)-heptene-4-ol), an aliphatic alcohol, is the compound identified as the main constituent of *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) pheromone. This beetle attacks mainly plants of the Arecaceae family and sugarcane [1-2]. It is attracted by the odor of fermentation sap of plants that suffered mechanical injury. When it finds the host plant, it releases an aggregation pheromone that attracts both males and females to the food source. The copulation occurs due to the presence of both sexes in the same environment. The females do postures in the newly opened wounds in the plant, restarting the entire life cycle. The larvae feed on the internal tissues of the plant crown, what may cause its death due to contamination by agents such as bacteria and fungi. In addition, the insect can cause an indirect fatal damage. This occurs because it is the main vector of the nematode *Bursaphelenchus (Rhadinaphelenchus) cocophilus*, causative agent of the red ring disease [1,3-5].

Several approaches based on pheromones have been used in agriculture for pest control due to several advantages, such as high specificity, biodegradability, and low toxicity, when compared to chemical control [6]. Considering *Rhynchophorus palmarum*, the bait based on rhynchophorol is the only way to control the beetle. In this case, the attraction of the insects to traps is used to capture *R. palmarum* adults. Thereby, different traps containing synthetic rhynchophorol have been tested [7-11]. The bucket trap has shown higher efficiency [7,8,10,11].

Generally, the bucket trap comprises a plastic bucket provided with a perforated cover, in which a funnel is inserted, allowing the passage of the insect, which will remain retained within the bucket [7-11]. The lures based on rhynchophorol used in these traps have been marketed as plastic bags [8,10] or plastic tubes, like Eppendorf safe-lock™ with a hole in the cap [11]. Both dispensers are classified as reservoir release systems. They have one disadvantage that is the dependence of the release rate on the particular fill level. As a result, there is a decrease in the rate with time and hence nonlinear release behavior [12]. Moreover, these dispensers are not environmental friendly, because plastic dispensers remain as waste in the field.

To overcome the disadvantages presented by the reservoir release systems, research efforts are directed towards the development of controlled release systems,

in which the pheromone is enclosed in “release carriers”, designed to release it in the appropriate dosage for a specified period of time [13]. Other advantages associated with this technique include reducing losses related to volatility and degradation, as well as longer time for replacement of the lure.

Various materials for the controlled release of a variety of bioactive agents for pest control can be found in the literature [14-18]. Among them, zeolites can be an efficient choice for pheromones release due to the characteristics of their structure. These aluminosilicates have a network of channels and cavities with pore diameters below 2 nm [19], which allows the diffusion of the pheromone. A simulation of its 3D structure obtained through ACD/3D Viewer™ software showed that the greater internuclear distance was 8.8 Å, which corresponds to the chain length of the rhynchophorol; the diameter was 2.2 Å; and molar volume was  $154.4 \pm 3.0 \text{ cm}^3 \text{ mol}^{-1}$ .

Some zeolites were already studied for this purpose, like zeolites A, X, Y, ZSM-5, beta, titanosilicate ETS-10 and mordenite [6,20,21], but no study using rhynchophorol was reported until this moment.

Jakus et al. [20] replaced polyethylene-bag verbenone dispensers by composite dispensers, consisting of zeolites (mordenite, Y or silicalite), verbenone and non-host volatiles, for controlling of *Tomicus piniperda* L. and *Ips typographus*. In conifer forest field, the experiments were partially successful for both species. The authors also evaluated the verbenone efficiency from commercial dispenser based on natural zeolite by aerial spraying. The experiments were unsuccessful due to low population of *I. typographus* and the large amount of dispenser remained in tree crowns.

Muñoz-Pallares et al. [6] evaluated the release of n-decanol and trimedlure through the following materials: zeolite X, Y, ZSM-5, beta, all in aluminosilicate forms. They concluded that these zeolites are suitable for application as dispenser for the studied semiochemicals. Furthermore, kinetic emission of the semiochemical can be easily changed by varying parameters such as: Si/Al molar ratio, compensating cations, presence of Brønsted acid sites, pore diameters and shape, and compression force; enabling approach to zero kinetics order, ideal feature for a dispenser.

Seo et al. [21] observed that a powder sample of zeolite A modified with cetyltrimethylammonium cations was an efficient matrix for the prolonged release of aggregation pheromones of *Riptortus pedestris*. This zeolite based matrix has presented superior results in the capture of the insects in soy and plum crops when compared to a commercial product.

Among the zeolites, ZSM-5 is a promising zeolite for application as rhynchophorol carrier, because its pore structure has the adequate dimensions to host the guest molecule and its pore system complexity could enable a slower and constant release for practical purposes. Zeolite ZSM-5 tridimensional framework (Figure 1) is constituted by two interconnected pore systems, one of them is a straight channel accessed by 10 membered rings of  $5.3 \times 5.6 \text{ \AA}$  in the crystallographic direction  $[010]$  and the other is a bi-dimensional sinusoidal channel, accessed by ellipsoidal 10 membered rings of  $5.1 \times 5.5 \text{ \AA}$  along  $[100]$  crystallographic direction [14]. This peculiar topology was designated as MFI (Mobil-Five) according to International Zeolite Association and describes not only ZSM-5 zeolite, but also isostructural pure silica materials, as silicalite-1.

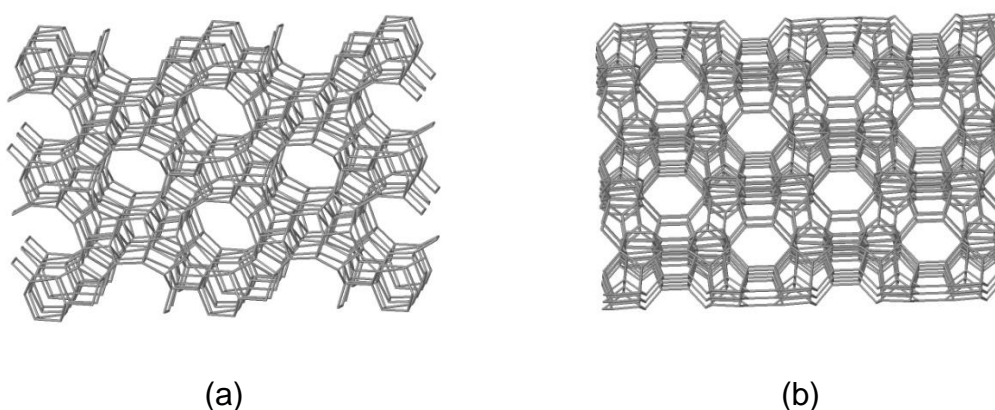


Figure 1. ZSM-5 framework (MFI topology) viewed along: (a)  $[010]$  and (b)  $[100]$  directions. Adapted from IZA Database Zeolite Structure.

Thus, the objective of this work is to evaluate the potential use of MFI zeolites (ZSM-5 and silicalite-1) as dispensers to prolong the release of rhynchophorol, aiming their application in coconut tree culture.

## EXPERIMENTAL

### Synthesis of MFI zeolites

ZSM-5 zeolite was prepared by adapting the procedures described by the International Zeolite Association (IZA) [22]. NaOH (Synth),  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (Synth), tetrapropylammonium hydroxide solution (TPAOH, Sigma-Aldrich, 20% in aqueous solution) and  $\text{SiO}_2$  Aerosil®200 (Degussa) were used as raw materials. Synthesis gels of the following general composition were prepared:  $0.30 \text{ NaOH}:\text{SiO}_2:x\text{Al}_2\text{O}_3:0.05\text{TPAOH}:30\text{H}_2\text{O}$  ( $x = 0.0125 - 0.033$ ).

The gels were transferred to PTFE-lined steel autoclaves and hydrothermally treated under non-agitated conditions. The reaction parameters were varied as described on Table 1.

For the synthesis of silicalite-1 (silica analogue of the ZSM-5 zeolite), a gel with the following composition was prepared:  $0.04\text{NH}_4\text{OH}:\text{SiO}_2:0.08\text{TPAOH}:20\text{H}_2\text{O}$  and crystallized under non-agitated conditions according to the conditions described in Table 1.

In order to eliminate the organic template, the samples were calcined from room temperature up to  $550^\circ\text{C}$ , at the rate of  $1^\circ\text{C min}^{-1}$ , under  $\text{N}_2$  flow of  $50 \text{ mL min}^{-1}$ . After the temperature reached  $550^\circ\text{C}$ , the  $\text{N}_2$  flow was substituted by synthetic air, remaining in this temperature for 6 h. The samples were cooled down under synthetic air and stored in plastic containers.

**Table 1.** Reaction parameters used in the hydrothermal synthesis of MFI zeolites.

Sample code	$\text{SiO}_2/\text{Al}_2\text{O}_3$	$\text{NaOH}/\text{SiO}_2$	$\text{TPA}/\text{SiO}_2$	$\text{H}_2\text{O}/\text{SiO}_2$	Time (days)	T ( $^\circ\text{C}$ )
IR14	30	0.30	0.05	30	6	150
IR17	50	0.30	0.05	30	6	150
IR21	80	0.30	0.05	30	6	150
IR07	$\infty$	0.04 <sup>a</sup>	0.08	20	17	180

<sup>a</sup>  $\text{NH}_4\text{OH}/\text{SiO}_2$



## Zeolite characterization

Powder diffraction patterns of the samples were collected on a Shimadzu XRD6000 diffractometer, using  $\text{CuK}\alpha$  radiation generated at 40 kV and 30 mA, with a scan speed of  $2^\circ \text{ min}^{-1}$  and a step of  $0.02^\circ$  in the range of  $1.4^\circ$  to  $80^\circ 2\theta$ , using divergence, scattering and reception slits of  $0.5^\circ$ ,  $0.5^\circ$  and 0.3 mm, respectively.

The semi-quantitative analysis of zeolite samples was obtained by Energy Dispersive X-ray Fluorescence spectroscopy (ED XRF). The powder was pressed and analyzed through Shimadzu EDX-720 equipment, with Rh radiation source at 15 KV (Na to Sc) or 50 KV (Ti to U), and collimating slit of 10 mm.

$^{27}\text{Al}$  MAS NMR spectra were obtained on a Bruker Advance 600 spectrometer (14.1 T magnetic field strength) at resonance frequency of 156.38 MHz, with a 4 mm MAS rotor and sample spinning rate of 12 kHz. Single-pulse excitation of 2  $\mu\text{s}$  and recycling times of 500 ms were used. Chemical shifts of aluminum were measured with reference to a  $1 \text{ mol L}^{-1} \text{ Al}(\text{NO}_3)_3$  aqueous solution at 0 ppm. To determine the relative amounts of tetraordinated ( $\text{Al}_{\text{Td}}$ ) and hexacoordinated aluminum ( $\text{Al}_{\text{Oh}}$ ) species, a simple integration of the respective peaks was applied. Results are shown in Supplementary Material as Figure S.1.

Fourier transformed infrared spectra (FTIR) of the samples were recorded in air at room temperature using a Shimadzu IR Prestige-21, with a resolution of  $2 \text{ cm}^{-1}$ , in the range of  $400\text{-}4000 \text{ cm}^{-1}$ , using 20 scans. The samples were prepared as 1.0 wt.% in KBr wafers. Results are shown in Supplementary Material as Figure S.2.

Thermogravimetric (TGA) and Derivative Thermogravimetric Analysis (DTG) were performed on a Perkin Elmer Pyris 1 TGA instrument from room temperature up to  $850^\circ\text{C}$ . A sample mass of 5 mg was heated under nitrogen flow of  $50 \text{ mL min}^{-1}$ , at a heating rate of  $10^\circ\text{C min}^{-1}$ , using a platinum cell. Results are shown in Supplementary Material as Figure S.3.

The morphology of the crystalline phase was examined using a Scanning Electron Microscope (SEM, Shimadzu, Superscan SSX-550) after metallization of the samples. For metallizing, the samples were deposited on the carbon tape adhered to the equipment support. Hereafter, they were metallized with gold vapor, using a Quick coater SC-701, Sanyu Electron, operating with a 5 mA current and voltage of 1 kV.

The process included an initial step of 5 min in vacuum, 15 min of metallization in argon atmosphere.

The adsorption-desorption isotherms of nitrogen were obtained at  $-196^{\circ}\text{C}$  with a Micromeritics ASAP 2020. The sample was previously treated for 180 min at  $350^{\circ}\text{C}$  under vacuum. The pore diameters, as well as the pore size distribution, were calculated by using the BJH (Barrett, Joyner and Halenda) method. The specific area was calculated with the BET (Brunauer, Emmett and Teller) equation in the low pressure region ( $P/P_0 < 0.3$ ), and the micropore area and micropore volume were estimated by using the t-plot method.

The profiles of the Temperature Programmed Desorption of ammonia (TPD- $\text{NH}_3$ ) were obtained with a Micromeritics Chemsorb 2720. The samples were pretreated for one hour at  $300^{\circ}\text{C}$ , under helium flow of  $25\text{ mL min}^{-1}$  followed by cooling down to room temperature in order to remove any physisorbed species on the surface of the sample. After this pretreatment, the samples were submitted to a chemisorption step using ammonia in helium (9.9%, mol/mol) flow of  $25\text{ mL min}^{-1}$  at room temperature, for 60 min. Thereafter, the system was purged with helium at room temperature for 60 min. In order to remove the physisorbed ammonia molecules, the material was treated for 60 min at  $150^{\circ}\text{C}$  under helium flow ( $25\text{ mL min}^{-1}$ ) and then cooled down to room temperature. This step was followed by the temperature programmed desorption analysis, in which the sample was heated from room temperature up to  $1000^{\circ}\text{C}$ , at a heating rate of  $10^{\circ}\text{C min}^{-1}$  and under helium flow ( $25\text{ mL min}^{-1}$ ). The amount of desorbed ammonia was monitored with a thermal conductivity detector.

### **Adsorption and recovery of rhynchophorol in zeolite**

For the adsorption process of rhynchophorol in zeolites, approximately 50 mg of the zeolite sample was weighted with a subsequent transfer to a glass tube. Then, approximately 7.5 mg of rhynchophorol (Interacta, Brazil) was added to the tube. The glass tube was sealed and the suspension was stirred during 1 min. The rhynchophorol-zeolite system obtained was kept at rest for 24 h. Five replicates were obtained by this method.

To measure the recovered rhynchophorol after 24 h of contact with the zeolite, 2 mL of n-hexane (Mallinckrodt, HPLC grade) were added to a glass tube containing the rhynchophorol-zeolite sample. The system, after sealed, was stirred during 1 min, resting for 4 h and stirred again for 1 min. The suspension was filtered using a 0.45  $\mu\text{m}$  Nylon membrane (13 mm Allcrom).

To determine rhynchophorol in hexane solution, a Perkin Elmer Clarus 680 gas chromatograph connected to a Perkin Elmer 600 C quadrupole mass spectrometer was used. The following conditions were used: Elite-5MS (30 m x 0.25 mm x 0.25  $\mu\text{m}$ ) column, helium flow = 1 mL  $\text{min}^{-1}$ , injection volume = 1  $\mu\text{L}$ , split = 50 mL  $\text{min}^{-1}$  and temperature of injector = 150°C. The temperature program used was: initial temperature = 50°C during 3 min, followed by increasing of temperature with heating rate of 10°C  $\text{min}^{-1}$  up to 200°C, remaining for 1 min. A volume of 10  $\mu\text{L}$  of 6-methyl-5 hepten-2-one was used as an internal standard (IS). The chromatographic data were analyzed using the Turbomass software, version 5.4.2.1617.

### **Rhynchophorol release and stability studies**

The release rate of rhynchophorol from silicalite-1 was measured by isothermal thermogravimetric method at 40°C. The weight loss was assumed to result from pheromone and residual moisture evaporation [14]. The tests were performed in the same thermobalance used for zeolite characterization. The following conditions were used: sample mass = 7 mg, nitrogen flow = 50 mL  $\text{min}^{-1}$ , heating rate = 2°C  $\text{min}^{-1}$ , during 600 min.

Long term stability of rhynchophorol adsorbed on silicalite-1 was performed by determining the pheromone recovery as described in the section 2.3 after different times of storage in an capeless Eppendorf<sup>TM</sup> at room temperature (25  $\pm$  1°C).

## RESULTS AND DISCUSSION

### Characterization of synthesized zeolites

The as-synthesized and calcined forms of MFI zeolites were analyzed by X-ray diffractometry in order to verify if the MFI structure was obtained and evaluate how the calcination step affected the crystallinity. Figure 2 shows the X-ray diffraction patterns of the samples obtained with different molar ratios  $\text{SiO}_2/\text{Al}_2\text{O}_3$ .

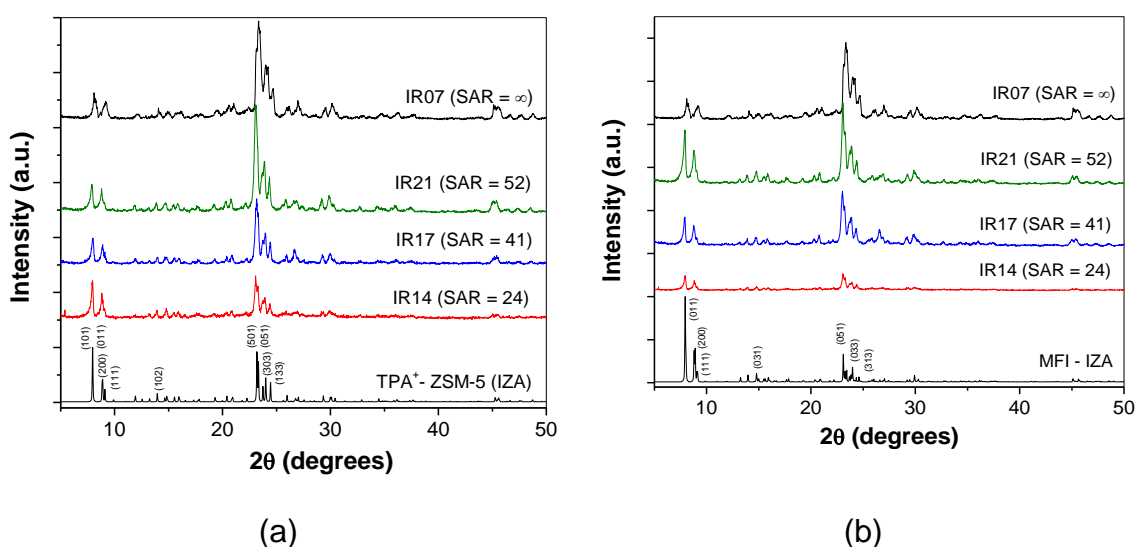


Figure 2. Powder X-ray diffraction patterns of MFI zeolites: (a) as-synthesized and (b) calcined forms.

As can be seen, all the samples are consistent with IZA pattern [22], confirming that the synthesized materials of different  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratio exhibit MFI topology. The diffraction patterns also show that the materials were kept intact after calcination process.

The relative crystallinity was calculated for calcined materials by comparison between the areas under the peaks in the range of  $22.5\text{-}25.06^\circ 2\theta$ , where usually appear the most intense peaks in X-ray diffraction pattern of ZSM-5 zeolite. The results are shown in Table 2. According to Table 2, the relative crystallinity increases with increasing of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratio. This should be considered for analysis of other chemical, structural and textural properties.

The experimental  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratio of synthesized zeolite was determined by ED XRF and by  $^{27}\text{Al}$  NMR, and the values are also shown in Table 2.

**Table 2.** Structural, chemical and textural properties of MFI zeolites.

Sample	Cl (%)	SAR		$S_{\text{BET}}$ ( $\text{m}^2 \text{g}^{-1}$ )	$S_{\text{external}}$ ( $\text{m}^2 \text{g}^{-1}$ ) <sup>a</sup>	$S_{\text{micropore}}$ ( $\text{m}^2 \text{g}^{-1}$ ) <sup>a</sup>	$V_{\text{micropore}}$ ( $\text{cm}^3 \text{g}^{-1}$ ) <sup>a</sup>	$V_{\text{mesopore}}$ ( $\text{cm}^3 \text{g}^{-1}$ ) <sup>b</sup>	$d_{\text{mesopore}}$ (nm) <sup>b</sup>
		ED XRF	<sup>27</sup> Al NMR						
IR14	35.6	24	24.3	225.3	50.5	174.8	0.0812	0.0154	57.21
IR17	61.4	41	41.3	342.1	87.9	254.2	0.1180	0.0737	98.29
IR21	82.1	52	53.3	388.0	120.8	267.2	0.1240	0.1326	98.37
IR07	100.0	$\infty$	-	319.0	288.3	30.7	0.0118	0.0614	147.31

Cl=Crystallinity

<sup>a</sup> *t-plot* method<sup>b</sup> BJH method

The experimental values of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio molar of zeolites are always lower than nominal values. This suggests that aluminum was more easily incorporated than silicon in the alkaline pH used for synthesis. The relatively high discrepancy between nominal and experimental SAR values shown in Table 2 can be explained by the silicon loss during the synthesis. The strongly basic medium favors the solubility of silicon due to increase of coordination number by hydroxyl ions, weakening Si-O bonds.

Figure 3 shows the images obtained with SEM of representative samples of MFI zeolites, all in calcined form.

SEM analysis showed that, with exception of IR07 (silicalite-1), the samples are formed by aggregates of approximately spherical morphology, that seem to be constituted by a great number of nanosized crystallites. This morphology is atypical for ZSM-5 zeolite, which is described in the literature as ellipsoidal particle formed by aggregates of prismatic crystallite [23] or prismatic crystal similar to coffins [24].

The sample IR14 (SAR = 24) showed agglomerates of various sizes, as pointed out in Figure 3a. The smaller aggregates have approximately 10  $\mu\text{m}$  of diameter, while the higher ones approximately 60  $\mu\text{m}$ .

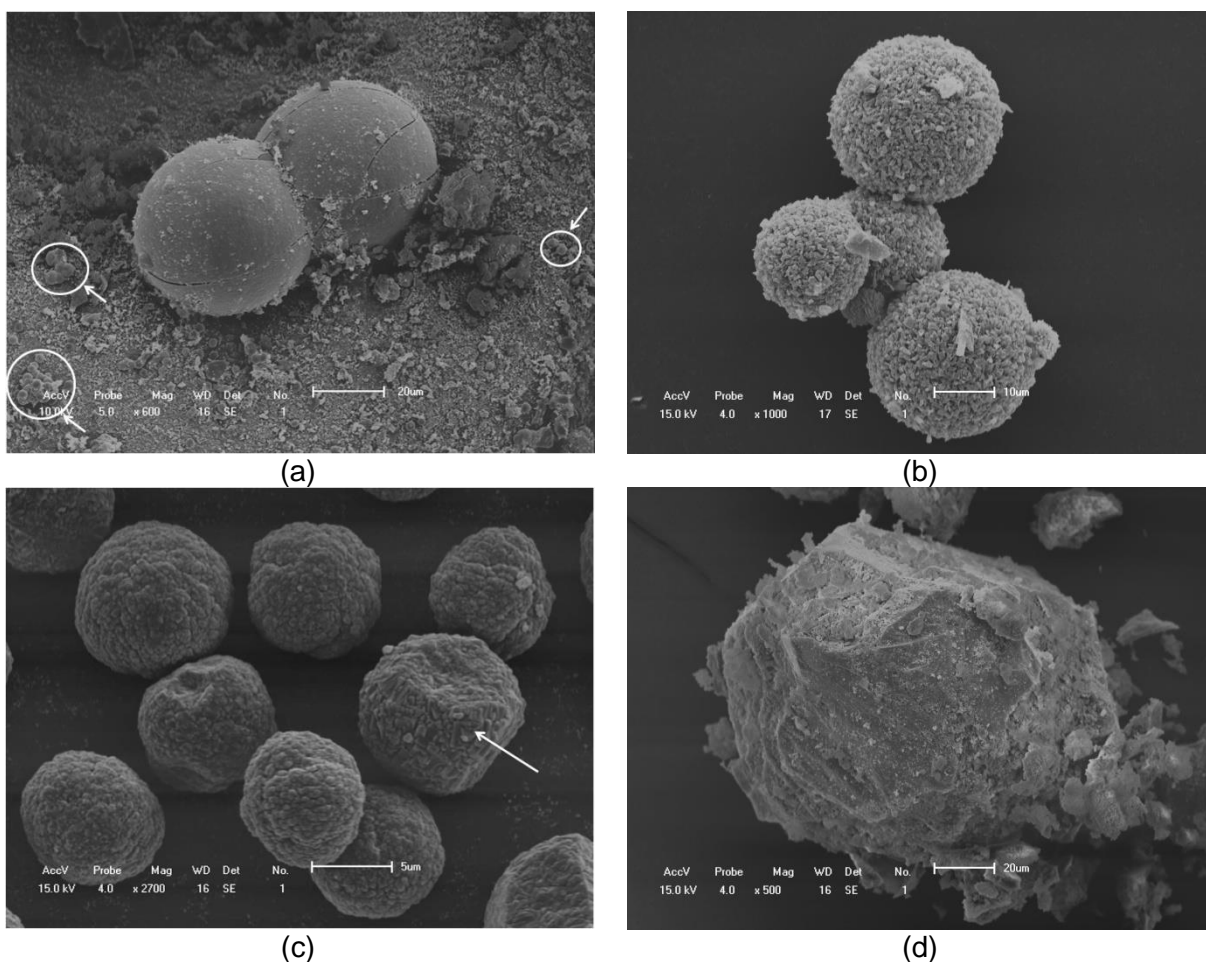


Figure 3. SEM micrographs of MFI family: (a) IR14 (SAR = 24); (b) IR17 (SAR = 41); (c) IR21 (SAR = 52); and (d) IR07 (SAR =  $\infty$ ).

The sample IR17 (SAR = 41), Figure 3b, showed homogeneous size agglomerates, varying between 19 and 29  $\mu\text{m}$ , but with rough surface. Similar morphology was observed by Derouane et al. [25], for a synthesis using sodium silicate, aluminum sulfate and tetrapropylammonium bromide (TPABr), as organic template. Thus, the morphological differences found in synthesized material can be explained by adaptations in standard synthesis method, which involves the use of gel seed and a different source of aluminum and silicon.

The morphology of the sample IR21 (SAR = 52), Figure 3c, is slightly different, because such particles are not spherical and resemble a small cauliflower, with various sizes between 8 to 10  $\mu\text{m}$ . However, in larger magnitudes, prismatic crystallites are observed in the aggregates, as indicated by the arrows in Figure 2.

The nitrogen adsorption isotherms for the calcined MFI zeolites are depicted in Figure 4.

The isotherms of the MFI zeolites can be described as hybrid type I / type IV patterns by IUPAC classification, typical of predominantly microporous materials, with secondary mesoporous, formed by stacking of particles. Furthermore, the isotherms in Figure 4 presented an H4 hysteresis loop [26]. This type of loop is associated with narrow slit-shaped pores [27].

The pore size distribution of the sample IR14, Figure 4b, shows that the sample is predominantly microporous, with almost no contribution of mesoporosity. Samples IR17 and IR21 (Figure 4c and 4d, respectively), on the other hand, have a bimodal distribution, with a maximum in the microporosity region and other in the mesoporosity region, confirming that this isotherm is a hybrid type. The wide distribution in the mesoporosity region confirms that the mesoporous are secondary.

The isotherm of IR07 (Figure 4a) is a non-conventional isotherm, with intermediate characteristics between type I and IV isotherms, suggesting that this material is a hierarchical material, with different pore sizes in the micro-, meso- and macroporosity regions. The presence of a step in the region of low relative pressures  $p/p^\circ$  was attributed to densification of the adsorbed phase, that corresponds to a phase transition from a lattice fluid-like phase to a crystalline-like solid phase [28, 29]. The microporosity is evidenced by inflexion point in low values of relative pressure ( $p/p^\circ$ ). The mesoporosity is characterized by hysteresis loop, from capillary condensation phenomena. This interpretation can be confirmed by pore size distribution as displayed in Figure 4e, in which a trimodal pore distribution can be seen (the microporous are evidenced by tendency of increase in adsorbed volume in regions of pores smaller than 2 nm).

The nitrogen adsorption isotherms displayed in Figure 4 were mathematically treated by BET, t-plot (for accessing information about microporosity of zeolites), and BJH (for obtaining data about the average diameter and volume of mesoporous) methods [29]. These results are summarized in Table 2.

The sample IR14 (SAR = 24) presents inferior textural properties both in micropore and mesopore regions, when compared to samples with higher  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratios. This was attributed to the low relative crystallinity observed for this material. IR07 (SAR =  $\infty$ ) sample showed the lowest micropore volume, and its surface area is constituted primarily by external area. However, this data must be cautiously evaluated, because t-plot uses BET surface area in calculation and BET method is not adequate for microporous materials [30,31].

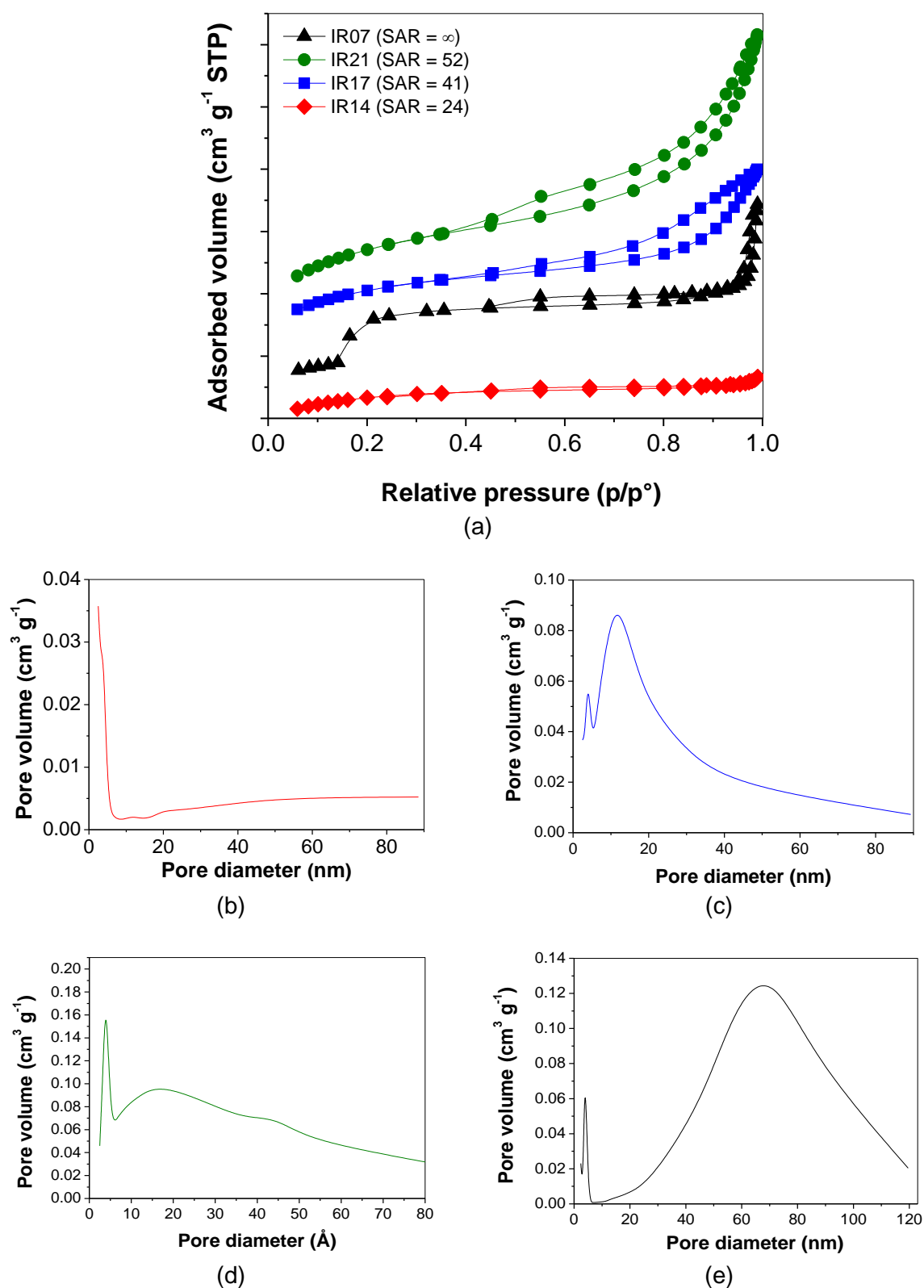


Figure 4. Nitrogen adsorption isotherms at  $-196^{\circ}\text{C}$  for MFI zeolites (a) and distribution of pore size for the samples: (b) IR14 (SAR = 24); (c) IR17 (SAR = 41); (d) IR21 (SAR = 52) and (e) IR07 (SAR =  $\infty$ ). Isotherms were shifted to ease visualization.



Acidity is not found in surface of materials in which only tetrahedral Si is present. However, Brönsted acid sites are observed in zeolites due to  $\text{Si}^{4+}$  species that are isomorphically substituted by trivalent metallic cation, as  $\text{Al}^{3+}$ . Thus, a negative charge is formed in zeolite framework, which is balanced by a proton ( $\text{H}^+$ ) or other counter ions, such as  $\text{NH}_4^+$ ,  $\text{K}^+$  or  $\text{Na}^+$ . As ZSM-5 zeolites were synthesized in sodium rich medium, at least a fraction of the exchange sites are occupied by  $\text{Na}^+$  cations in the final structure [32].

The acidity of zeolite can be related to aluminum content, and is expected a linear increase in total acidity with an increase of aluminum content in zeolite [32,33]. Additionally to Brönsted acidity nature, the aluminosilicates also have Lewis acidity, due to the presence of low coordination aluminum compound or  $\equiv\text{Si}^+$  ions, formed by dehydroxylation during calcination step in temperatures greater than  $500^\circ\text{C}$  [33]. Although the  $\text{NH}_3$  molecule is not an adequate probe molecule for distinguishing between acid sites of Brönsted and Lewis, the temperature desorption can be a criterion in the evaluation of strength of acid sites.

The  $\text{NH}_3$ -TPD profiles of calcined zeolites and their respective curves generated after deconvolution can be viewed in Figure 5. As shown in Figure 5, the simulated curves showed  $r^2 > 0,9909$  and error lower than 6.7%.

The  $\text{NH}_3$ -TPD profiles were quantified and the data shown in Table 3. Ammonia desorbs in three main regions: below  $250^\circ\text{C}$ , between  $250$ - $350^\circ\text{C}$ , and above  $350^\circ\text{C}$ , corresponding to weak, moderate, and strong acid sites, respectively [34]. For ZSM-5 samples, two peaks were observed in low temperature region, suggesting the presence of two types of weak acid sites.

Analyzing the data of Table 3, it is possible to verify that there was not a decrease in density of total acid sites when the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratio increased of 24 up to 41, as expected. Unlike, occurs a slight increase in total acid sites content, probably due to differences in crystallinity of materials. On the other hand, there is a

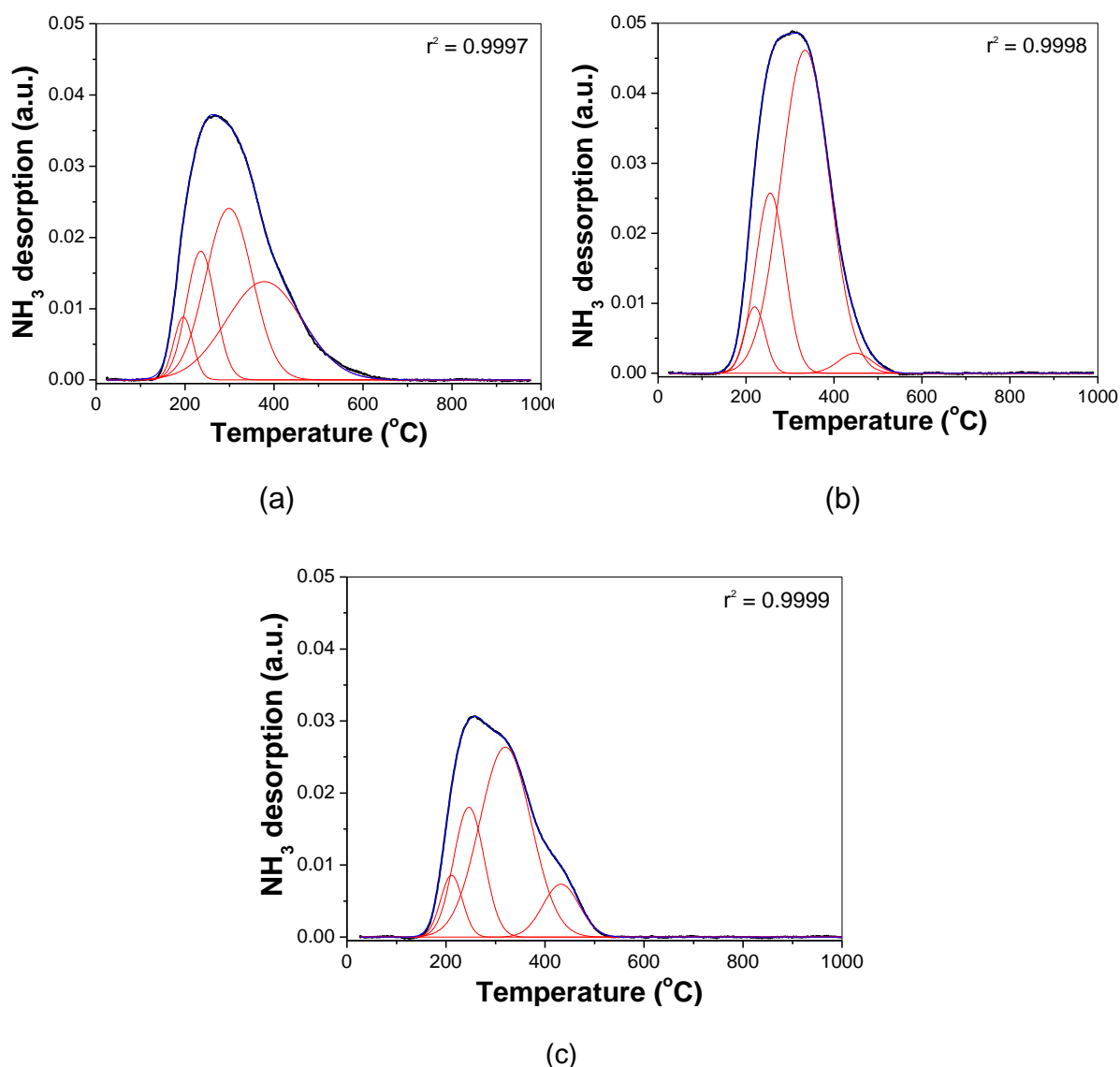


Figure 5.  $\text{NH}_3$ -TPD profile of MFI zeolites: (a) IR14 (SAR = 24); (b) IR17 (SAR = 41); and (c) IR21 (SAR = 52). Profile for sample IR07 (SAR =  $\infty$ ) was not shown because lead to a very low consumption of  $\text{NH}_3$ .

significant decrease in strong acid sites content and an increase in moderate acid sites content, which is consistent with the reduction in aluminum content framework. Increasing  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratio from 41 to 52, materials that have similar relative crystallinities, a decrease in total acid sites content is observed, but the distribution of sites by acid strength is quite similar.

**Table 3.** Quantitative analysis of NH<sub>3</sub>-TPD profiles of MFI zeolites

Sample	Acid site strength	T <sub>m</sub> (°C)	Acid sites density (mmol g <sup>-1</sup> )	
			Partial	Total
IR14	Weak	196.0	0.41 (5.9%)	
		235.4	1.25 (18.1%)	
	Moderate	298.9	2.71 (39.2%)	
	Strong	378.2	2.53 (36.6%)	
IR17	Weak	219.5	0.46 (6.0%)	
		255.0	1.80(23.6%)	
	Moderate	334.3	5.28 (69.2%)	
	Strong	448.5	0.23 (3.0%)	
IR21	Weak	211.2	0.42 (7.4%)	
		246.3	1.24 (21.7%)	
	Moderate	319.9	3.07 (53.7%)	
	Strong	432.5	0.60 (10.5%)	
IR07	Weak	185	0.038 (100%)	

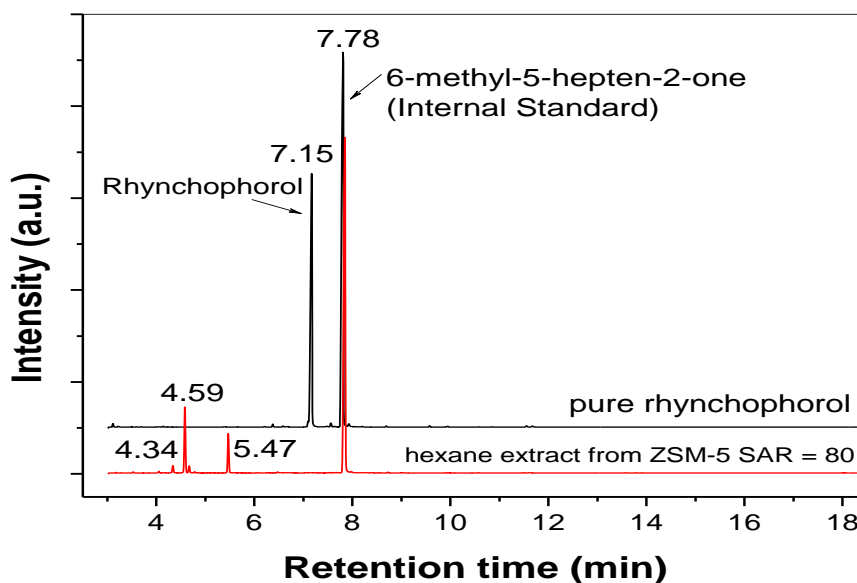
The effect is more pronounced for silicalite-1 (sample IR07), whose experimental SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> molar ratio was ∞. This result was confirmed by the absence of signals in the regions of Al<sub>Td</sub> (δ = 55 – 62 ppm) or Al<sub>Td</sub> (δ ~ 0 ppm) of the <sup>27</sup>Al NMR spectrum (Supplementary Material Figure S.1). The residual weak acidity observed for this sample can be attributed to terminal silyanol groups [35].

### Recuperation, stability and controlled release of adsorbed rhynchophorol

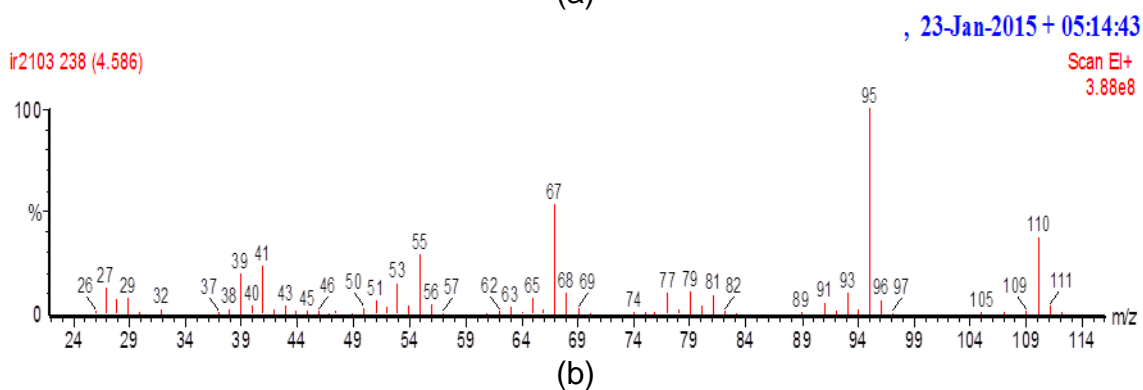
Immediately after the adsorption of rhynchophorol, the zeolite materials did not present any visual alteration. However, after 24 h, the samples IR17 (SAR = 41) and IR21 (SAR = 52) changed from white to an orange color. This color indicates chemical interactions between zeolite and rhynchophorol. Thus, in order to verify the stability of the pheromone, extractions of rhynchophorol from rhynchophorol-zeolite systems were carried out using hexane, since the pheromone is soluble in this solvent.

After 24 h of contact between the rhynchophorol-zeolite system and hexane, it was observed that the extracts were colorless; indicating that the degradation products that promote color were not completely solubilized in hexane and remained into the zeolite network.

Figure 6 shows the chromatograms comparing pure rhynchophorol and rhynchophorol obtained from rhynchophorol-ZSM-5 (SAR = 52) sample. The profiles obtained for hexane extracts from rhynchophorol-ZSM-5 (SAR = 24 and 42) were similar to that obtained with sample IR21(SAR = 52).



(a)



(b)

Figure 6. (a) Chromatograms obtained after extraction of rhynchophorol from IR17 (SAR=52) zeolite and (b) mass spectra obtained for degradation product referring to retention time  $t_r = 4.59$  min.

From Figure 6.a, it is noticed the absence of rhynchophorol peak ( $t_r = 7.15$  min) and the generation of new peaks in chromatogram of hexane extract from IR21 (SAR = 52). This indicates that the adsorbed rhynchophorol was converted to other products.

Rhynchophorol was only recovered from silicalite-1, sample IR07 (SAR =  $\infty$ ). This result is consistent with the permanence of white color observed for this material.

Although sample IR14 (SAR=24) remained white after 24 h after contact with the pheromone, peaks of degradation products were detected in the hexane extract chromatogram, with subsequent decrease in the rhynchophorol content. When the experiment was performed using a commercial zeolite ZSM-5 (Degussa, SAR = 42), the sample changed from white to orange after 24 h of contact with rhynchophorol, indicating that the low crystallinity of the sample IR14 (SAR = 24) was crucial in the final result.

IR14 (SAR = 24) is a material that has a high density of acid sites ( $6.92 \text{ mmol g}^{-1}$ ) and presents a high percentage of moderate and strong acid sites (75.8%). In contrast, silicalite-1 (IR07) has not presented aluminum in its framework and for this reason, presents a very low acid sites density ( $0.038 \text{ mmol g}^{-1}$ ), mainly constituted of very weak acid sites, that desorb under  $200^\circ\text{C}$ . These results suggest that acidity is clearly responsible for the degradation of the pheromone. However, textural and diffusion properties of the materials should be taken into account.

The release rate of rhynchophorol from silicalite-1 matrix was determined by isothermal TG experiments. From the TG curve (not shown) it was possible to see two different regions: i) an initial and fast mass loss (initial release rate =  $0.42 \text{ \% h}^{-1}$ ), probably due to the desorption of rhynchophorol adsorbed on the external surface of zeolite crystals; and ii) a linear region, in which the release rate is very low (linear release rate =  $5.14 \times 10^{-2} \text{ \% h}^{-1}$ ), probably due to the desorption of rhynchophorol from the internal pores of MFI topology or weakly interacting with internal silanol groups or defects.

Compared to liquid rhynchophorol, to which the initial release rate is  $5.54 \text{ \% h}^{-1}$  and the linear release rate is  $1.66 \text{ \% h}^{-1}$ , one could observe a significant decrease in both release rates when the pheromone is adsorbed in the zeolite matrix.

Figure 7 shows the rhynchophorol recovery as a function of time in a laboratory simulated stability test at room temperature.

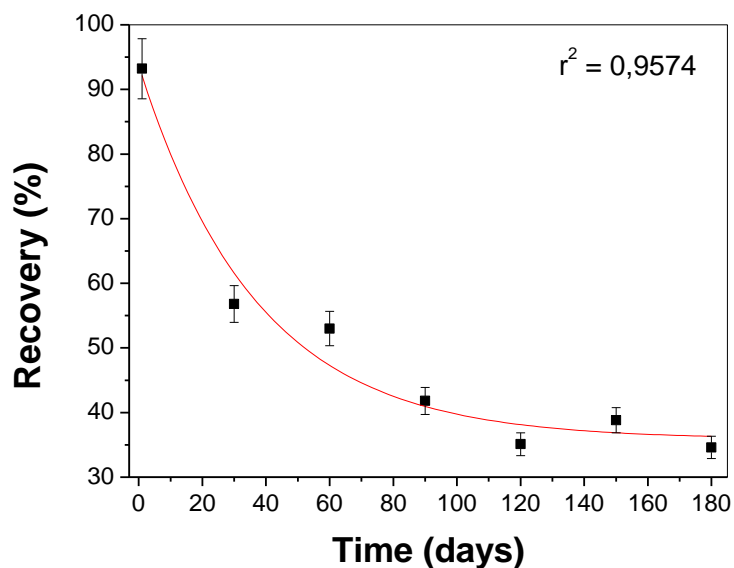


Figure 7. Long time stability test for rhynchophorol adsorbed on silicalite-1 matrix (IR07).

From the analysis of the curve in Figure 7, rhynchophorol content in the silicalite-1 matrix decay exponentially with time in 180 days. As shown by thermogravimetry simulation, a fast initial release was also observed. The initial release rate was calculated as being  $1.48\% \text{ h}^{-1}$  and this rate decreases to a linear release rate of  $1.24 \times 10^{-2} \% \text{ h}^{-1}$ , which is in the same order of magnitude of that estimated by TG.

Thus, silicalite-1 material is a promising dispenser for rhynchophorol prolonged release and could be used in insect trapping systems based on capeless plastic tubes, like Eppendorf™, only by substituting the pure pheromone by the rhynchophorol adsorbed on the solid matrix. Figure 8 presents a prototype of a simple trapping system which was developed by Interacta Química Ltda. to evaluate the efficiency of silicalite-1 matrix as a rhynchophorol delivery system in field studies.

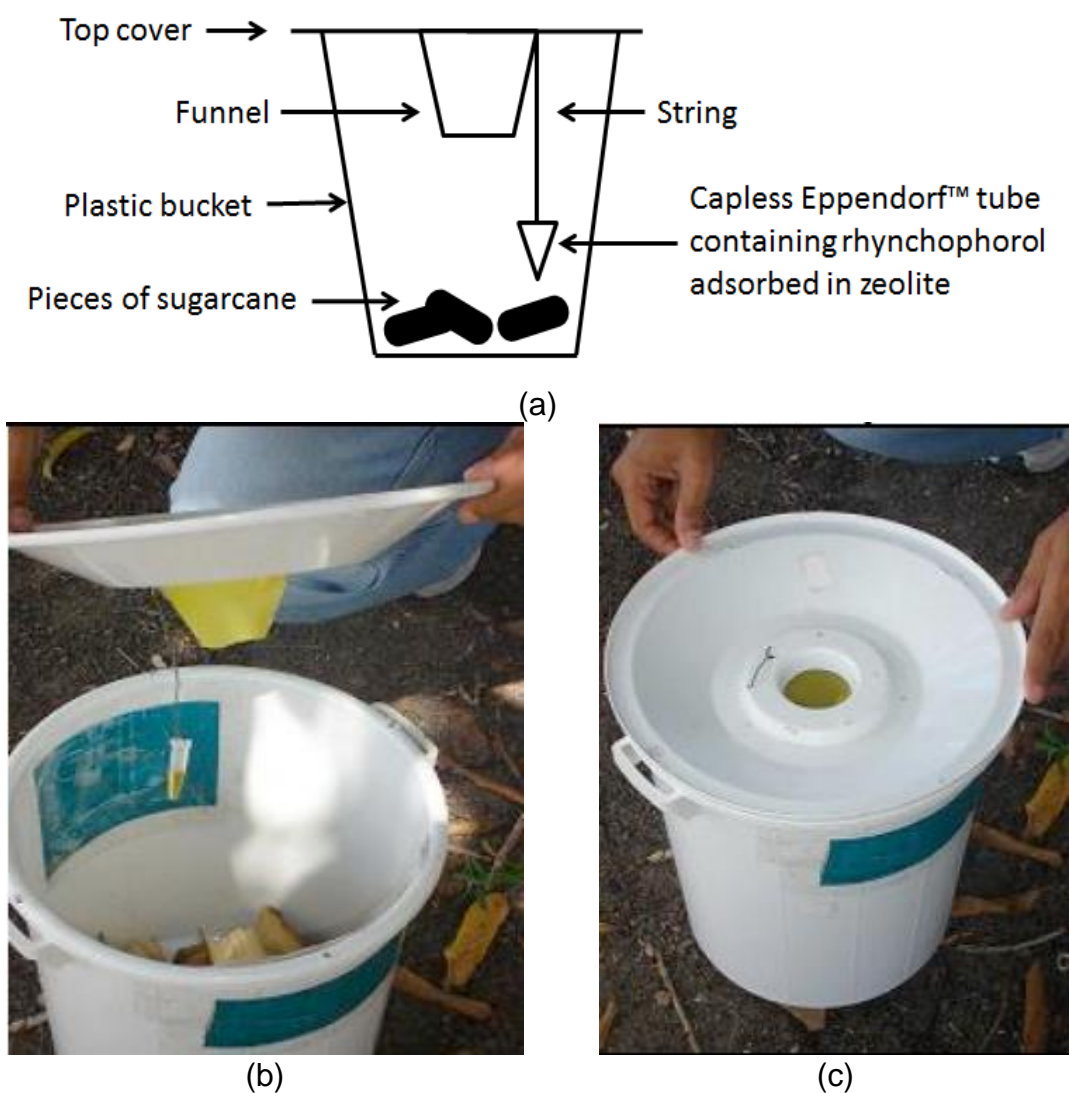


Figure 8. (a) Schematic diagram of a prototype of the insect trapping system fabricated using pheromone based zeolites; (b) Insect trapping system manufactured by Interacta Química Ltda (open lid view) and (c) closed lid view.

### Degradation products formed due to interaction between rhynchophorol and ZSM-5 zeolite

A typical reaction of secondary alcohol, such as (*E*)-6-methyl-2-hepten-4-ol is the E<sub>1</sub> intramolecular elimination, catalyzed by acids, producing alkenes. This elimination occurs by acid-catalyzed dehydration.

The first step of this reaction is the protonation of alcohol, converting the hydroxyl in a good leaving group (H<sub>2</sub>O). Next, the carbocation is formed with

subsequent elimination of proton from a carbon adjacent to the carbon positively charged. Rearrangement of carbocation can occur in this reaction, producing the more substituted (more stable) alkene [36].

Studies indicate that ZSM-5 zeolite can promote the alcohol dehydration at low temperatures, and the increase of crystallinity of these materials causes a higher yield for mentioned reaction [37]. Rhynchophorol degradation products produced by interaction with acid sites of substrate were analyzed in a gas chromatograph linked to mass spectrometer. The results of mass spectrometry (Figure 6(b)) of more volatile peaks than rhynchophorol ( $r_t$  between 4.34 and 5.47 min) confirmed that the reaction of elimination in acidic medium occurred.

The Figure 9 shows the proposed mechanism for rhynchophorol degradation in ZSM-5 samples.

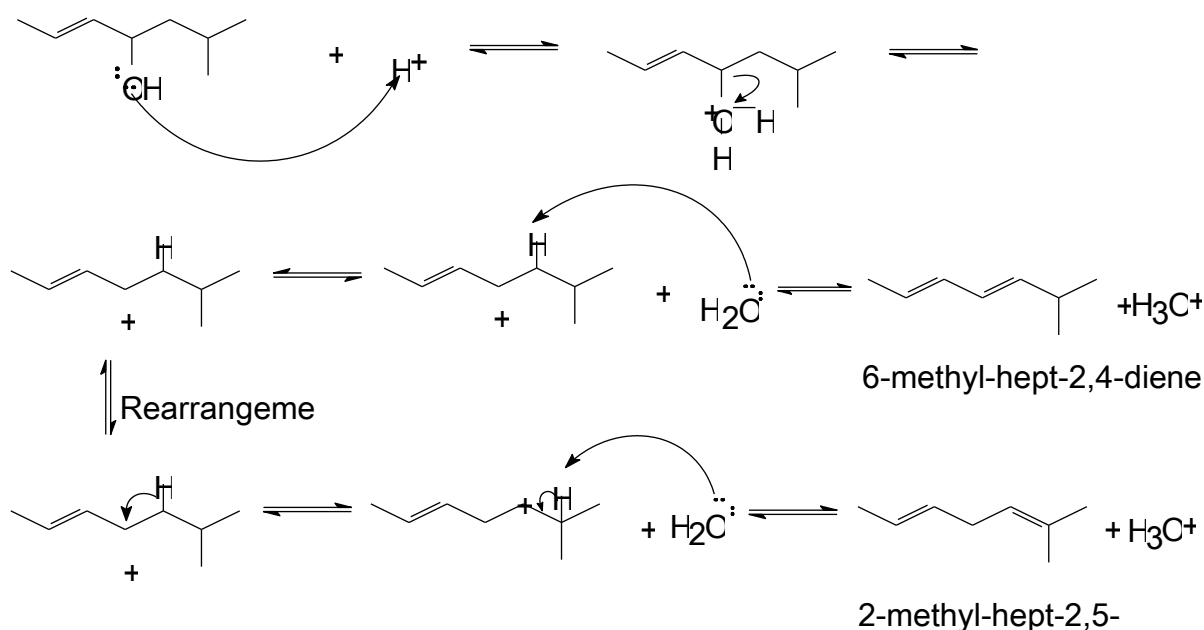


Figure 9. Mechanism proposal of rhynchophorol dehydration in acidic medium based on GC MS analysis.

Among the possible alkadienes which could be formed and cannot be distinguished by mass spectrometry are the isomers: 6-methyl-hept-2,4-diene and 2-methyl-hept-2,5 diene.



## CONCLUSION

Through this study it was possible to conclude that there was strong chemical interaction between rhynchophorol and acid sites of ZSM-5 zeolites promoting the degradation of this pheromone and making it unfeasible to use these materials as matrixes for rhynchophorol prolonged release. This degradation occurred mainly by E<sub>1</sub> intramolecular elimination of the alcohol and produced more volatiles compounds than rhynchophorol, among them, the 2-methyl-hept-2,5-diene and the 6-methyl-hept-2,4-diene isomers.

On the other hand, the rhynchophorol was recovered with levels above 90% only for silicalite-1. As silicalite-1 has no acidic sites in amounts sufficient to promote the reaction that leads to rhynchophorol degradation and has high complexity of the porous network because of MFI topology, it may be considered to be a promising material to be used as a matrix for prolonged release of rhynchophorol. Field test will be performed to evaluate the efficiency of silicalite-1 as rhynchophorol dispenser in real conditions.

## ACKNOWLEDGMENT

The authors thank to Interacta Química Ltda. for helping in the manufacture of rhynchophorol pheromone and the insect trapping system; and to Dr. Edson de Souza Bento (IQB-UFAL) for the <sup>27</sup>Al NMR spectra.

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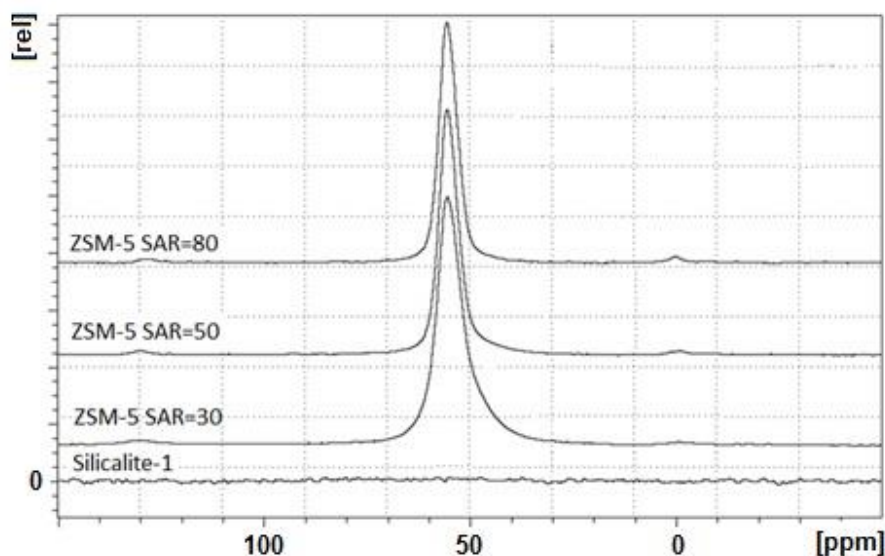
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## SUPPLEMENTARY MATERIAL

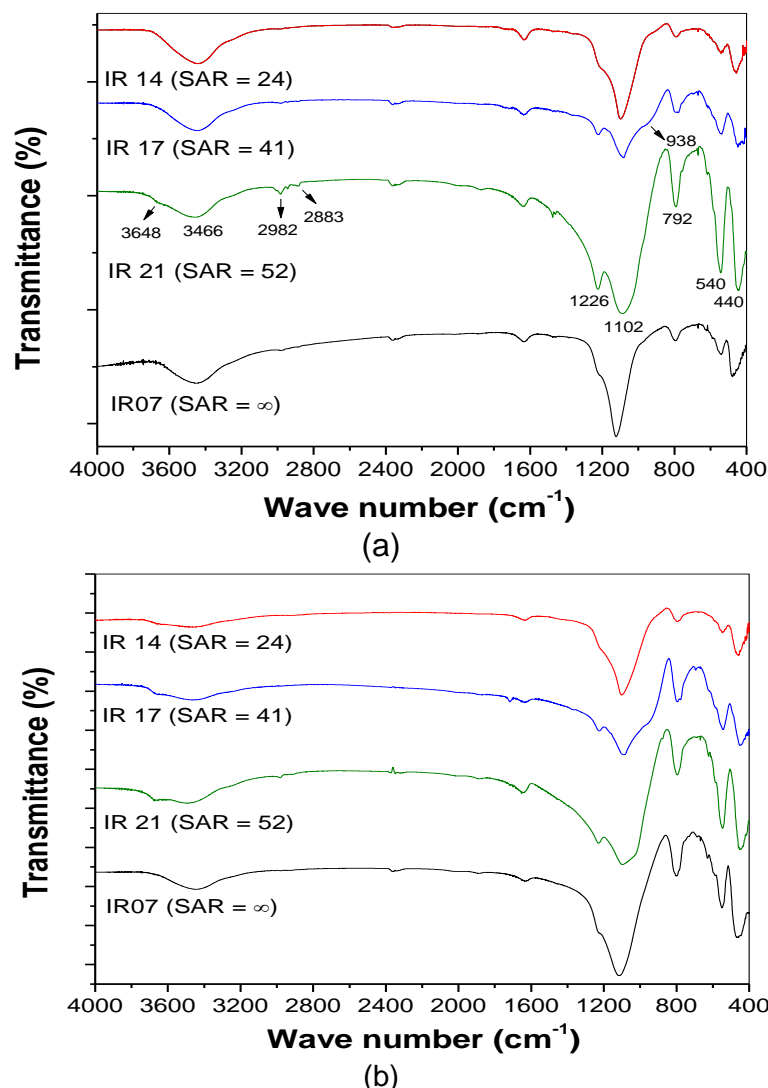
### *Characterization of MFI zeolites*

The  $^{27}\text{Al}$ -MAS-NMR spectra are shown in Figure S.1. In general, chemical shifts at 0 and 55 ppm are assigned to hexacoordinated and tetraordinated aluminum, respectively [1]. It is possible to observe only a very small peak of hexacoordinated extra-framework aluminum species. Based on integration data, framework  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratios were calculated and are shown in Table 2.



**Figure S.1.**  $^{27}\text{Al}$ -MAS-NMR spectra of MFI zeolites.

Figure S.2 shows the infrared spectra of ZSM-5 and silicalite-1 zeolite in as-synthesized and calcined form. Well-defined bands in spectral range 1200-550  $\text{cm}^{-1}$  exhibited in infrared spectra, are due to vibrations of secondary building units (SBU), characteristic of the zeolite framework [2]. These spectra are consistent with the vibrations of framework region described for MFI zeolite systems.



**Figure S.2.** FTIR spectra of MFI zeolites in: (a) as-synthesized and (b) calcined forms.

In Figure S.2.a, weak bands at 2982 and 2883  $\text{cm}^{-1}$ , due to the C-H stretching of the organic template can be seen. These bands disappeared in the spectra of Figure S.2.b. In the range 3670-3460  $\text{cm}^{-1}$  there are broad bands, attributed to OH group stretching of silanol or Si(OH)Al group stretching, in strong interaction, via hydrogen bond, with physisorbed water [3].

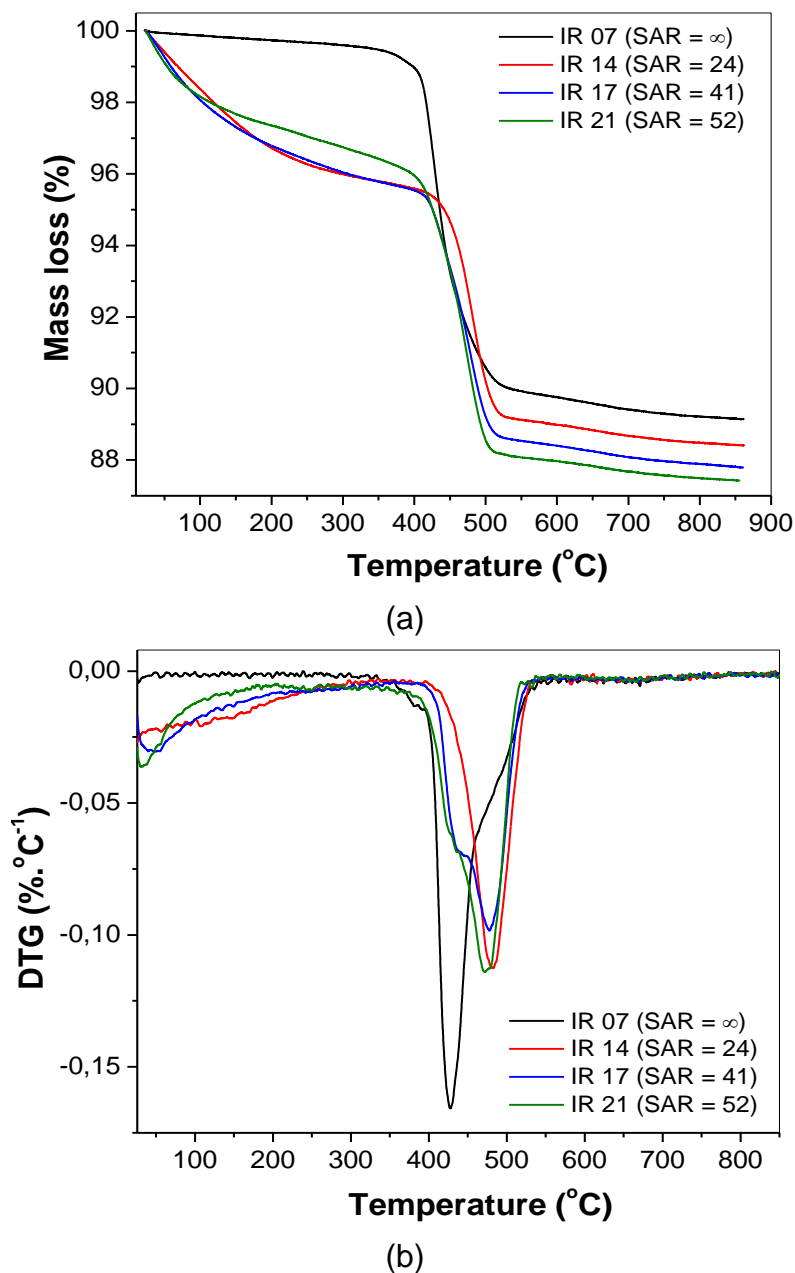
The absorption bands around 1226, 1102 and 792  $\text{cm}^{-1}$  are characteristic of  $\text{SiO}_4$  tetrahedral units [3]. The external asymmetric stretching vibration near 1226  $\text{cm}^{-1}$  is due to the presence of structures containing chains of five-membered rings arranged around a two-fold screw axis [2,3]. The absorption band around 1080  $\text{cm}^{-1}$  is attributed to the internal asymmetric stretching vibration of Si-O-T linkage [3], while the absorption near 792  $\text{cm}^{-1}$  is assigned to the symmetric stretching of the external linkages among tetrahedron [3].

The absorption bands in the range 650-500  $\text{cm}^{-1}$  are assigned to double four, five and six membered ring in framework of zeolite, which are characteristic for zeolites and many silicates [2]. Therefore, the absorption band verified in 540  $\text{cm}^{-1}$  was classified as a sensitive vibration related to the MFI topology, caused by external vibrations of double five-membered ring [3]. The absorption band observed in 440  $\text{cm}^{-1}$  refers to internal linkage vibrations (T-O) to  $\text{SiO}_4$  and  $\text{AlO}_4$  tetrahedrons.

The TG and DTG curve for the as-synthesized ZSM-5 and silicalite-1 samples are shown in Figure S.3. Two different regions of mass loss are verified in Figure S.3.a: i) between 100 and 250°C, assigned to dehydration of zeolite samples; and ii) above 300°C, attributed to decomposition, in several gas products, of  $\text{TPA}^+$  cations occluded in MFI framework [4,5]. The DTG curves (Figure S.3.b) revealed an asymmetric peak in the TPA loss region, separating clearly in two regions of different temperatures. In silicalite-1 sample, the maximum temperature of peak was 426°C, with a shoulder in higher temperature regions ( $\approx$  480°C). According the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  decrease, this shoulder becomes the main peak centered in 478°C, so that, in IR14 (SAR = 24) sample a single asymmetric peak centered in 482 °C is observed.

A possible interpretation is the decomposition of  $\text{TPA}^+$  cation in different interactions with zeolite structure: one with exchange sites generated by aluminum (stronger interaction), the other by interaction with the silanol groups within the channel of ZSM-5, but not in the vicinity of exchange site (weaker interaction). Parker et al [6] also obtained similar results studying the  $\text{TPA}^+$  cation decomposition, using thermal analysis linked with mass spectrometry. They found that the  $\text{TPA}^+$  ions that do not have proximity to aluminum decompose first, in similar temperature to that found for silicalite-1.

Data on Table S.1 show the mass loss obtained in thermal analysis of MFI materials in as-synthesized form.



**Figure S.3.** TG and DTG curve for MFI zeolites in as-synthesized form, in following conditions: mass  $\approx$  5 mg,  $N_2$  flow = 50 mL  $min^{-1}$ , heating rate = 10°C  $min^{-1}$ .

**Table S.1.** Mass loss obtained in thermal analysis of ZSM-5 and silicalite-1 in as-synthesized form.

Sample	Mass loss (%)		
	Region I	Region II	$T_m$ (°C)
IR14 (SAR = 24)	4.00	7.01	483.7
IR17 (SAR = 41)	3.94	7.64	478.7
IR21 (SAR = 52)	3.20	8.77	472.9
IR07 (SAR = $\infty$ )	0.42	9.85	426.8

From data of Table S.1, it is possible to observe that as the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio increases, the mass loss in region I decreases, relative to dehydration of zeolite. This is consistent, because the material becomes less hydrophilic when the aluminum content on the network decreases. On the other hand, an increase of mass loss in region II, due to  $\text{TPA}^+$  cation decomposition, was verified. This indicates that decreases of aluminum content and, hence, water content, implies an increase of organic template content within the zeolite pores, what is important to stabilize the aluminosilicate porous structure. Similar results were obtained by Ali et al. [3] and Shirazi et al. [4].

After calcination, TG curves were carried out for ZSM-5 and silicalite-1 zeolites. The analysis of these curves allowed to check the efficiency of calcination process and to calculate the hydration degree. The profile of TG curves showed only the event of dehydration, indicating that the organic template was totally removed. The results of moisture are shown in Table S.2.

**Table S.2.** Moisture determined by TG of MFI zeolites in calcined forms

<b>MFI zeolites</b>	<b>Mass loss (%)</b>
IR 14 (SAR = 24)	6.71
IR 17 (SAR = 41)	7.89
IR 21 (SAR = 52)	5.41
IR 07 (SAR = $\infty$ )	1.37

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4.2. MANUSCRIPT 01: VALIDATION OF ANALYTICAL METHOD FOR RHYNCHOPHOROL QUANTIFICATION AND DEVELOPMENT OF INORGANIC COMPOSITE FOR THE CONTROLLED RELEASE OF THIS PHEROMONE

(Conforme normas do periódico Chemistry Central Journal. Submetido em: 17/08/2017)

VALIDATION OF ANALYTICAL METHOD FOR RHYNCHOPHOROL  
QUANTIFICATION AND DEVELOPMENT OF INORGANIC COMPOSITE FOR THE  
CONTROLLED RELEASE OF THIS PHEROMONE

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## ABSTRACT

A fast method for the identification and stability evaluation of the aggregation pheromone rhynchophorol, which is the main substance used for chemical communication by the beetle *Rhynchophorus palmarum* L, was validated. In addition, the technique was applied to the evaluation of two inorganic matrices, with the objective of using them as controlled-release devices. The analytical method showed good linearity ( $R^2 = 0.9978$ ), precision ( $CV\% < 1.79$ ), recovery (84 – 105%) and limits of detection ( $0.2 \text{ mg.mL}^{-1}$ ) and quantification ( $0.3 \text{ mg.mL}^{-1}$ ); in compliance with the validation legislation established by ANVISA (2017). In the interaction study, the inorganic matrices zeolite L and Na-magadiite showed high rates of pheromone recovery without promoting its degradation for a period of 180 days, which is not reported in the literature for other matrices. The structures of the zeolite L/rhynchophorol and Na-magadiite/rhynchophorol composites showed slower release kinetics during the storage period when compared with pure pheromone, which is desirable since it extends the period of rhynchophorol release and decreases the negative effects caused by the environmental parameters.

**Keywords:** Semiochemical, zeolite, clay, controlled release, *Rhynchophorus palmarum* L.

**HIGHLIGHTS:**

- Fast and low cost analytical method for the quantification and stability evaluation of the pheromone rhynchophorol;
- Analytical method for the adsorption and recovery of pheromone using inorganic matrices;
- Elaboration of an inorganic matrix/pheromone composite aimed at pest control through controlled pheromone release.

## INTRODUCTION

The beetle *Rhynchophorus palmarum* L. is an insect of the family Dryophthoridae, subfamily Rhynchophorina and class Rhynchophorinire (1).

This insect is a recurrent pest, which attacks mainly sugarcane (*Saccharum officinarum*) and coconut (*Cocos nucifera* L.) plantations, damaging the stalks of these plants in the search for food and reproduction sites, and laying eggs which will later hatch (2). However, the highest risk posed by this beetle is its use as a vector by the nematode *Bursaphelenchus cocophilus*. This nematode is the main agent responsible for causing the disease in coconut trees known as red ring, which rapidly leads to the death of the plant. In order to control the populations of this nematode, the main strategy is to eliminate the insect *Rhynchophorus palmarum* L. and its larvae, so that the number of individuals is maintained at acceptable levels and the economic viability of coconut cultivation is preserved (3).

The aggregation pheromone 6-methyl-2-hepten-4-ol (rhynchophorol), released by *R. palmarum* L. at the time of feeding to attract other individuals and also promote reproduction, has been used as an alternative for the control of this pest, due to its potential use together with biological traps (3,4).

The control of *Rhynchophorus ferrugineus*, an insect of the same genus as *R. palmarum* L., can be carried out using natural enemies such as viruses, bacteria, fungi, yeasts, nematodes and mites, of which the use of fungi is the most common. However, the use of these natural enemies is not effective against all insects of the Rhynchophorus genus, since the success of this strategy is influenced by the insect dispersion and environmental variations (5).

Some materials have been studied for the controlled release of pheromones, including zeolites, nanoencapsulates and nanosensors (6). The choice of the adsorbent matrix

must be made cautiously, aimed at guaranteeing the maximum efficiency of the composite formed (matrix/pheromone) without contributing to the degradation of the pheromone during its preparation or storage (7). In the selection process, some characteristics of the matrix should be observed, such as: pheromone release kinetics as close to zero as possible, sufficient number of traps to attract the pests, low production cost, and easy installation.

Some structures for the pheromone controlled release matrix have been studied, such as: sepiolite clay (8); whey protein with acacia gum for microencapsulation (9); plastic pipette tips (10); zeolites ZSM-5, silicalite-1, faujasite and beta zeolite (7,11). The use of pheromone rhynchophorol together with mass-traps has been studied and implemented over the years, seeking to improve the efficiency of the application of this technique and enable the capture of the highest number of insects during the period of control (12-14).

The aims of this study were to validate an analytical method for the identification and quantification of the aggregation pheromone rhynchophorol and to develop a composite comprised of an inorganic matrix and rhynchophorol (Figure 1) for the chemical attraction of the beetle *R. palmarum* L. A controlled release study was carried out and the interaction of the pheromone with the Na-magadiite and zeolite L matrices was investigated.

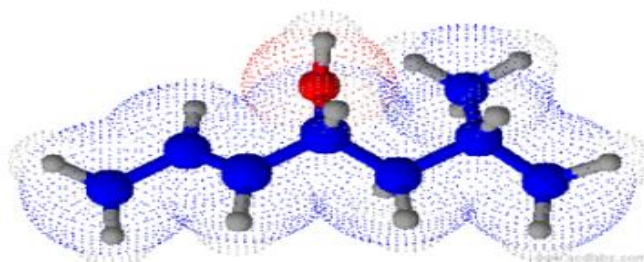


Figure 1: 3D structure of rhynchophorol generated by ACDLABS 12.0™.

## MATERIALS AND METHODS

### Chemicals

The rhyngophorol (2-methyl-5(E)heptenone-4-ol) standard, with a purity greater than 99%, was donated by Interacta Química Ltd (Alagoas, Brazil). HPLC-grade n-hexane (Mallinckrodt ChromAr) was used as the organic solvent. The substance 6-methyl-5-hepten-2-one with 99% purity (Sigma-Aldrich) was used as the internal standard.

As starting reagents for the Na-magadiite synthesis, the following materials were used: NaOH (Synth), hexamethyleneimine (HMI, Sigma-Aldrich, 99%), Aerosil 200 silica (Degussa) and NaCl (Sigma-Aldrich).

### Inorganic Structures

The Na-magadiite lamellar structure was obtained through the synthesis method proposed by ELYOSSI (2014) (15) for the obtainment of zeolite ITQ-1 with modifications. In this synthesis, the hydrothermal process was carried out in the static form over a period of 7 days, the gel formed being described by:  $\text{SiO}_2 : 0.31\text{HMI} : 0.15\text{NaCl} : 0.31\text{NaOH} : 44\text{H}_2\text{O}$ . In addition, the TMAOH was replaced by NaOH.

### Characterization of the Samples of the Inorganic Matrices

X-ray diffraction (XRD) was carried out with a Shimadzu diffractometer (model XRD6000), with  $\text{CuK}\alpha$  radiation at 40 kV and 30 mA, carrying out the reading from  $5^\circ$  up to  $55^\circ 2\theta$  at a velocity of  $2^\circ\text{min}^{-1}$ . The identification of the clay composition was

performed with the aid of an energy dispersive X-ray spectrometer (Shimadzu EDX-720) with a rhodium radiation source, operating at 15 kV (Na to Sc) or 50 kV (Ti to U) with a collimating slit of 10 mm (7). FTIR spectra were obtained with the use of a Spectrum Two spectrometer, manufactured by PerkinElmer (USA), with an ATR probe.

### **Methodology for the determination of rhynchophorol by CG-MS**

Prior to performing the analytical method, the best evaluation parameters were sought in order to aid the identification, separation and quantification of the pheromone and the internal standard with the equipment used. Conditions for the heating rate of the ramp, injection temperatures, flow velocities, and analysis time were optimized.

This analytical method validation was based on the category II classification of the Guide for Validation of Analytical and Bioanalytical Methods of ANVISA (2017), aimed at quantitative or limit tests for the determination of the impurities and degradation products in pharmaceutical products and raw materials (16). The parameters of linearity, specificity, recovery, precision, detection limit and quantification limit were evaluated.

### **Linearity and Specificity**

Seven concentrations of the pheromone rhynchophorol, varying from 0,86 up to 43 mg, were prepared in triplicate. The samples were diluted in 1 mL of HPLC-grade n-hexane together with 10  $\mu$ L of 6-methyl-5-hepten-2-one, used as an internal standard (IS). The areas for each substance were obtained through the peak integration with the aid of the TurboMass software program (version 5.4.21617), along with the retention time.



The analytical curve for the correlation between the rhynchophorol / IS areas was constructed.

### **Recovery and Precision**

In order to evaluate the rhynchophorol recovery, triplicate samples containing 10 µL of pheromone were adsorbed onto 50 mg of the zeolite L inorganic structure. The system was shaken for 1 min. After being left to stand for 4 h, at ambient temperature, 2 mL of n-hexane was added to the system followed by shaking for 1 min. The system was then left to stand for 4 h. After this period, the system was shaken again for 1 min and left to stand 1 min again, where the supernatant was removed and filtered through a nylon membrane of 0.45 µm (Allcrom/Brazil). The supernatant was later analyzed by GC-MS.

### **Detection Limit (DL) and Quantification Limit (QL)**

In order to determine the DL and QL values, samples of the pheromone rhynchophorol were prepared and evaluated with the aid of the signal-to-noise ratio tool, provided in the TurboMass software program (version 5.4.2.1617), installed in the equipment used. To obtain the DL and QL values, signal-to-noise ratios of 3:1 and 10:1, respectively, were considered as established by ANVISA (16,17).

### **Preparation of composites of inorganic Matrix and rhynchophorol**

Composites were formed through the interaction of the inorganic matrices used in this study with the pheromone rhynchophorol. The methodology described by Ramos et al.

(2017) (7) was applied in the preparation procedure. 50 mg of the lamellar structured Na-magadiite or zeolite L was placed in an Eppendorf (2 mL) and 10  $\mu\text{L}$  (~8.1 mg) of rhynchophorol was added. The system was shaken for 1 min and later kept under storage at ambient temperature for 24 h.

### **Evaluation of the Stability of Composites**

The stability of the pheromone adsorbed onto the matrix was evaluated through the extraction and recovery of the rhynchophorol. In this procedure, 2 mL of n-hexane was added to the system, which was shaken for 1 min and then left to stand for 4 h. After this period, the system was shaken again for 1 min and the supernatant was removed and filtered through a nylon membrane of 0.45  $\mu\text{m}$  (Allcrom/Brazil).

### **Quantification of the recovered rhynchophorol by CG-MS**

The amount of rhynchophorol recovered was determined using a gas chromatograph (Clarus 680), coupled to a mass spectrometer detector (Clarus 600C), with an ELITE-5MS capillary column (Perkin Elmer/USA). Samples (1  $\mu\text{L}$ ) were injected through a CTC Combipal automatic injector (Pal System/Switzerland). The run conditions were: helium carrier gas with 1  $\text{mL}\cdot\text{min}^{-1}$  flow, 50 mL split, and injector temperature of 150  $^{\circ}\text{C}$ . The initial temperature of the oven was 50  $^{\circ}\text{C}$  for 3 min with a heat ramp of 10  $^{\circ}\text{C}\cdot\text{min}^{-1}$  up to 200  $^{\circ}\text{C}$ , held for 1 min. The mass spectrometry detector was configured to operate with ionization of 70 eV in scanning mode (SCAN), in the mass range of 25-500  $m/z$ . The temperatures were fixed at 200  $^{\circ}\text{C}$  for the ionization source and 180  $^{\circ}\text{C}$  for the quadrupole. The interface with the mass detector was kept at 200  $^{\circ}\text{C}$ .

## RESULTS AND DISCUSSION

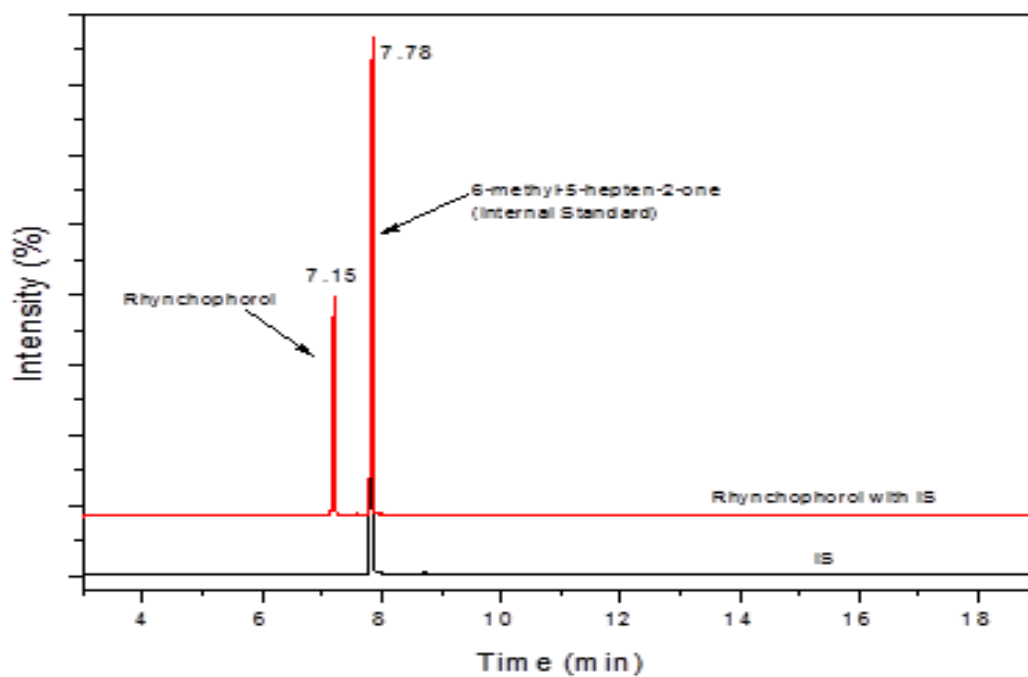
### **Specificity and Linearity**

The result obtained for the correlation coefficient was  $R^2 = 0.9978$ , demonstrating good linearity for the calibration curve. This result is in compliance with the standard value required by ANVISA (2017) (16), which establishes acceptable linearity as an  $R^2$  value above 0.99 and an analytical curve of  $y = 0.062x + 0.1249$  (Figure 2).

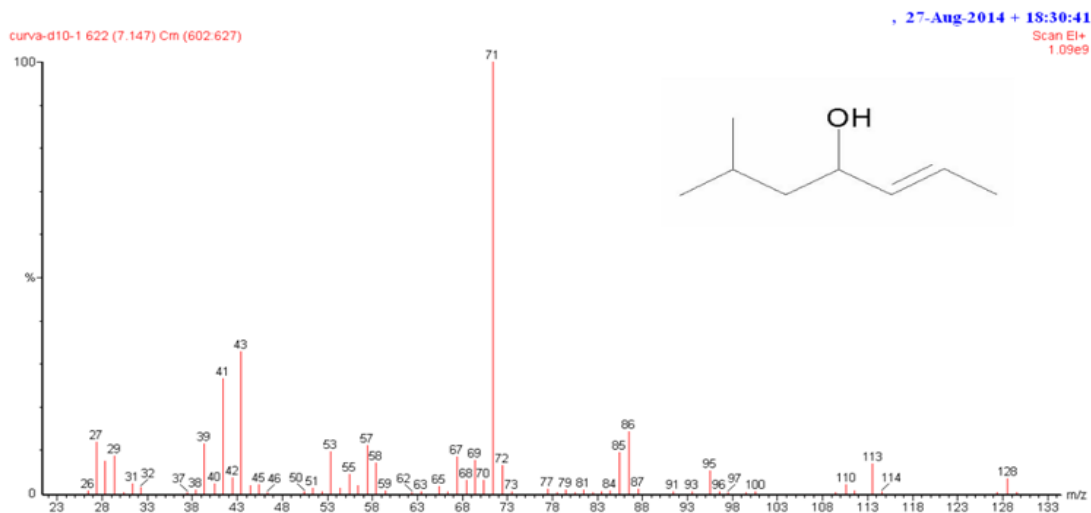
The specificity of a method relates to its ability to accurately measure an analyte in the presence of other components that may be present in the sample, such as impurities, degradation products and other matrix components (Eurachem, 2014)(18). In this method, mass spectrometry was used for the detection of the pheromone. Ions characteristic of rhynchophorol were used for the identification:  $m/z$  (%)  $M^+$  41 (2), 53 (13), 57 (12), 71(100) and 128 (2)(19). The software program NIST Mass Spectral Search (version 2.0f), was used to aid the confirmation of the identification, and similarity above 80% was observed for rhynchophorol.

### **Precision, Recovery, Detection Limit (DL) and Quantification Limit (QL):**

The precision, considering the values for the coefficient of variance (CV%) and standard deviation (STD), obtained for the pheromone rhynchophorol are given in Table 1. The results show CV and STD values lower than 5%, satisfying the requirements established by ANVISA (16).



(a)



(b)

Figure 2: Chromatograms obtained under the analytical conditions of the method: (a) hexane solution containing rhyncophorol and IS; (b) mass spectrum obtained for rhyncophorol.

Table 1: Intermediary precision for the analytical method to determine the pheromone rhynchophorol.

Concentration	Average	STD	CV%
1	0.0836	0.0013	1.59
20	1.2723	0.0040	0.31
50	2.4445	0.0165	0.68

The percentage recovery of the absorbed rhynchophorol from the composite (CR%) varied from 84% to 105%. These results are also in compliance the current legislation, which establishes recovery rates within the theoretical concentration range of 80% to 120%.

Values of  $0.2 \text{ mg.mL}^{-1}$  for DL and  $0.3 \text{ mg.mL}^{-1}$  for QL were obtained as the operational limits of the device used.

### **Characterization of the Synthesized Na-Magadiite**

The Na-Magadiite formation was confirmed through comparison of the XRD result with the standard provided by IZA (2017), as shown in Figure 3.

Intensity peaks can be observed on the diffractogram for the angles characterizing the Na-magadiite formation: 5.62, 11.32, 17.06, 25.9, 26.96, 28.32, and 50.02.

In the EDX elemental analysis carried out on the synthesized Na-magadiite, a predominantly  $\text{SiO}_2$  (97.87% of the total composition) matrix was observed, with 1.89% of  $\text{Al}_2\text{O}_3$ . Trace levels of ZnO and CuO were also present.

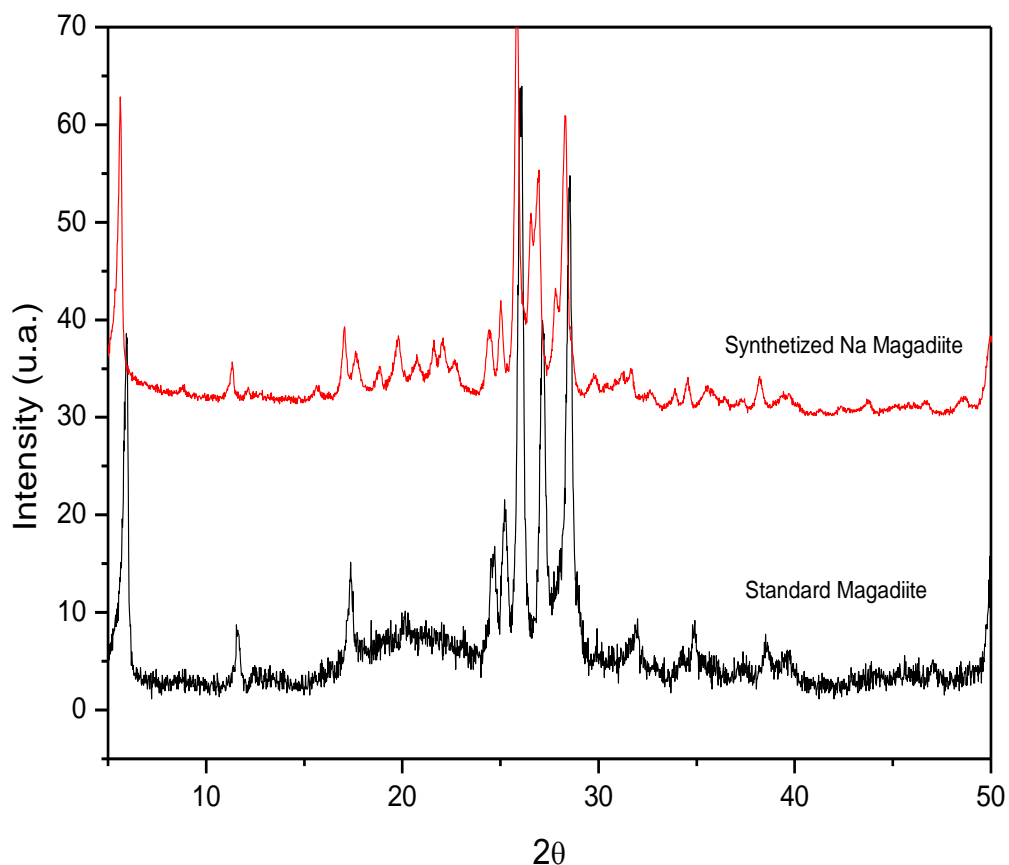


Figure 3: X-ray diffraction pattern for the synthesized and standard Na-magadiite.

### **Rhynchophorol Interaction with Na-Magadiite and Zeolite L**

According to Ramos et al. (2017), one of the main reactions that demonstrates rhynchophorol degradation in the rhynchophorol+magadiite interaction is the color change of the material, which can be seen with the naked eye. Figure 4 shows the rhynchophorol+magadiite and rhynchophorol+zeolite matrices after 24 h of interaction. It can be observed that the pheromone was not degraded in these interactions.

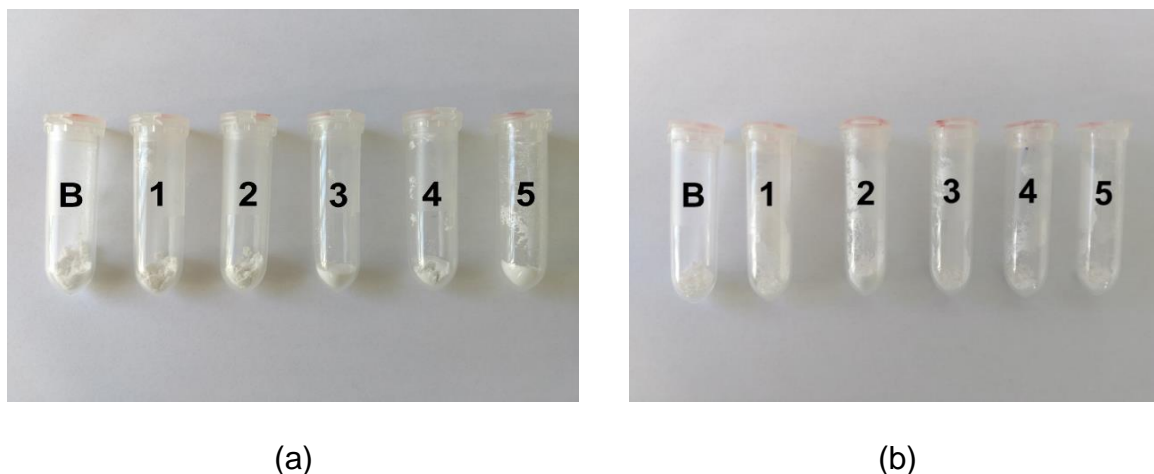


Figure 4: Matrices after a period of 24 h rhynchophorol adsorption: (a) Na-magadiite and (b) zeolite L.

The integrity of the rhynchophorol in the matrices studied was confirmed by the FTIR spectra obtained (Figure 5). Rhynchophorol contains functional groups in its structure, which can be observed through the FTIR technique, confirming the integrity of the structure. The main groups are: -OH (3442); -CH<sub>2</sub> (2929); -CH (2872); -CH<sub>3</sub> (2962); -CH (1384) with the doublet effect, and -CH<sub>2</sub>(1384) with angular deformation (20). The presence of these functional groups was observed in the complexes studied. On the other hand, the Na-magadiite lamellar structure shows a wavelength between 1300 and 700, since it has predominantly Si-O bonds, for the formation of the bidimensional structure Figure 5(b).

The evaluation of the material after the rhynchophorol/Na-magadiite adsorption demonstrated that the wavelengths in the range of 3700 to 1300 were maintained, highlighting the stability of the adsorbed pheromone. The other functional groups of pheromone were not observed due to the superposition of the Na-magadiite characteristic groups (Figure 5(C)).

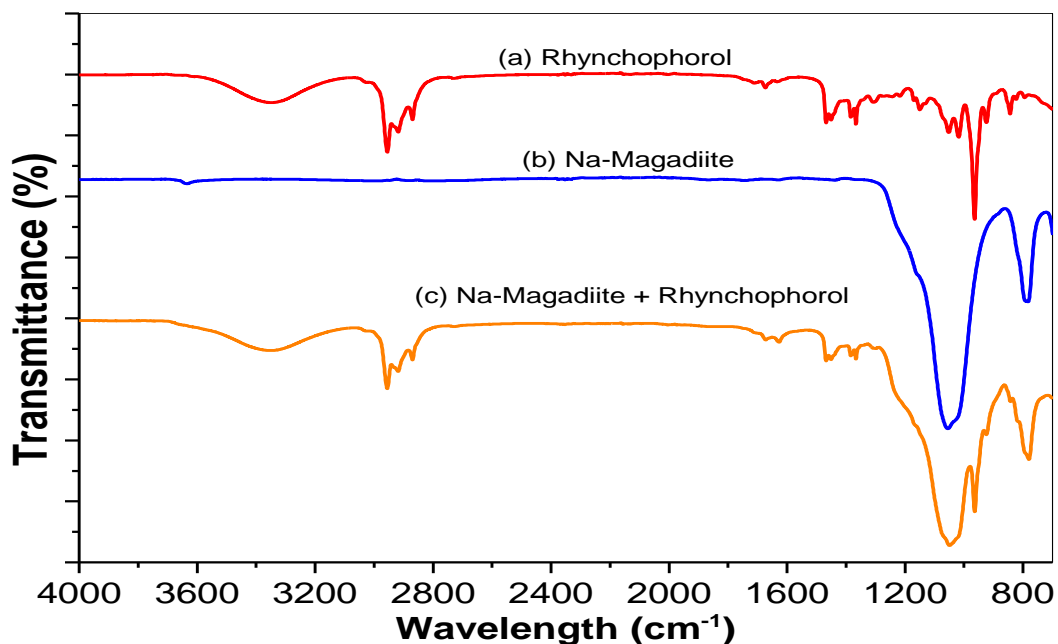


Figure 5: FTIR spectra for rhynchophorol (a), Na-magadiite (b), and Na-magadiite/rhynchophorol composite (c).

### **Study on the controlled release of the rhynchophorol adsorbed on the magadiite and zeolite**

In order to confirm the presence of rhynchophorol in the composite formed with the Na-Magadiite, the pheromone was recovered by extraction with n-hexane and quantified by the validated method. Typical chromatograms for the extracts obtained are given in Figure 6.

It can be observed that the n-hexane solution obtained in the extraction process is similar to the standard of pure rhynchophorol. After 24 h of adsorption and the subsequent extraction, it was possible to recover 89.05% of rhynchophorol, which highlights the protection of this pheromone in the matrix studied. It was also observed that the formation of new peaks did not occur, indicating that rhynchophorol degradation products were not generated. In a study carried out by Ramos et al (2017),



zeolites with an MFI topology (ZSM-5 and silicalite-1) were used as a device to for the controlled release of rhynchophorol and it was verified that the characteristics of the adsorbent matrix are essential factors in avoiding the pheromone degradation during the adsorption process. Structures with high Al contents in the network formation promote higher acidity and an increase in the diameter of the channels, facilitating the access of pheromone to the interior of the structure, leading to greater degradation of the pheromone studied.

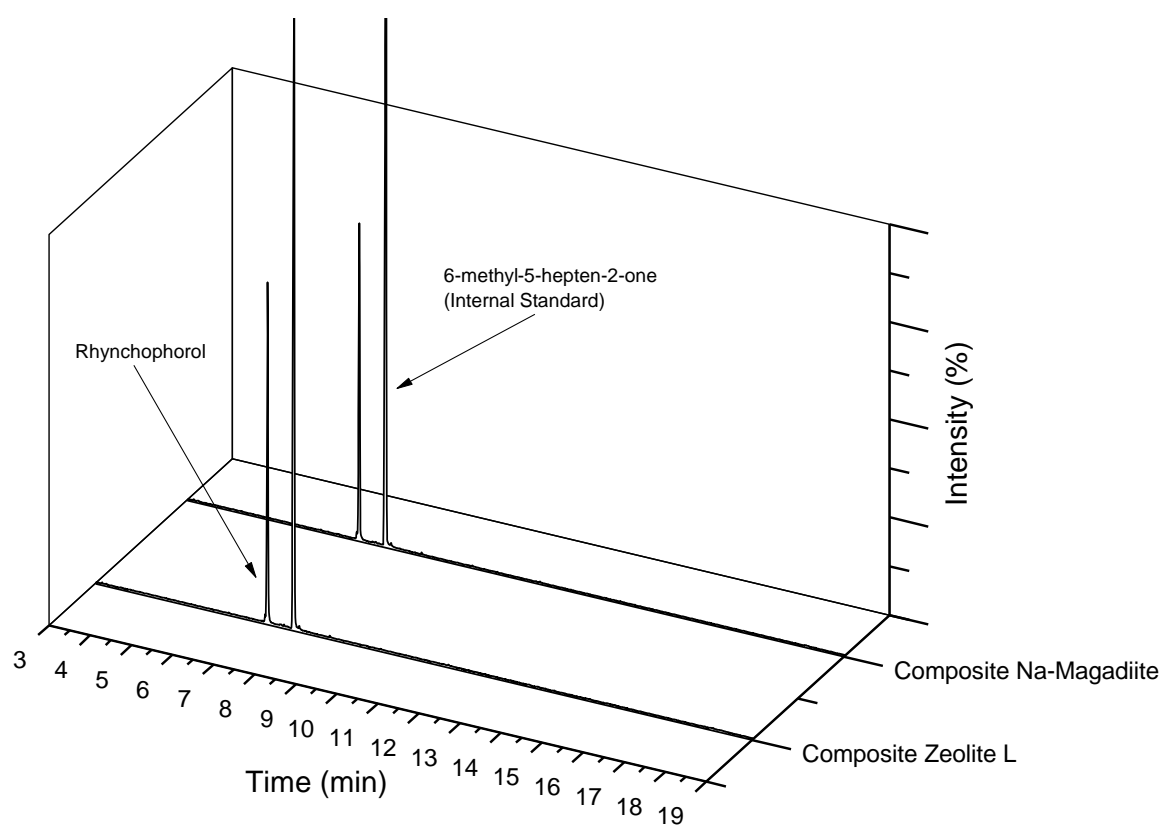
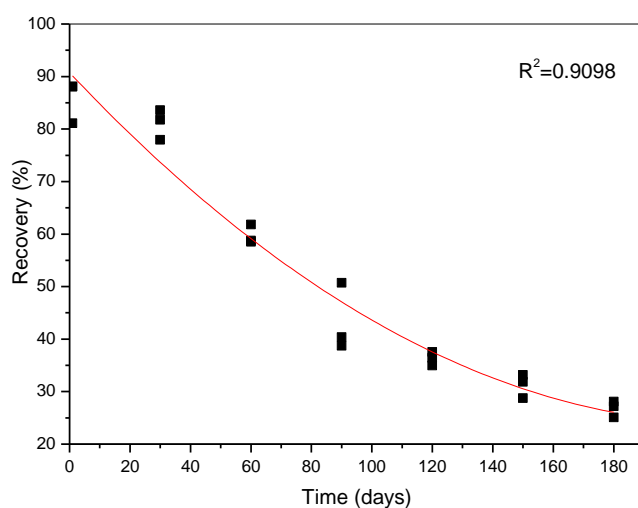


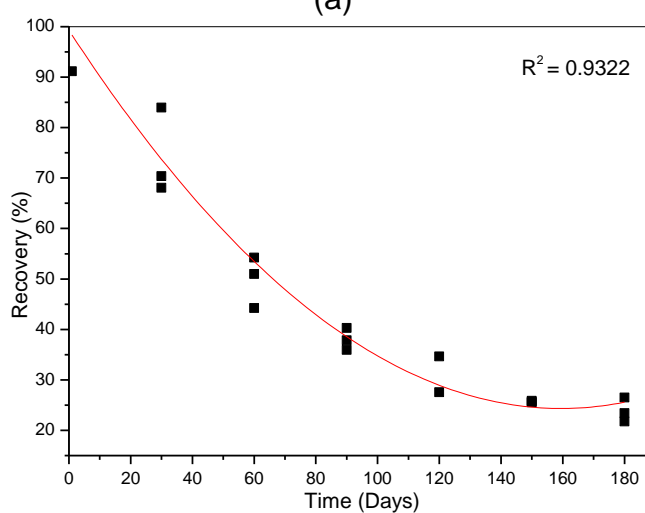
Figure 6: (a) Chromatograms of the solutions recovered from the Na-magadiite and zeolite L composites.

Figure 7 shows the values for the rhynchophorol adsorbed on Na-magadiite as a function of the storage time, simulating the stability condition at ambient temperature.

The rhynchophorol adsorbed on the matrix shows an exponential mass loss behavior during storage for 180 days (Figure 7). The pheromone release rate was  $0.89 \pm 0.41$  mg day<sup>-1</sup> in the first 30 days, due to the dispersion of the pheromone in the matrix. After 30 days, the release rate decreased to approximately  $0.046 \pm 0.008$  mg day<sup>-1</sup>, with the controlled release of the pheromone being observed throughout the period evaluated. The same behavior was noted for the composite formed with zeolite L, which showed a release rate of  $0.517 \pm 0.68$  mg day<sup>-1</sup> in the first 30 days, reducing to an average rate of  $0.0539 \pm 0.0154$  mg day<sup>-1</sup> for the remainder of the period.



(a)



(b)

Figure 7: Long-term stability test for rhynchophorol adsorbed on Na-magadiite (a) and zeolite L (b).

Stipanovic et al. (2004) (21) carried out controlled release tests on the pheromone codlemone adsorbed on cellulose derivatives surrounded by a polymeric membrane, aimed at its application in the control of Lepidopteran pests (moths). They obtained release rates of around  $0.784 \text{ mg day}^{-1}$ , which was similar to the value obtained in this study for the composite formed with the Na-magadiite. Since zeolite L is a three-dimensional network of channels, the release of the rynchophorol adsorbed on this matrix was slower. This was also observed by Ramos et al. (2017) for the zeolite silicalite-1. Release rates for the pheromone rynchophorol varying from  $0.002592$  to  $0.2592 \text{ mg day}^{-1}$  are favorable for the identification and the attraction of *R. palmarum* L., showing that the two matrices used in this study have the potential for application together with traps for periods of up to 180 days (22).

In order to evaluate the stability of the rynchophorol adsorbed on the Na-magadiite during its long-term storage, the composite was evaluated considering the possibility of degradation with the formation of new compounds. In Figure 8, the maintenance of rynchophorol ( $t_r=7.18 \text{ min}$ ) and the IS ( $t_r=7.84 \text{ min}$ ) can be observed.

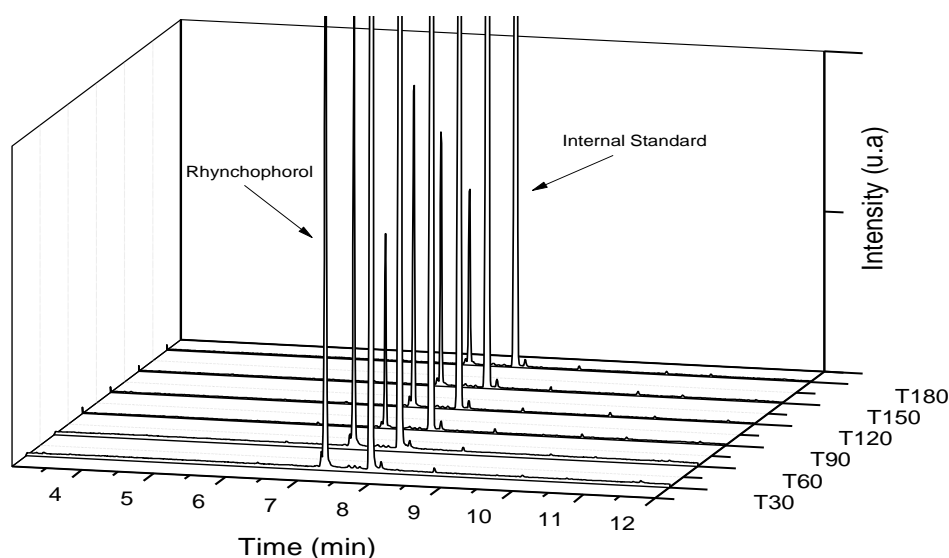


Figure 8: Storage of Na-magadiite/rynchophorol composite for intervals of up to 180 days.

During the storage period, new peaks were not observed in the analysis, confirming that the reduction in the rhynchophorol values for the studied matrices was due to release and not to the degradation of this pheromone (Figure 8). Ramos et al. (2017) observed that pure silica zeolites, of the type silicalite-1, were also able to store rhynchophorol for long periods without its degradation. In contrast, in the case of zeolite ZSM-5, when used for the same purpose, pheromone degradation was observed within less than 30 days of storage. The cited authors attributed the degradation to acids in the matrix and diffusion within the structure, leading to access to Brønsted acid sites.

## CONCLUSIONS

In this study, stable matrices of Na-magadiite and zeolite L containing rhynchophorol were successfully prepared. The analytical methodology for the determination of rhynchophorol was considered adequate with regard to the proposed application, since it showed good values for recovery, linearity, DL and QL. The characterization of the matrix highlights that rhynchophorol remained stable and did not degrade on interaction with the inorganic matrix. The study confirmed that the controlled release of the pheromone occurred at rates that enable the identification and the attraction of the target insect. It was possible to obtain a stable complex for the controlled release of the pheromone, which could be used in the future for the control of *Rhynchophorus palmarum* L, insects that can cause the destruction of cultures such as coconut trees and oil palm trees. This approach can be applied in the form of tablets or in plastic Eppendorf™ tubes or similar materials, as described in the patent request

BR1020150326041, registered at the National Institute for Industrial Property – INPI/BR.

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## COMPETING INTERESTS

The authors declare that they have no competing interests.

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4.3. MANUSCRIPT 02: RELEASE OF RYNCHOPHOROL AGGREGATION  
PHEROMONE FROM THE MONTMORILLONITE CLAYS AND KAOLIN



## RELEASE OF AGREGATION FEROMONE RHYNCHOPHOROL FROM CLAY MINERALS MONTMORILLONITE AND KAOLIN

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### ABSTRACT

The development of lures based on pheromone for pest control in agriculture requires a release with sufficient concentration for insect perception, but without excess, to be economically viable. Considering this, the objective of this work was to evaluate the clay minerals montmorillonite and kaolin as carriers for rhynchophorol prolonged release. The clay minerals were characterized by EDX RF, XRD, FTIR, TG/DTG and textural analysis. The composites rhynchophorol-clay minerals were characterized by FTIR and TG/DTG. Recovery tests have shown the complete degradation of rhynchophorol in the two types of montmorillonites studied. This degradation was attributed to presence of acidic sites of Brønsted and Lewis. The degradation has occurred via an intramolecular E1 elimination and electrophilic substitution. Rhynchophorol recovery from Kaolin was approximately 63%. Long-term studies have shown continuous degradation of pheromone from Kaolin composite up to 50 days. TG in isothermal mode was used to evaluate the release kinetic of rhynchophorol from Kaolin. The results were promising, comparing with the volatilization of pure rhynchophorol.

### 1. Introdução

The *Rhynchophorus palmarum* beetle, pest that attacks the coconut tree (*Cocos nucifera*), palm tree (*Elaeis guineenses*) and pineapple tree (*Ananas comosus*), among others, [1,2] is responsible for causing high economic losses to these crops. Due to the use of the stem of these crops by beetles as a source of feed (direct attack) [2] and also as a place for laying their eggs, the insect can transmit the nematode *Bursaphelenchus cocophilus*, which causes the red ring disease, lethal to plants of the *Arecaceae* family [1,2,3].

When finding food, the male of *R. palmarum* releases an aggregation pheromone named rhynchophorol ((2E)-6-methyl-2hepten-4-ol), an unsaturated aliphatic alcohol

of 8 carbons [1,2,4]. Currently the joint use of bucket-type traps containing rhynchophorol lure and attractive is the main form for control and monitoring of *R. palmarum* [5,6,7]. The chemical control, although often attempted, is not feasible due to the high cost and low effectiveness [8].

The lures based on rhynchophorol pheromone have been marketed as Eppendorf safe-lock® tubes, containing a 1 mm diameter hole in the cover. Navarro and colleagues [1] determined an evaporation rate of  $4.3 \text{ mg day}^{-1}$  (equivalent to  $5 \mu\text{L}$ ) of rhynchophorol from this device.

For using pheromones as pest control in agriculture, its release in environment must occur in appropriate and constant concentration (order zero kinetic). This concentration must be high enough to be detected by insect plague, but not excessive, making its application economically unfeasible. Moreover, its efficiency should not be influenced by environmental conditions [9,10].

Release matrix systems of semiochemicals have been developed for the purpose of prolonged release [9,10,11,12,13,14]. In these systems, the pheromone is adsorbed in the matrix, releasing the active compound with the rate determined by diffusion. Thus, the rate is largely dependent of geometric parameters of matrix [9].

Inorganic materials are an expanding area, especially with regard to compounds that present ordered structure, designed to perform functions of specific interest. Among the inorganic materials with potential use as matrix for the release of semiochemicals, zeolites stand out due to their structural characteristics, which allows modulation in the release kinetics [9,13,14]. The rhynchophorol has already been released from silicalite-1 and promising results were obtained, since the release of integral pheromone was kept during up to 180 days [14]. However, this material is not commercially available because its application in large scale is a step to be overcome.

As well as the zeolites, the clay minerals have adjustable physical-chemical properties, allowing its use as semiochemical dispensers.

Among the several classes, the clay minerals belonging to the filissilicates family have a greater interest in the chemistry of materials. These materials are constituted by continuous two-dimensional tetrahedral layers with composition  $T_2O_5$  ( $T = Si, Al, Be...$ ). The tetrahedral layers are linked in structure to the octahedral layers, or to groups of coordinated cations, or to individual cations [15]. The organization of tetrahedral and octahedral layers leads to classification of clay minerals: the structure 1:1 is formed by a tetrahedron layer (T) condensed with a octahedron layer (O), being named structure OT; the structure 2:1 is formed by a octahedron layer between two tetrahedral layers, being named structure TOT [15].

The montmorillonite is a planar filissilicate 2:1 belonging to dioctahedral smectite [16]. Theoretically, the structural formula is  $[Si_{4,0}]^{IV}[Al_{2,0}]^{VI}O_{10}(OH)_2$ , in which IV and VI indicates the cations coordinated tetrahedrally and octahedrally, respectively [17]. It has a negative electric charge due to the isomorphic substitution of cations with lower valence, such as  $Al^{3+}$  by  $Mg^{2+}$  and/or  $Fe^{2+}$  in octahedral layers. The cation  $M^+$  which balances the negative charge, usually  $Na^+$  or  $Ca^{2+}$ , is called exchangeable cation, since it can be reversibly exchanged for other cations [17,18,19]. The basal interplanar distance (considers the dimensions of exchangeable interleaved cations and the presence of hydration water molecules of exchangeable cations) is of magnitude of 15,4 Å, and the thickness of layer 2:1 is of magnitude of 8,97 Å [20].

The montmorillonite clay minerals are available in large scale under the name K10 and KSF. Such modified materials from the natural montmorillonite have similar physical-chemical characteristics, but very different surface area. The montmorillonite K10 has surface area around  $250 \text{ m}^2 \text{ g}^{-1}$ , while the KSF has around  $10 \text{ m}^2 \text{ g}^{-1}$  [21].

The Kaolin clay is constituted mainly by clay mineral kaolinite. The Kaolinite is a hydrated aluminum silicate, formed by regular stacking of layers 1:1, presenting asymmetric layers electrically neutral [20]. One side consists of an octahedral structure of alumina,  $[\text{Al}_2(\text{OH})_4]^{+2}$ . The other side consists of a tetrahedral structure of silica,  $[\text{Si}_2\text{O}_5]^{-2}$  [22]. Its structural formula of unit cell is  $\text{Al}_2\text{Si}_4\text{O}_{10}(\text{OH})_8$  and its the percentage composition is:  $\text{SiO}_2$  - 46,54%,  $\text{Al}_2\text{O}_3$  - 39,50% e  $\text{H}_2\text{O}$  - 13,96%.10-13 [23]. It presents low content of isomorphic substituents (usually  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  in the  $\text{Al}^{3+}$  site) and consequently, low exchange capacity [24].

The clay minerals montmorillonite and kaolin have already been used as a matrix for controlled release of fertilizers [25,26,27], pesticides [28,29], drugs [30], herbicides [31,32], among others. The kaolinite has already been used with success as dispenser of semiochemicals [33]. But, no application with pheromones was found.

Considering the chemical and structural characteristics described for clay minerals montmorillonite and kaolin, and the low acquisition cost of these materials, they become suitable candidates for use as a prolonged release matrix of pheromones. Therefore, the objective of this work was to evaluate the rhynchophorol release from clay minerals montmorillonite (K10 e KSF) and Kaolin.

## 2. Material and Method

The clays Kaolin (PN: K7375), Montmorillonite K10 (PN: 281522) and KSF (PN: 281530) were acquired from Sigma-Aldrich. The rhynchophorol sample was donated by Química Ltda (Brasil). N-hexane HPLC degree (Mallinckrodt ChromAr) was used for extractions of rhynchophorol. The substance 6-methyl-5-hepten-2-one with 99% purity (PN: M48805) was used as internal standard of chromatographic tests.

## 2.1. Composites preparation

For release kinetic analysis of composite formed by clay mineral and rhynchophorol using thermal analysis, a volume of 10  $\mu\text{L}$  ( $8.0 \pm 0.2$  mg) of rhynchophorol was mixed 25 mg of clay mineral. The composites produced were named CK10 (composite montmorillonite K10/rhynchophorol), CKSF (composite montmorillonite/rhynchophorol) and CKAO (composite kaolin/rhynchophorol).

For recovery tests, the composite was formed by 50 mg of clay mineral and 10  $\mu\text{L}$  ( $8.0 \pm 0.2$  mg). The sample was mixed by approximately 1 min and kept at rest, during 24 h, at room temperature.

## 2.2. Clay minerals characterization

The semi-quantitative analysis of montmorillonite (K10 and KSF) and Kaolin samples was obtained by Energy Dispersive X-ray Fluorescence spectroscopy (ED XRF). The powder was pressed and analyzed through Shimadzu EDX-720 equipment, with Rh radiation source at 15 KV (Na to Sc) or 50 KV (Ti to U), and collimating slit of 10 mm.

Powder diffraction patterns of the samples were collected on a Shimadzu XRD6000 diffractometer, using  $\text{CuK}\alpha$  radiation generated at 40 kV and 30 mA, with a scan speed of  $2^\circ \text{min}^{-1}$  and a step of  $0.02^\circ$  in the range of  $5^\circ$ - $80^\circ 2\theta$ , using divergence, scattering and reception slits of  $0.5^\circ$ ,  $0.5^\circ$  and 0.3 mm, respectively.

Fourier transformed infrared spectra (FTIR) of the samples were recorded in air at room temperature using a Perkin Elmer Spectrum Two equipment, with a resolution of  $1 \text{ cm}^{-1}$ , in the range of  $4000$ - $400 \text{ cm}^{-1}$ , using 20 scans. The samples were prepared as 1.0 wt% in KBr wafers.

Thermogravimetric (TGA) and Derivative Thermogravimetric Analysis (DTG) were performed on a previously calibrated Perkin Elmer Pyris 1 TGA instrument from room temperature up to 210 °C. A sample mass of 7 mg was heated under nitrogen flow of 50 mL min<sup>-1</sup>, at a heating rate of 2 °C min<sup>-1</sup>, using a platinum cell. For isothermal analysis, the temperature of 35 °C was maintained during 10 h and 9 mg of sample mass was used. The other parameters were kept unchanged.

The adsorption-desorption isotherms of nitrogen were obtained at -196 °C with a Micromeritics ASAP 2020. The sample was previously treated for 180 min at 350 °C under vacuum. The pore diameters, as well as the pore size distribution, were calculated by using the BJH (Barrett, Joyner and Halenda) method. The specific area was calculated with the BET (Brunauer, Emmett and Teller) equation in the low-pressure region ( $P/P_0 < 0.3$ ), and the micropore area and micropore volume were estimated by using the t-plot method.

### 2.3. Recovery and Stability assessment

The rhynchophorol present in the composite was recovered after 24 h in order to evaluate its stability. For this, 2 mL of n-hexane was added to tubes containing the composite. The tube was shaken during 1 min and kept at rest by 4 h. After this period, the tube was shaken again by 1 min. The suspension was filtered using 0.45 µm nylon membrane.

To determine rhynchophorol in hexane solution, a Perkin Elmer Clarus 680 gas chromatograph connected to a Perkin Elmer 600 C quadrupole mass spectrometer was used. The following conditions were used: Elite-5MS (30 m 0.25 mm x 0.25 mm) column, helium flow = 1 mL min<sup>-1</sup>, injection volume using CTC Combipal automatic injector = 1 mL, split = 50 mL min<sup>-1</sup> and temperature of injector = 150 °C. The

temperature program used was: initial temperature was 50 °C during 3 min, followed by temperature increase under heating rate of 10 °C min<sup>-1</sup> up to 200 °C, after reaching 200 °C, this temperature was maintained for 1 min. A volume of 10 µL of 6-methyl-5 hepten-2-one was used as an internal standard (IS). The chromatographic data were analyzed using Turbomass software, version 5.4.2.1617.

### 3. Results and Discussion

#### 3.1. Characterization

Table 1 presents the chemical analysis data (ED XRF) of K10 (K10-MT) and KSF (KSF-MT) montmorillonite, as well as Kaolin. The K10 montmorillonite and Kaolin have presented a low concentration of Fe<sub>2</sub>O<sub>3</sub>, whereas KSF montmorillonite has presented appreciable concentration. For all clay samples, the Mg isomorphic substitution was small.

Table 1 - Chemical composition of clay minerals: K10 and KSF Montmorillonite (% mass/mass)

Composition	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	SO <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	K <sub>2</sub> O	MgO	TiO <sub>2</sub>	BaO
K10	50.79	44.63	0.13	1.4	0.09	0.32	-	2.10	0.369
KSF	43.43	17.19	16.36	13.83	2.54	2.48	2.42	0.708	0.306
Kaolin	68.8	19.9	0.07	5.6	0.29	2.61	1.55	0.75	0.24

Note: The values below 0.03% are not presented.

The XRD pattern of K10, KSF montmorillonite and Kaolin are shown in Figure 1. The peaks found at 6.14°, 19.82° and 35.04° in K10 and KSF montmorillonite are all consistent with this clay mineral [34]. The presence of kaolinite, illite and quartz in both types of montmorillonite was verified [35,36,37]. The characteristic diffraction peaks for kaolinite (2θ nearly 12.3° and 24.8°) [111,38,39] were also found, besides others, as

pointed in Figure 1. The presence of montmorillonite and quartz was noticed in Kaolin sample.

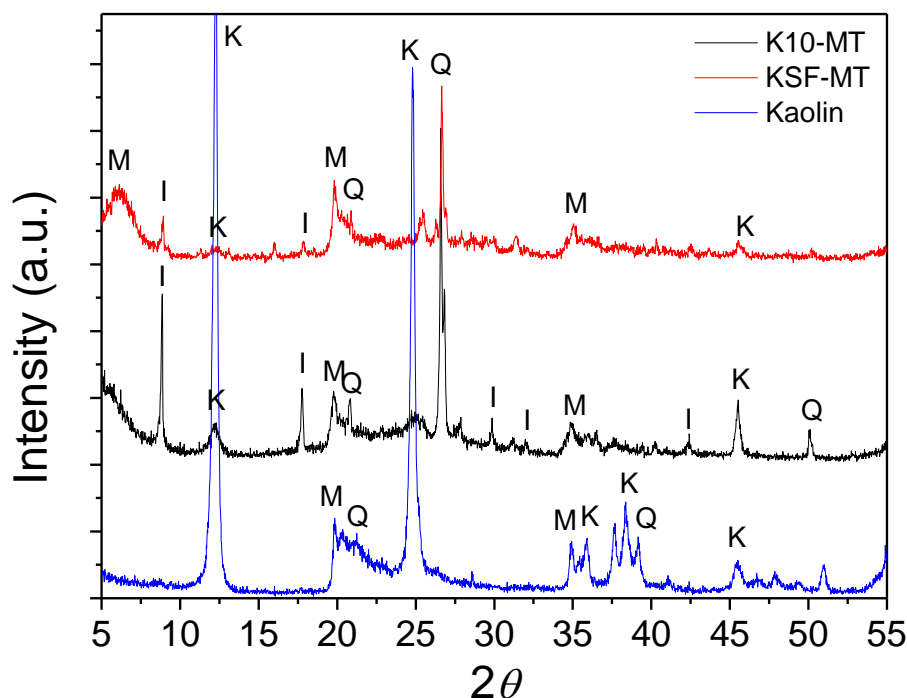


Figure 1 - X-ray diffraction patterns of montmorillonites (K10-MT and KSF-MT) and Kaolin. M = montmorillonite, I = Illite, K = kaolinite and Q = quartz.

The FTIR spectra obtained for K10-MT, KSF-MT and Kaolin can be seen in Figure 2. For K10-MT (Figure 2-a), characteristic absorptions are observed in the low energy region. An intense and broad band around  $1044\text{ cm}^{-1}$  is attributed to the Si–O stretching vibrations of the tetrahedral layer [40,41,42]; whereas in the region between  $913$  and  $796\text{ cm}^{-1}$  is attributed to the octahedral layers of the aluminosilicate [43]. The bands that appear at  $694$ ,  $528$  and  $470\text{ cm}^{-1}$  refer to Si-O-Al bending, Si-O bending and Si-O-Si bending, respectively [40,43]. The bands referring to OH stretching were noticed at  $3624$ ,  $3436$  and  $1634\text{ cm}^{-1}$  corresponding to the  $\text{Al}_2\text{OH}$  group of the octahedral layer, stretching vibrations of water molecules and bending vibrations of  $\text{H}_2\text{O}$ , respectively [44,45]. The FTIR spectrum of KSF-MT (Figure 2-b) has presented similar FTIR profile.



The infrared spectra obtained for Kaolin (Figure 2-c) exhibited profile similar to theoretical kaolinite [46]. The bands referring to OH stretching were noticed at 3696, 3653 and 3620  $\text{cm}^{-1}$  and OH deformation band at 914  $\text{cm}^{-1}$ . Bands attributed to Si-O stretching were observed at 795, 754 and 697  $\text{cm}^{-1}$ .

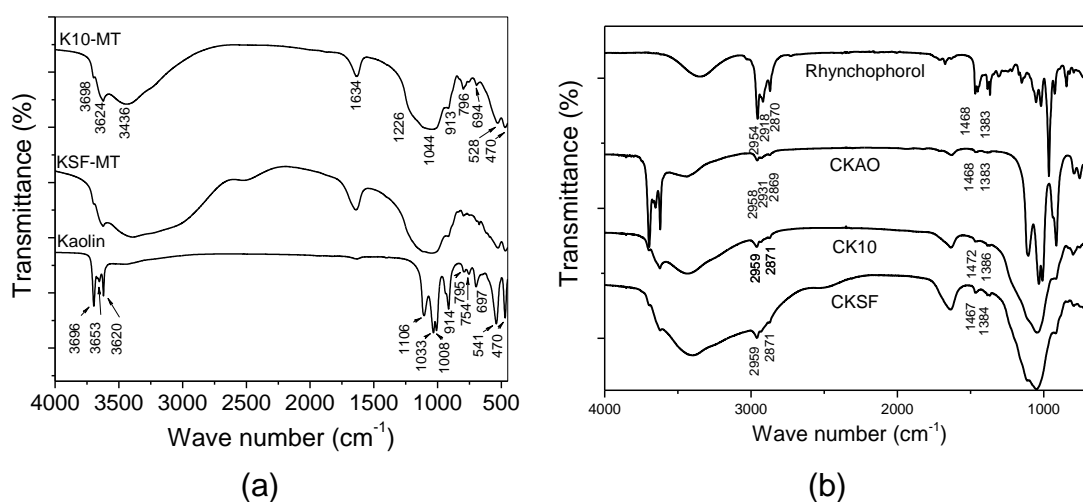


Figure 2 - FTIR spectra of clay minerals (a), rhynchophorol and composites (b).

Figure 3 shows the TG and DTG curves obtained for the clay mineral samples. All samples of clay minerals studied presented one step of dehydration. The clay minerals have presented the following humidity up to 210  $^{\circ}\text{C}$  in ascending order: 0.4% < 6.4% < 6.8%, for Kaolin, K10-MT and KSF-MT, respectively.

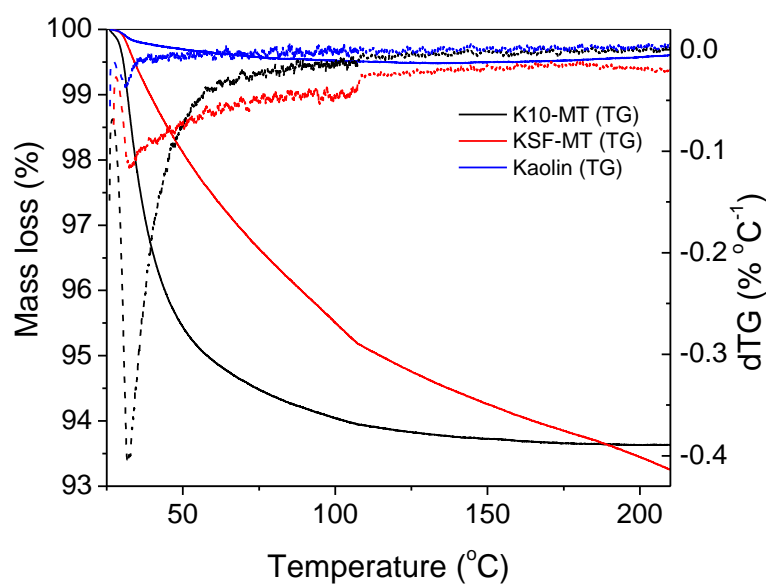


Figure 3 – TG/DTG of clay minerals.

The isotherms of K10-MT and KSF-MT can be described as hybrid type I/type IV patterns by IUPAC classification, typical of predominantly microporous materials, with secondary mesoporous, formed by stacking of particles [47]. The K10-MT has presented an H3 hysteresis loop, indicating existence of non-rigid aggregates of plate-like particles or assemblages of slit-shaped pores [48]. The KSF-MT, meantime, has presented H4 hysteresis loop. This type of loop is associated to narrow slit-shaped pores [47] and limited amounts of mesopores limited by micropores [49]. The kaolin could be labeled as a typical type-IV isotherm, presenting H3 hysteresis loop, suggesting aggregates of platelike particles forming slit-like pores [50,51].

The pore size distribution of the sample K10-MT and KSF-MT (Figure 4-b and 4-d, respectively), shows that the sample is predominantly microporous, with almost no contribution of mesoporosity. The kaolin, meantime, shows different pore sizes in the micro-, meso- and macroporosity regions.

The K10-MT has presented superior textural properties, as expected. Its surface area is constituted almost entirely (95.8%) by external area. However, this data must be cautiously evaluated, because t-plot uses BET surface area in calculation and BET method is not adequate for microporous materials [47]. The kaolin has presented the lowest surface area and very large pore diameters, indicating formation of large inter particle cavities [51].

The nitrogen adsorption isotherms displayed in Figure 4 were mathematically treated by BET, t-plot (for accessing information about microporosity of clay minerals), and BJH (for obtaining data about the average diameter and volume of mesoporous) methods. These results are summarized in Table 2.

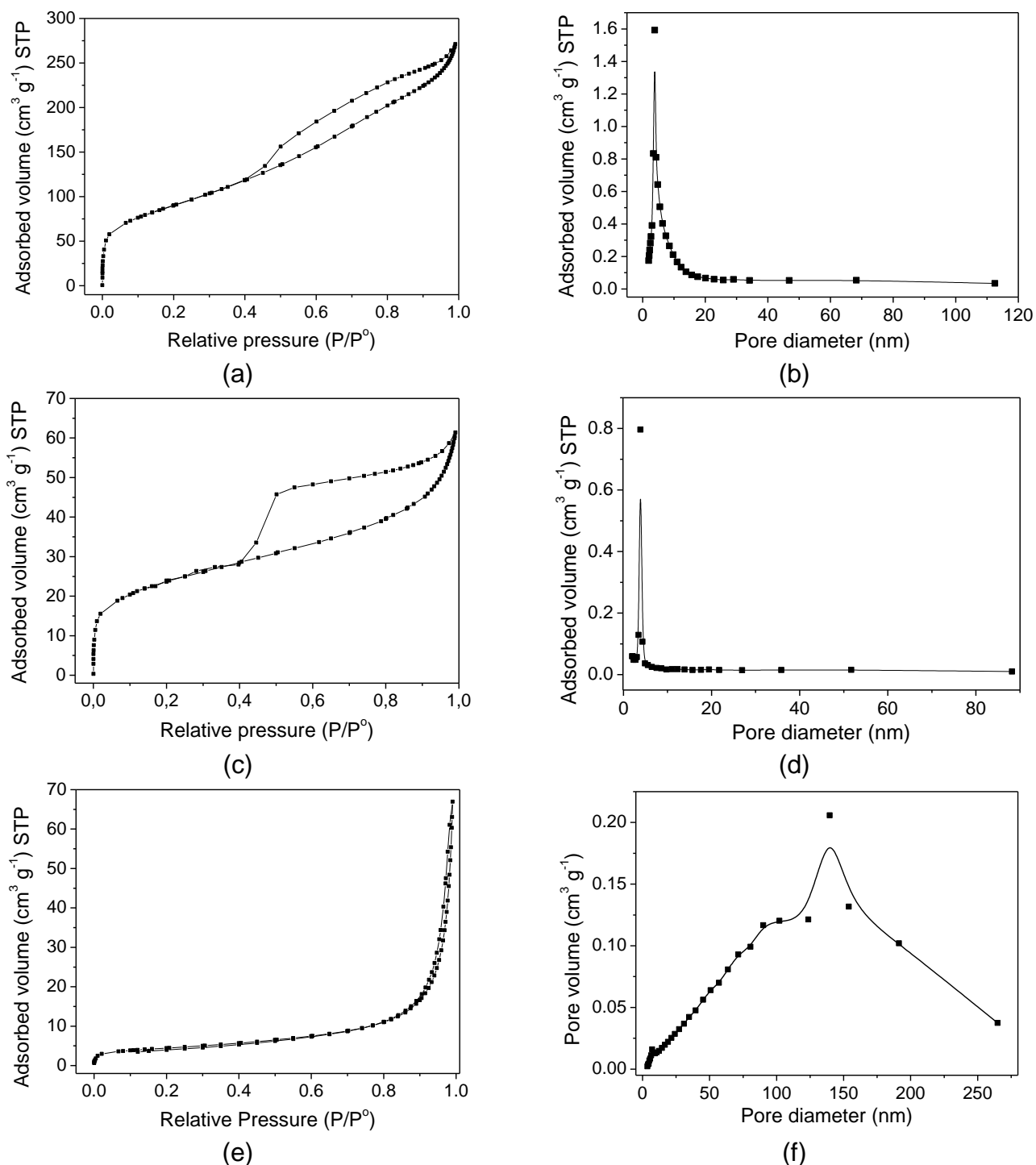


Figure 4 - Nitrogen adsorption isotherms at 196 °C for clay minerals and distribution of pore size for the samples: K10-MT (a) and (b), KSF-MT (c) and (d), and Kaolin (e) and (f).

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method is not adequate for microporous materials [47]. The kaolin has presented the lowest surface area and very large pore diameters, indicating formation of large inter particle cavities [51].

Table 2 - Textural properties of clay minerals

Sample	$S_{\text{BET}}$ ( $\text{m}^2 \text{g}^{-1}$ )	$S_{\text{external}}$ ( $\text{m}^2 \text{g}^{-1}$ ) <sup>a</sup>	$S_{\text{micropores}}$ ( $\text{m}^2 \text{g}^{-1}$ ) <sup>a</sup>	$V_{\text{micropores}}$ ( $\text{cm}^3 \text{g}^{-1}$ ) <sup>a</sup>	$V_{\text{mesopores}}$ ( $\text{cm}^3 \text{g}^{-1}$ ) <sup>b</sup>	$d_{\text{mesopores}}$ (nm) <sup>b</sup>
K10-MT	320.8	307.3	13.6	0.0050	0.4406	10.93
KSF-MT	81.9	74.9	7.0	0.0040	0.0923	13.98
Kaolin	15.7	13.4	2.3	0.0010	0.1038	53.79

<sup>a</sup> t-plot method.

<sup>b</sup> BJH method.

### 3.2. Characterization and stability of composites

The FTIR spectra of rhynchophorol and composites formed by clay minerals and rhynchophorol are shown in Figure 2-b. The presence of rhynchophorol is verified in the composites through the bands:  $2954 \text{ cm}^{-1}$ ,  $2918 \text{ cm}^{-1}$ ,  $2870 \text{ cm}^{-1}$ , attributed to the C-H stretching;  $1468 \text{ cm}^{-1}$ , which refers to  $\text{CH}_2$  bending vibration; and  $1383 \text{ cm}^{-1}$ , attributed to doublet for the  $\text{CH}_3$  bending [52]. This FTIR technique did not provide information about interaction between rhynchophorol and the clay minerals due to overlapping bands.

Figure 5 shows the TG and DTG curves obtained for rhynchophorol and composites formed by the clay minerals and the pheromone. The rhynchophorol has presented two steps of mass loss. This can be seen in DTG profile (Figure 5-a). The first step can be attributed to residual solvent, since previous chromatographic studies have shown that no degradation occurs at temperature lower than  $120 \text{ }^\circ\text{C}$ . The second thermal event is due to rhynchophorol evaporation. Mass loss, temperature ranges and a description of thermal events observed in the studies are summarized in Table 3.

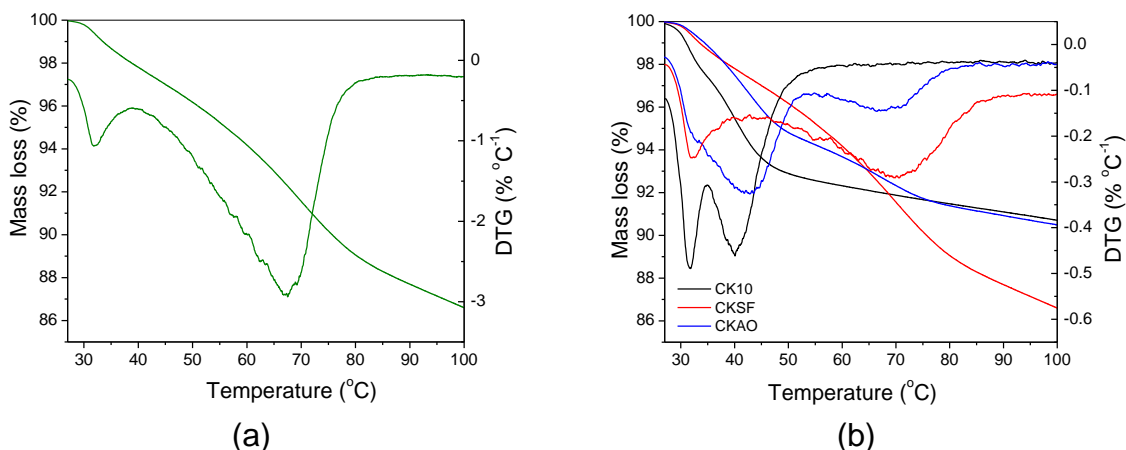


Figure 5 – TG and DTG of rhynchophorol (a) and composites (b).

Table 3 – Mass loss observed for DTG of rhynchophorol and composites

Sample	1° thermal event			2° thermal event			3° thermal event		
	T <sub>initial</sub> (°C)	T <sub>end</sub> (°C)	Mass loss (%)	T <sub>initial</sub> (°C)	T <sub>end</sub> (°C)	Mass loss (%)	T <sub>initial</sub> (°C)	T <sub>end</sub> (°C)	Mass loss (%)
Rhyn.	26.3	39.0	2.0	39.0	90.0	10.3	-	-	-
CK10	26.2	34.8	2.6	34.8	57.8	5.0	-	-	-
CKSF	26.3	35.8	1.5	46.1	90.0	9.1	-	-	-
CKAO	26.0	33.4	1.0	33.4	55.0	4.8	55.0	82.1	3.0

Rhyn. = rhynchophorol

The thermal event attributed to residual solvent of rhynchophorol is observed in all composite samples, with small displacement for lower temperatures. The DTG profile of CKAO sample has presented three mass losses. Whereas the composites CK10 and CKSF have presented two (Figure 5-b), as well as rhynchophorol.

The CK10 composite presented the final temperature of the second thermal event 4.2 °C smaller than the final temperature of the second thermal event of the rhynchophorol. The CKSF composite was the material that has shown the most similar DTG profile compared to rhynchophorol. However, in this composite, the second mass loss starts at higher temperature (46.1 °C) than the second mass loss in rhynchophorol DTG profile (39.0 °C).

In DTG profile of CKAO, the event attributed to residual solvent of rhynchophorol is overlapped with the second thermal event, almost showing a single loss of mass. The second thermal event ends at approximately 55 °C, very close the final temperature of CK10 second mass loss. However, for this composite, an additional thermal event is verified between 55-96 °C.

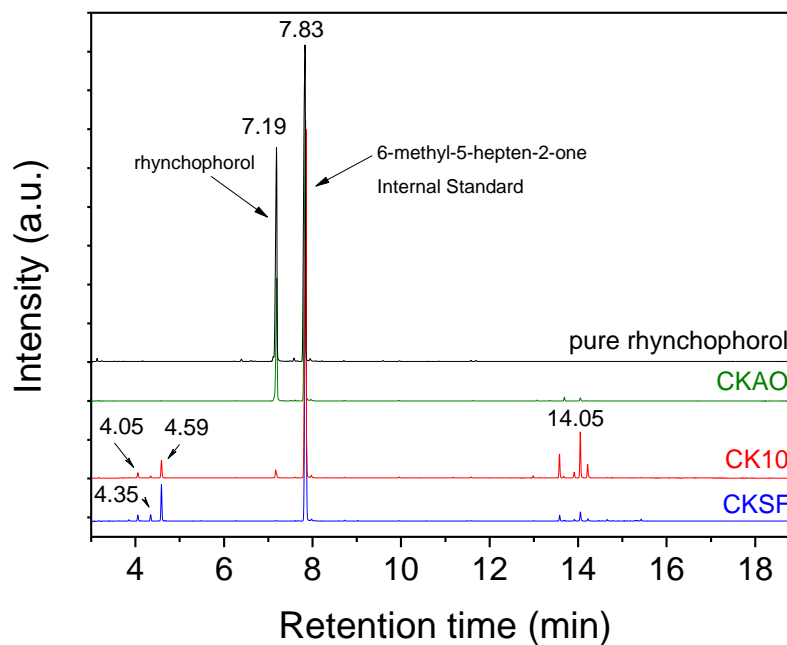
As the thermal analysis technique does not distinguish rhynchophorol evaporation and degradation products evaporation from composites, the stability of pheromone adsorbed was evaluated through its extraction with hexane, since the pheromone is soluble in this solvent.

Figure 6 shows the chromatograms comparing pure rhynchophorol and rhynchophorol recovered from composites. On Figure 6-a, the presence of rhynchophorol peak ( $t_r = 7.19$  min) is noticed only for CKAO, with 62.54% of recovery. It is important to note that, the expressive loss of rhynchophorol was due to volatilization, since very little degradation product is observed in the chromatogram. This volatilization occurred because the storage container was not hermetically sealed.

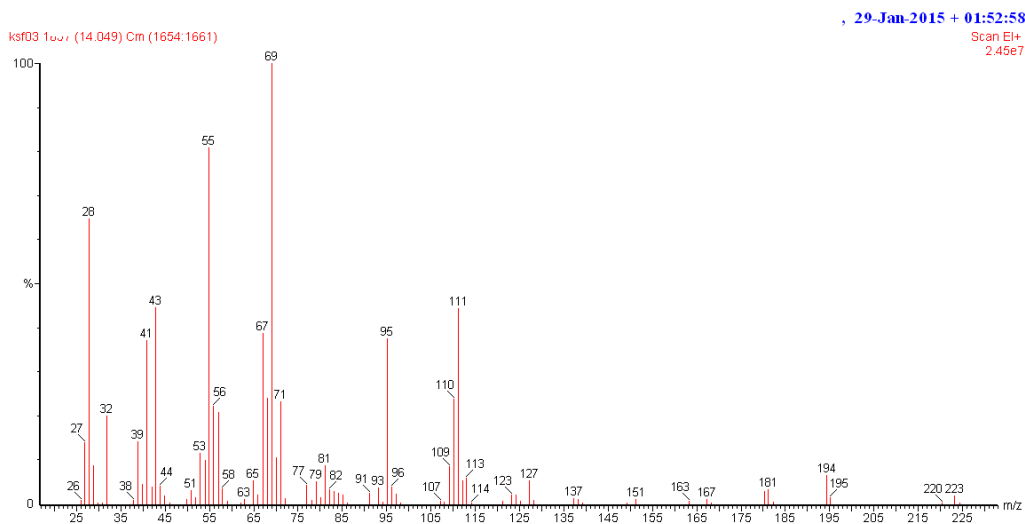
For the composites CK10 and CKSF it is verified the absence of rhynchophorol peak and the emergence of new peaks in the range of 4-5 min and 13-15 min. This indicates that the adsorbed rhynchophorol was converted to other products, due to chemical interaction between rhynchophorol and both montmorillonites.

The same degradation products in range 4-5 min present in hexane solution from extraction of rhynchophorol were noticed for composites rhynchophorol/ZSM-5 zeolites with different  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratio. The formation of these degradation products was due to dehydration reaction catalyzed due to high density of acidic sites [14]. In fact, the montmorillonite possesses Lewis acidity, associated with the metal atoms at the edge sites; and Brönsted acidity, associated with the interlamellar water

coordinated to exchangeable cations [53]. The kaolin, on the other hand, has low acidity [54], which justifies the stability of rhynchophorol at CKAO.



(a)



(b)

Figure 6 – Chromatograms obtained after extraction of rhynchophorol from composites (a) and mass spectra obtained for degradation product referring to time  $t_r = 14.05$  min (b).

As already mentioned, the composites CK10 and CKSF also produced compounds less volatile than rhynchophorol (Figure 6-a,  $t_r = 13-15$  min). These degradation products were analyzed by gas chromatography linked to mass spectrometer and the formation of compounds resulting from etherification reaction was verified. Figure 6-b shows the mass spectra obtained from compound eluted at 14.05 min.

The acidic sites also are responsible by electrophilic substitution, with the formation of ethers. The mechanism is similar to dehydration in acidic medium, shown in work of Ramos and colleagues [14]. In this case, the carbocation is formed, but binds to another molecule of the alcohol to form the ether (Figure 7). This reaction has lower activation energy than that intramolecular dehydration [55].

From Figure 6 it was observed that the etherification products (54.27%) have stood out in relation to dehydration products (45.73%), for CK10 composite. Meantime, for CKSF, the magnitude of degradation products resultant of dehydration reaction (55.84%) was higher than the products resultant of etherification reaction (44.16%).

The data above suggests that the dehydration reaction is associated with the Lewis sites and the etherification reaction at the Brönsted sites. The KSF-MT has higher  $\text{SiO}_2/\text{Al}_2\text{O}_3$  molar ratio (2.53) than K10-MT (1.14). Thus, less aluminum is present in the silicate network of KSF-MT and, consequently, the number of Brönsted acidic sites is lower for this clay mineral. On the other hand, the KSF-MT has an expressive amount Fe in its structure (Table 1), being able to act as Lewis acidic sites and catalytically active.



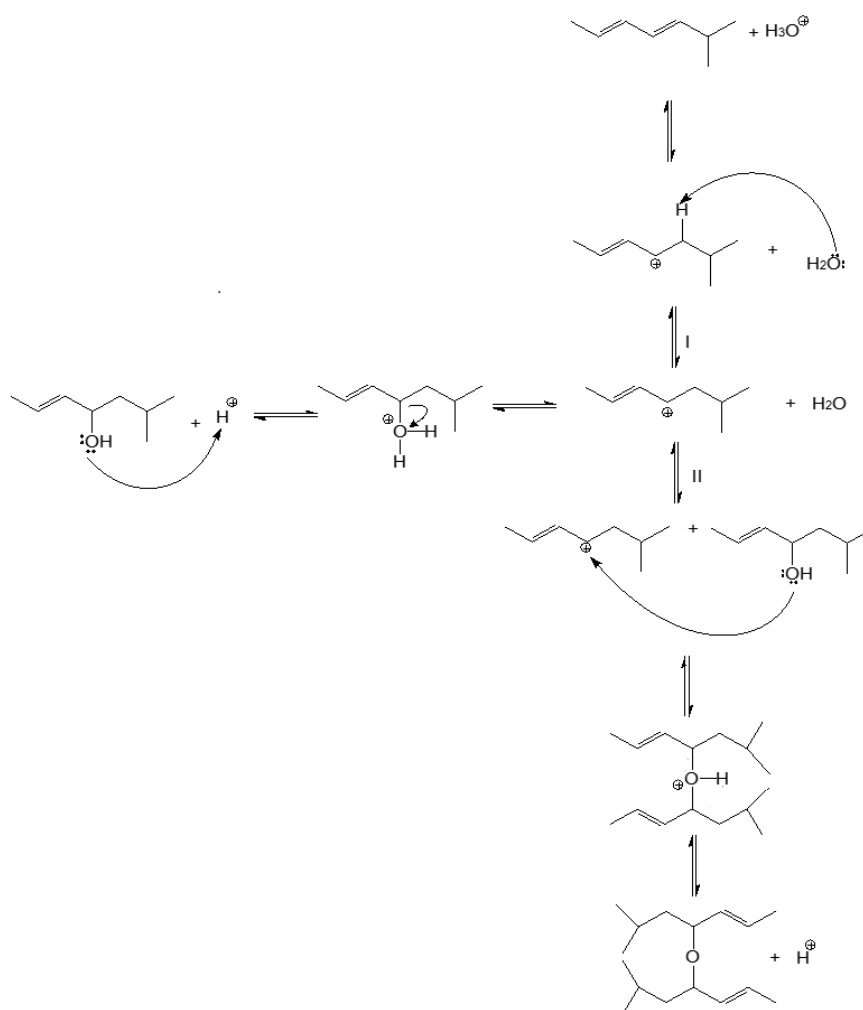


Figure 7 – Mechanism proposal of rhynchophorol etherification in acidic medium based on GC MS analysis.

The K10 and KSF montmorillonite are widely used as catalysts for reactions of dehydration of alcohols of short chain. However, this reaction occurs at temperatures above 100 °C. In this work, the dehydration and etherification reactions have occurred around 25 °C.

On Figure 6-a, the presence of very small peaks referring to degradation products due to etherification reaction in hexane solution after 1 day is verified. Thus, a long-term study was carried out in order to evaluate the decreases of rhynchophorol over time and the formation of these degradation products.

Figure 8 shows the exponential decrease of rhynchophorol over 180 days. However, this decrease is not accompanied with formation of degradation products. The degradation products increase up to 50 days, and then decrease. This can be explained considering that the reaction of etherification reached equilibrium only after 50 days of contact between rhynchophorol and KAO-MT. After this period, only volatilization of degradation products occurs. No product concerning the dehydration reaction was formed for CKAO.

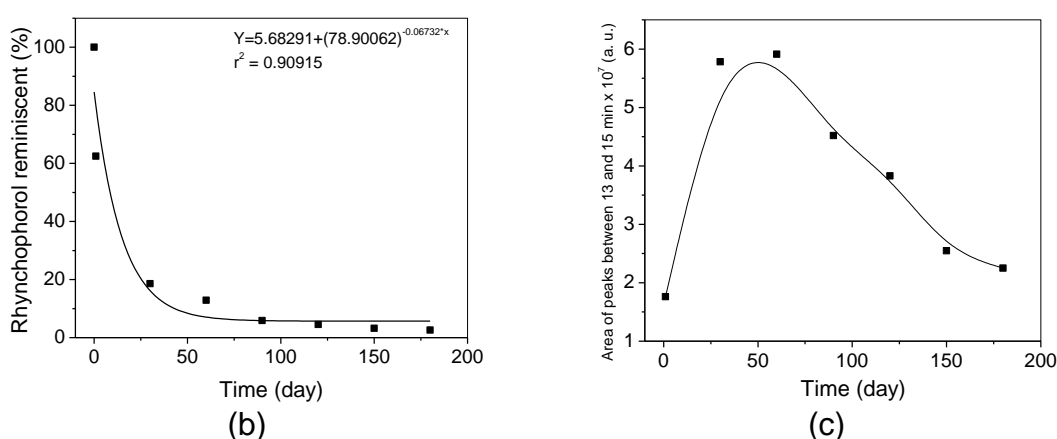


Figure 8 – Degradation profile of rhynchophorol (a) and formation of degradation products (b) from CKAO.

### 3.3. Release of rhynchophorol from CKAO

There are several methods that can be used to evaluate the rate of pheromone release. Among them, the thermogravimetry stands out as an *in vitro* technique that allows the rapid characterization of controlled release profiles from matrix systems [56]. Despite TG isothermal method does not differentiate water loss and rhynchophorol loss, this analysis was able to show the trend of release of pheromone.

Thus, in order to evaluate the kinetic release of rhynchophorol from CKAO, this method was used. The Figure 8 shows the profiles of release of this composite. The CKAO composite has shown 14.3% of mass loss in 10 h, representing a release of 59.2% of rhynchophorol, an expressive retention comparing with pure rhynchophorol.

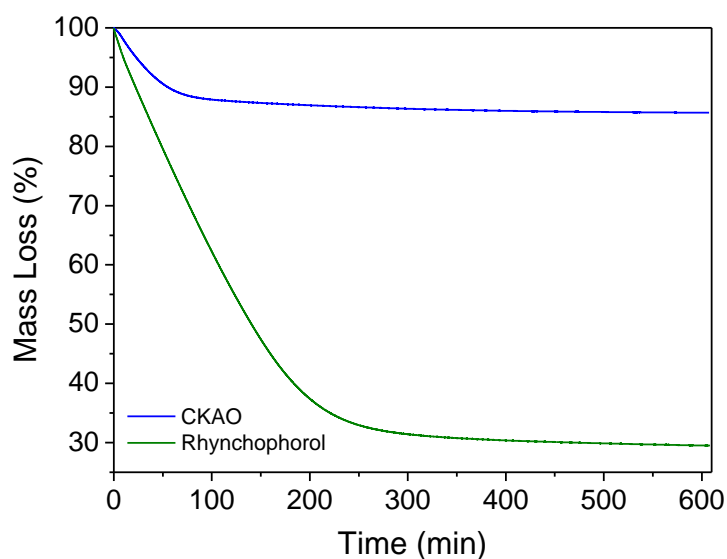


Figure 9 - Profiles of release of rhynchophorol from CKAO using thermal analysis

The release rate of rhynchophorol was calculated for the CKAO from thermogravimetric curves presented in Figure 8 in two steps. For the first step (initial average rate) the mass loss from the end of dynamic heating (near 7.7 min) up to the beginning of linear trend was considered. For the second step the linear trend was considered. The results are shown in Table 4.

Table 4 – Average release rate of rhynchophorol from composites

Composite	Initial average release rate (% h <sup>-1</sup> )	Average release rate at linear step (% h <sup>-1</sup> )
Rhynchophorol	11.39	0.26
CKAO	4.59	0.21

The initial release rate is important information for controlled release matrix systems, since for these systems it is desirable to have zero order release kinetic [9]. Considering that in the same analysis conditions the rhynchophorol has presented initial evaporation average rate of 11.39 % h<sup>-1</sup>, the CKAO was promising as prolonged release matrix with regard to the initial retention.

#### 4. Conclusions

It was concluded that both montmorillonites (K10 and KSF) were not appropriate as carrier for rhynchophorol prolonged release because degradation products were formed immediately after adsorption of pheromone. This degradation occurred due to acidic sites of Brønsted and Lewis along two paths: E1 intramolecular elimination of the alcohol, producing more volatile compounds than rhynchophorol; and electrophilic substitution, producing less volatile compounds than rhynchophorol.

The Kaolin, despite the considerable recovery value after 24 h, has presented degradation products due to etherification reaction over 50 days. As this material has shown expressive retention of rhynchophorol during isothermal thermogravimetric studies, modifications in kaolin are suggested for adjust it to carrier of this pheromone.

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## 5. CONCLUSÃO GERAL

Através dos resultados obtidos, foi possível realizar a validação de um método analítico para identificação e quantificação do feromônio de agregação rinfoforol, conforme Resolução - RE nº 166, de 29 de julho de 2017 da ANVISA/Brasil. Conjuntamente o mesmo método foi adaptado para identificação e quantificação do rinfoforol e seus subprodutos oriundos de sua degradação, recuperado após adsorção nas matrizes inorgânicas e orgânica.

O teste de estabilidade do rinfoforol realizado no tempo de 24 horas, demonstrou promissora utilização para algumas matrizes utilizadas no presente estudo. Taxas de recuperação do rinfoforol forma obtidas: Silicalita-1 (93,17%); Zeólito L (93,62%); ZSM-5 SAR30 (91,26%); Na-Magadiíta (89,05%), caulim (88,3%) e amilose de batata (95,23%). As demais matrizes utilizadas promoveram degradação total do rinfoforol adsorvido, sendo produzidos novas substâncias através de reações de desidratação e eterificação.

Durante a avaliação de estabilidade visando seu armazenamento, as matrizes Na-Magadiíta, Silicalita-1 e zeólito Y foram as que apresentaram melhores resultados, mostrando uma liberação do rinfoforol no ambiente durante todo o período avaliado de 180 dias sem promover a degradação do princípio ativo. A argila Caulim não foi adequada nesta etapa, por ocasionar degradação parcial do rinfoforol e sua elevada perda já nos primeiros 60 dias de armazenamento, sendo mais adequada seu uso para períodos menores de utilização e armazenamento.

Os resultados permitiram a proteção dos processos e produtos desenvolvidos, através do registro de 02 patentes junto ao INPI. Estes registros poderão favorecer a transferência de tecnologia para futuras empresas.

Com os resultados da presente Tese, podemos concluir que é possível a utilização como dispositivo para liberação controlada do feromônio de agregação rinfoforol para as matrizes inorgânicas silicalita-1, zeólito L, Na-magadiíta e argila Caulim, como também a matriz orgânica amilose de batata.

**ANEXOS**

## Anexo A – Exame Preliminar Patente BR102015032604-1



**SERVIÇO PÚBLICO FEDERAL  
MINISTÉRIO DA INDÚSTRIA, COMÉRCIO EXTERIOR E SERVIÇOS  
INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL**

**EXAME PRELIMINAR**

**N.º do Pedido:** BR102015032604-1      **N.º de Depósito PCT:**  
**Data de Depósito:** 17/12/2015

O pedido atende formalmente as disposições legais, especialmente quanto ao Art. 19 da LPI e o Instrução Normativa nº 31/2013, estando apto a ser protocolado.

Condições do Pedido	S	N
Requerimento de depósito com os campos obrigatórios preenchidos	X	
Idioma Português	X	
Relatório Descritivo	X	
Reivindicações	X	
PI e C – Apresenta desenhos citados ou não cita nem apresenta desenhos. MU – Apresenta desenhos.	X	
Resumo	X	
Formatado no padrão exigido	X	
Valor correto de Recolhimento	X	

Rio de Janeiro, 24 de fevereiro de 2017.

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Andrea Massad Fonseca Barbosa  
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## Anexo B – Exame Preliminar Patente BR102017012450-9



**SERVIÇO PÚBLICO FEDERAL**  
**MINISTÉRIO DA INDÚSTRIA, COMÉRCIO EXTERIOR E SERVIÇOS**  
**INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL**

**EXAME PRELIMINAR**

**N.º do Pedido:** BR102017012450-9      **N.º de Depósito PCT:**  
**Data de Depósito:** 12/06/2017

O pedido atende formalmente as disposições legais, especialmente quanto ao Art. 19 da LPI e o Instrução Normativa nº 31/2013, estando apto a ser protocolado.

Condições do Pedido	S	N
Requerimento de depósito com os campos obrigatórios preenchidos	X	
Idioma Português	X	
Relatório Descritivo	X	
Reivindicações	X	
PI e C – Apresenta desenhos citados ou não cita nem apresenta desenhos. MU – Apresenta desenhos.	X	
Resumo	X	
Formatado no padrão exigido	X	
Valor correto de Recolhimento	X	

Rio de Janeiro, 22 de novembro de 2017.

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