UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Nísia Cé

Utilização de filmes de quitosana contendo nisina e natamicina para cobertura de kiwis e morangos minimamente processados.

Dissertação de Mestrado

Porto Alegre, Brasil
Janeiro 2009

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Utilização de filmes de quitosana contendo nisina e natamicina para cobertura de kiwis e morangos minimamente processados.

Nísia Cé

Dissertação submetida ao Programa de Pós-graduação em Ciência e Tecnologia de Alimentos como requisito parcial para obtenção do grau de Mestre em Ciência e Tecnologia de Alimentos.

Orientador: Prof. Dr. Adriano Brandelli
Co-Orientador: Prof. Dr. Caciano Pelayo Zapata Noreña

Porto Alegre, janeiro de 2009

Nísia Cé

(Nutricionista UNISC/RS)

DISSERTAÇÃO

Utilização de filmes de quitosana contendo nisina e natamicina para cobertura de kiwis e morangos minimamente processados.

Submetida como parte dos requisitos para a obtenção do grau de

MESTRE EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Programa de Pós Graduação em Ciência e Tecnologia de Alimentos (PPGCTA)

Universidade Federal do Rio Grande do Sul – Porto Alegre, RS, Brasil.

Aprovada em:...../...../.....
Pela Comissão Examinadora:

Prof. Dr. Adriano Brandelli
Orientador – PPGCTA/UFRGS

Prof. Dr. Caciano Pelayo Zapata Noreña
Co-orientador – PPGCTA/UFRGS

Homologada em: ...../...../.....
Por:

Prof. Dr. Eduardo Tondo
PPGCTA/UFRGS

José Maria Wiest
Coordenador do Programa de Pós Graduação em Ciência e Tecnologia de Alimentos (PPGCTA)

Prof. Dr. Renar João Bender
Departamento de Hortifruticultura e Silvicultura Faculdade de Agronomia – UFRGS

Prof. Dr. Pedro Luiz Manique Barreto
Departamento de Ciência e Tecnologia de Alimentos – UFSC

ADRIANO BRANDELI
Diretor do Instituto de Ciência e Tecnologia de Alimentos ICTA/UFRGS

AGRADECIMENTOS

Agradeço ao orientador professor Dr. Adriano Brandelli.

Ao co-orientador professor Dr. Caciano Pelayo Zapata Noreña.

Aos colegas de mestrado pelo companheirismo e amizade, especialmente a Gisele Schmidt e a Simone Weschenfelder, que lembrarei com muito carinho.

Aos professores e funcionários do PPGCTA.

Aos amigos do laboratório 218, especialmente a Simone Pieniz e a Cássia R. Nespolo.

A amiga e ex. professora Simone Luísa Berti, que me introduziu ao mundo da pesquisa.

E a todas as pessoas que acompanharam de alguma forma esta caminhada.

Dedico este trabalho a minha família em especial a minha irmã Dra. Deisy Cé, pelo carinho e apoio financeiro. A meu amor Maurício Zuchetti, que esteve muito presente neste momento.

CÉ, N. **Utilização de filmes de quitosana contendo nisina e natamicina para cobertura de kiwis e morangos minimamente processados.** 95 p. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) - Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, (2009).

RESUMO

As frutas fornecem um aporte de vitaminas, de minerais e açúcares, além de terem substâncias com propriedades funcionais. Atualmente, por parte dos consumidores há uma crescente preocupação para encontrar no mercado alimentos que tenham sido minimamente processados, sendo que produtos com essa característica podem ser obtidos através de aplicações tecnológicas como emprego do frio, de sistemas de atmosferas controladas ou modificadas, e uso de embalagens comestíveis antimicrobianas. A perspectiva de conservação de alimentos busca a aplicação de sistemas antimicrobianos naturais onde se enfatiza a ação sinérgica de vários elementos. O objetivo geral deste trabalho foi avaliar o efeito de cobertura de quitosana em combinação com nisina e natamicina, sob refrigeração, na conservação de kiwi e morango minimamente processados. Foi avaliado o emprego de diferentes combinações de quitosana, nisina e natamicina durante o armazenamento das frutas sob refrigeração. Determinou-se a variação da atividade de água, vitamina C, pH, acidez titulável, perda de peso, sólidos solúveis, além da avaliação microbiológica das frutas, na condição de armazenamento em temperatura de refrigeração a $4 \pm 2^{o}C$ durante os intervalos de 1 (tempo 0), 7 e 14 dias. Os filmes com a combinação dos antimicrobianos foram eficientes para evitar a deterioração microbiológica nas frutas, mas não foram capazes de afetar positivamente os resultados das análises físico-químicas. Em kiwi, os índices de sólidos solúveis, vitamina C e pH foram os únicos afetados positivamente pelos filmes. E em morango, apenas a umidade e a vitamina C foram as variáveis que apresentaram menores perdas significativas com adição das coberturas. A aplicação dos filmes de quitosana para preservação de alimentos, além da estabilidade das frutas frente à deterioração microbiana, aumenta a garantia de obtenção de kiwis e morangos sadios minimamente processados por mais tempo.

Palavras – chave: biofilmes, frutas, antimicrobianos, vida de prateleira.

CÉ, N. **Application of chitosan coatings nisin and natamicyn on kiwi and strawberry minimally processed.** 95 p. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) - Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, (2009).

ABSTRACT

Fruits are sources of vitamins, sugars and minerals, and contain substances with functional properties. Currently, for consumers there is a growing concern in the market to find foods that are minimally processed. The products with this feature can be obtained through technological applications such as: the use of refrigerated storage, use of or modified controlled atmospheres or applying edible antimicrobial packaging. The prospect of keeping food seeking the application of natural antimicrobial systems which emphasizes the synergistic action of several elements. This work intended to coatings of chitosan in combination with nisin and natamycin, under refrigeration in the conservation of kiwi ana strawberry minimally processed. The use of different combinations of chitosan, nisin and natamycin, in combination with temperatura refrigeration were as well evaluated. Ranges of water activity, vitamin C, pH, acidity, fresh weight loss, soluble solids, than the microbiological evaluation of fruit containing biofilms on the condition of storage temperature of refrigeration at 4 ± 2ºC, during the intervals of 0, 7, and 14 days. The biofilms in antibiotic combination were effective in reduction of microbial deterioration in the fruits, but they were not able to act positively on physical and chemical variables. In kiwi, the contents of soluble solids, vitamin C and pH were the only variables positively affected by films. And in strawberry, only the moisture and vitamin C were the variables that showed reduced losses when coatings were added. Furthermore, with the application of biofilms as food preservatives, and the stability of fruit in contrast to microbial deterioration, increase the guarantee of obtaining in minimally processed and healthy strawberries and kiwi fruit.

Key words: biofilms, fruits, antimicrobial, shelf life.

SUMÁRIO

LISTA DE FIGURAS

LISTA DE TABELAS

Tabelas do Artigo 1 - Evaluation of minimally processed kiwi fruit covered with chitosan films incorporating nisin and natamycin

Tabelas do Artigo 2 - Evaluation of minimally processed strawberry fruit covered with chitosan films incorporating nisin and natamycin

1 - INTRODUÇÃO

O Brasil é o terceiro pólo mundial de fruticultura, com uma produção anual de cerca de 38 milhões de toneladas. O Estado do Rio Grande do Sul é o primeiro produtor nacional de uva, pêssego, figo, pêra, nectarina e kiwi. A fruticultura propicia a geração de empregos e fixação de famílias no meio rural, contribui na alimentação e saúde das populações urbanas e rurais, assim como, na sustentabilidade ambiental. Assim, o conhecimento, por parte dos produtores, de novas tecnologias de armazenamento pós-colheita na cadeia de comercialização das frutas, visa ampliar o espaço do Estado como fornecedor de produtos de qualidade para os mercados nacional e internacional (EMATER, 2007).

Atualmente está implantado no Estado do Rio Grande do Sul o Programa Estadual de Fruticultura - PROFRUTA/RS. Um dos objetivos do Programa é o de coordenar ações das instituições públicas e privadas com o intuito de propiciar o desenvolvimento de uma fruticultura moderna, sustentável e competitiva (RIO GRANDE DO SUL, 2006).

As frutas além do aporte das vitaminas, minerais e açúcares, possuem substâncias com propriedades funcionais. Entende-se por propriedade funcional aquela relativa ao papel metabólico ou fisiológico que o nutriente ou não nutriente tem no crescimento, desenvolvimento, manutenção e outras funções normais do organismo humano, bem como a prevenção e a redução de algumas doenças como, por exemplo, problemas cardiovasculares e surgimento de tumores malígnos.

Atualmente, por parte dos consumidores existe uma crescente preocupação por encontrar no mercado alimentos que tenham sido minimamente processados, sendo que produtos com essa característica podem ser obtidos pelo emprego do frio, sistemas de atmosferas controladas, modificadas e embalagens antimicrobianas.

Tem-se observado o grande interesse no desenvolvimento de embalagens antimicrobianas usando polímeros biodegradáveis e/ou renováveis. A quitosana é um polímero biodegradável obtido a partir da retirada da capa dos crustáceos e conchas, sendo a maior constituinte do exoesqueleto dos crustáceos. Ela tem recebido uma significativa atenção por ser um polímero renovável, não tóxico, apresentar excelente

biocompatibilidade com outras substâncias e, portanto, vem sendo empregada em áreas da medicina, agricultura, na indústria química e de alimentos (DIAB et al. 2001).

A perspectiva de conservação de alimentos busca a aplicação de sistemas antimicrobianos naturais onde se enfatiza a ação sinérgica de vários elementos. Portanto, foram aplicados neste estudo bacteriocinas, as quais são peptídeos produzidos por bactérias, que possuem seu potencial de ação determinado como antimicrobiano; e também um conservante natural com propriedade antifúngica. A natamicina é um antifúngico produzido por *Streptomyces natalensis* e outros *Streptomyces* spp. relacionados. Ela mostra atividade contra fungos e leveduras, mas não apresenta efetividade contra bactéria (DELVES-BROUGHTON et al. 2006). Então estas substâncias oferecem uma possibilidade viável para a aplicação como bioconservantes em alimentos.

Nesse sentido o presente trabalho visa contribuir com o setor frutícola do Estado, na área de armazenamento através da cobertura das frutas com substâncias orgânicas, como são os biopolímeros de quitosana, contendo os antimicrobianos nisina e natamicina para dessa forma aumentar a estabilidade das frutas frente à deterioração e assim aumentar a sua vida de prateleira.

2 - REVISÃO BIBLIOGRÁFICA

2.1 - Quitosana

A quitosana é um polímero natural derivado do processo de desacetilação da quitina, biopolímero abundante no exoesqueleto de crustáceos e moluscos, também na estrutura da parede celular de certos fungos e insetos (VARGAS et al. 2004). É assumido como o segundo polissacarídeo mais abundante da natureza, sendo que sua estrutura é formada pela repetição de unidades beta (1→4) 2-amino-2-deoxi-D-glucose (ou D-glucosamina) apresentando uma cadeia polimérica similar à da celulose, como ilustrado na Figura 1 (KOIDE, 1998). É definida também como sendo um polissacarídeo catiônico de alto peso molecular, solúvel em ácido orgânico, usada como material preventivo em cobertura de frutas (CONG et al. 2007).

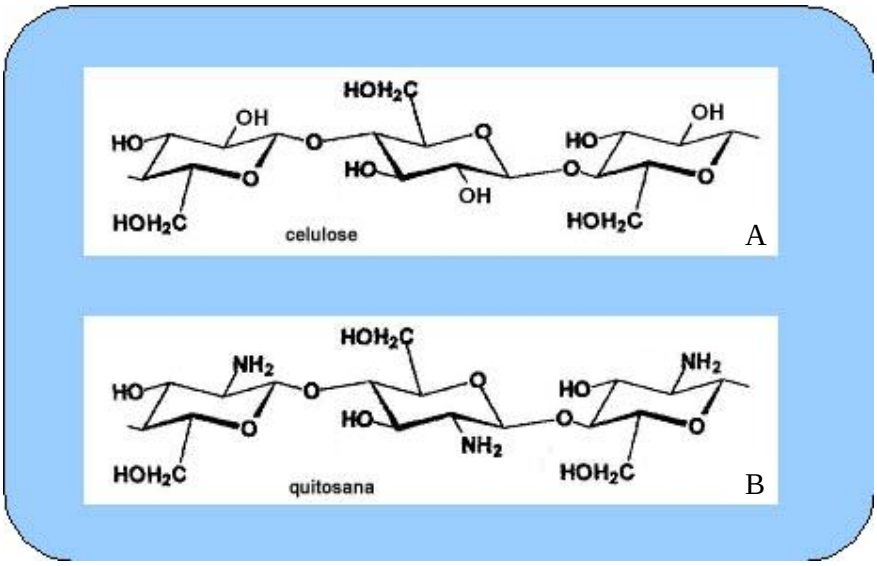


Figura 1. Comparação da estrutura química da quitosana (B) com a da celulose (A).

Devido a suas características atóxicas e de fácil formação de géis, a quitosana tem sido considerada há décadas como um composto de interesse industrial e especialmente de uso farmacêutico (CAMPANA FILHO e DESBRIÈRES, 2000 e DEVLIEGHERE et al. 2004). Os pesquisadores NO et al. (2002), KENDRA et al. (1989) e PARK et al. (2004)

relataram que a quitosana possui também, atividade antifúngica e antibacteriana, mostrando sua potencial utilização sobre as superfícies cortadas ou nos frutos que possuem alta taxa de maturação após colheita.

A parte do efeito antimicrobiano, a quitosana é também utilizada em alimentos como agente clarificante em suco de maçã (BOGUSLAWSKI et al. 1990; ROOT e JOHNSON, 1978; SOTO-PERALTA et al. 1989), antioxidante em lingüiças (XIE et al. 2001), inibidora do escurecimento enzimático em sucos de pêra e maçã (SAPERS, 1992) e em tomates (DORNENBURG e KNORR, 1997).

A atividade antimicrobiana da quitosana depende de vários fatores, tais como: o tipo de quitosana (grau de desacetilação e peso molecular) usado, o pH do meio, a temperatura e a presença de componentes alimentares. O mecanismo da atividade antimicrobiana não é bem definido até o momento, mas várias hipóteses têm sido sugeridas. A hipótese mais provável é a mudança na permeabilidade celular devido às interações entre a quitosana policatiônica e as cargas eletronegativas na superfície da célula. Esta interação gera um escapamento de eletrólitos e constituintes protéicos intracelulares (PAPINEAU et al. 1991; SUDARSHAN et al. 1992; FANG et al. 1994; CHEN et al. 1998; YOUNG et al. 1982).

Alguns estudos têm sido publicados caracterizando o uso da quitosana como cobertura de alimentos ou revestimento protetor em frutas e legumes processados (SHAHIDI et al. 1999; JIANG e LI, 2001; COMA et al. 2002). Ela tem sido usada para manter a qualidade no pós–colheita de frutas e vegetais tais como citrus (CHIEN et al. 2007c), pêssego, pêra e kiwi (DU et al. 1997), morango (EL GHAOUTH et al. 1991), tomates (El GHAOUTH et al. 1992), maçãs (IPPOLITO et al. 2000) e lichia (ZHANG e QUANTICK, 1997; JIANG e LI, 2001). Ainda em filmes ela tem o potencial de prolongar a vida de prateleira em frutas como morango, pêra e uva de mesa (ROMANAZZI et al. 2003).

Outros trabalhos avaliam o efeito combinado da ação dos agentes antimicrobianos, antioxidantes, nutrientes, corantes e flavorizantes quando adicionados nos filmes elaborados a partir da quitosana (PARK et al. 2004). CHEN et al. (1996) incorporaram conservantes de alimentos, tais como sorbato de potássio e benzoato de sódio em filmes de quitosana e compararam o efeito inibidor dessa matriz no crescimento de microrganismos. LEE et al. (2003) relataram que o uso de nisina e quitosana pode melhorar a estabilidade microbiana em suco de laranja e em leite, quando armazenados a 10^0C.

ZIVANOVIC et al. (2005) estudaram as propriedades antimicrobianas e físico-químicas de filmes de quitosana enriquecidos com óleos essenciais em carnes. A solução quitosana pode ser usada para outras finalidades ainda não citadas, como é o caso do estudo a seguir: ela tem sido empregada como cobertura em sementes com o intuito de aumentar a taxa de germinação e a resistência aos agentes patogênicos (FREEPONS, 1997).

SATHIVEL (2005) estudou o efeito da quitosana, da albumina de ovo, da proteína concentrada de soja e da proteína concentrada de salmão, quando empregadas como coberturas, na qualidade de filetes de salmão armazenados sob congelamento durante três meses. Esses autores relataram que o emprego da quitosana reduziu a perda de umidade e retardou a oxidação de lipídios. DEBEAUFORT et al. (1998) observaram que a função dos polissacarídeos e/ou proteínas, quando empregados como cobertura em alimentos congelados, é a de agir como barreira no controle da transferência de umidade e do oxigênio.

Em morangos (*Fragaria x ananassa Duch*), a quitosana apresenta alto potencial aplicativo. HAN et al. (2005) estudaram o emprego da quitosana no armazenamento de morangos, observando que é um conservante ideal quando usada como material de barreira, devido a suas propriedades antifúngicas. Porém a quitosana, ao ser dissolvida em soluções ácidas, desenvolve adstringência e amargura no sabor das frutas.

EL GHAOUTH et al. (1991) avaliaram o efeito da cobertura de quitosana 1.0 e 1.5% m/v (massa/volume) no controle da deterioração do morango, a 13°C, quando comparado ao efeito do fungicida iprodione (Rovral®). Esses autores observaram que não houve diferença significativa entre os tratamentos empregados aos 21 dias de armazenamento.

Ainda em frutas, as pesquisas citadas a seguir indicam a ampla finalidade da aplicação de diferentes concentrações da solução de quitosana em filmes de cobertura. CHIEN et al. (2007a) estudaram o efeito de soluções de quitosana a concentrações de 0,5%, 1% e 2% como cobertura em manga fatiada e armazenada a 6°C. Os autores constataram que houve um forte decréscimo na perda de água pelo produto e o aumento nos teores de sólidos solúveis, acidez e ácido ascórbico, assim como, a inibição do crescimento de microrganismos.

Em outro estudo, CHIEN et al. (2007b) avaliaram o efeito da quitosana como cobertura, sobre a estabilidade das pitaiaias (fruta escamosa, também chamada de fruta-dragão, Flor-da-Lua ou Dama da Noite espécie, nativa do México e América do Sul) cortadas em rodelas e armazenadas a 8°C. Esses autores constataram a diminuição na perda de água por evaporação e não ocorreram mudanças nos teores de sólidos solúveis, acidez titulável e ácido ascórbico. DONG et al. (2004) determinaram que a aplicação da cobertura de quitosana na lichia (*Litchi chinensis Sonn* – fruta nativa da Ásia) descascada e armazenada a -1°C retardou a perda de peso, observando o aumento no teor dos sólidos solúveis, acidez e ácido ascórbico, assim como a diminuição da atividade da polifenoloxidade e da peroxidase. EL GHAOUTH et al. (1992) mostraram que o emprego da quitosana como revestimento em concentrações de 1% e 2% reduz a deterioração do tomate causada principalmente por *Botrytis cinerea*.

A partir destas informações a quitosana nos mostra que pode ser útil como matéria–prima de base em filmes comestíveis, incorporados ou não, de outros agentes antimicrobianos e antifúngicos com grande potencial de aplicabilidade em frutas minimamente processadas.

2.2 - Bacteriocinas

Bacteriocinas são considerados peptídeos biologicamente ativos que têm atividade antimicrobiana contra bactérias, geralmente, relacionadas à bactéria produtora (TAGG et al. 1976). Estes pesquisadores as caracterizam como substâncias de estreito espectro de atividade, possuidoras de uma fração protéica ativa, com atividade bactericida, com mecanismo de ação ocorrendo pela ligação a receptores específicos na parede celular das células sensíveis.

Há uma diversidade de outras substâncias com atividade antimicrobiana que não, necessariamente, apresentam todas estas características. O termo semelhante à bacteriocina (*bacteriocin-like*) engloba os compostos antimicrobianos de natureza protêica que ainda não estão completamente definidos ou não possuem todas as características de bacteriocinas. Estas substâncias, geralmente, possuem um espectro de ação maior, atuando contra uma variedade de bactérias Gram-positivas, Gram-negativas e contra alguns fungos (DE VUYST e VANDAMME, 1994).

As bacteriocinas são classificadas em lantibióticos (classe I) e não lantibióticos (classe II). Na classe dos lantibióticos, os peptídeos são pequenos (< 5 kDa), possuem de 19 a 50 aminoácidos, apresentam alguns aminoácidos pouco comuns como a lantionina, β-metil-lantionina e deidroalanina, que se formam devido a modificações posteriores ao processo de tradução. Esta classe é subdividida em Classe Ia e Classe Ib.

A Classe Ia, que inclui a nisina, consiste de peptídeos hidrofóbicos e catiônicos que formam poros na membrana da célula alvo e possuem uma estrutura flexível, quando comparados com uma estrutura mais rígida dos peptídeos da Classe Ib. A classe 1b de bacteriocinas possui peptídeos globulares que podem ter carga negativa ou que não possuem carga (ALTENA et al. 2000).

A Classe II é caracterizada pelas bacteriocinas de peso molecular variado, que contém aminoácidos regulares não modificados, estáveis ao calor, e também pode ser subdividida em três classes. A Classe IIa inclui os peptídeos como a pediocina, ativos contra *Listeria*. A Classe IIb, é constituída das bacteriocinas compostas de 2 peptídeos diferentes, onde ela necessita de ambos para ser totalmente ativa. A Classe IIc é proposta para separar as bacteriocinas secretadas pelo sistema *sec*-dependente (NES et al. 1996).

Como alternativa para substituição, ao menos parcialmente, dos agentes químicos, a introdução de bacteriocinas com o objetivo de conservar o alimento e, portanto, agregar valor nutricional e econômico, vem sendo hoje utilizada como uma técnica para proporcionar melhora na qualidade dos alimentos (MARTINEZ-GONZÁLEZ et al. 2003). Filmes comestíveis para a conservação de produtos minimamente processados, submetidos ao armazenamento sob refrigeração são um dos métodos mais efetivos de manutenção da qualidade destes alimentos, e contribuem para estender a sua vida de prateleira (LI e BARTH, 1998; COELHO et al. 2003 e LI et al. 2006). Eles podem ser usados como um veículo para a incorporação de ingredientes funcionais, tais como: antioxidantes, pigmentos, sabores, agentes antimicrobianos e nutracêuticos (DIAB et al. 2001).

O uso de filmes contendo agentes antimicrobianos apresenta vantagens sobre os métodos tradicionais de adição direta dos conservantes nos alimentos, visto que podem ser liberados de maneira controlada, estando, portanto, em menores quantidades no alimento, e atuando principalmente na superfície do produto. Também pode ocorrer inibição ou redução da atividade do antimicrobiano, quando adicionado de forma tradicional, por diversas substâncias do próprio alimento (QUINTAVALLA e VICINI, 2002).

2.2.1 - Nisina

A nisina é uma bacteriocina produzida a partir de uma linhagem de *Lactococcus lactis*, que possui um potencial de aplicação prática em alimentos (Figura 2). É uma substância considerada GRAS (*Generally Regarded as Safe*) e sua utilização está aprovada pelo *Food and Drugs Administration* (FDA), (APHA, 1992; CODEX ALIMENTARIS, 1995); sendo a primeira bacteriocina permitida para a aplicação em alimentos. É usada por mais de 50 anos e em mais de 40 países como antimicrobiano de uso alimentar (CLEVELAND et al. 2001). A nisina tem atividade antimicrobiana contra um amplo espectro de bactérias Gram-positivas, e tem sido usada na indústria de alimentos como conservante seguro e natural (O'SULLIVAN et al. 2002).

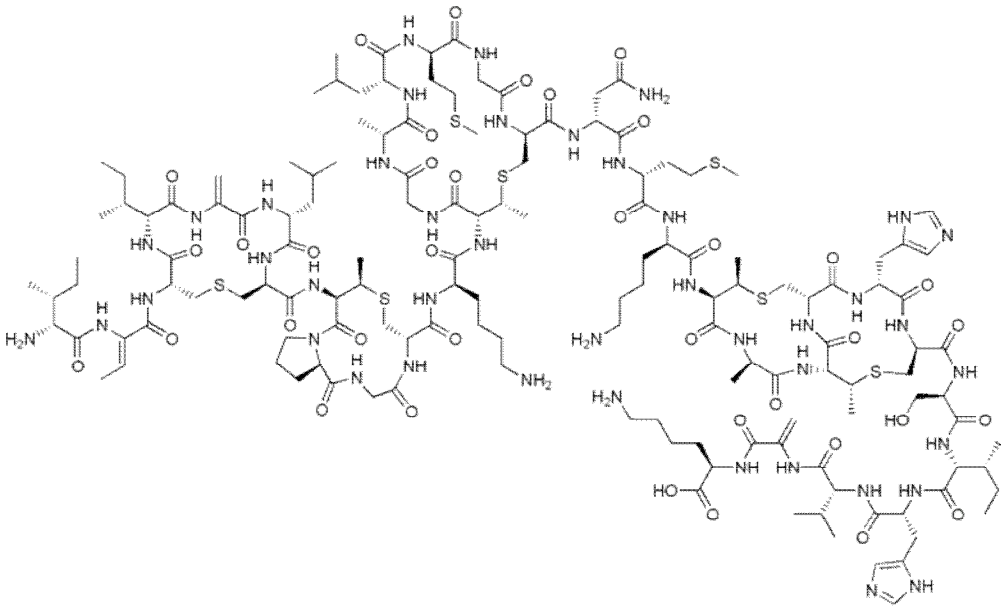


Figura 2. Estrutura química da nisina

Estudos de toxicidade aguda e crônica, bem como, sensibilidade e reprodução, *in vitro* e estudos de resistência cruzada, mostraram que a nisina é segura para o consumo humano a um valor de 2,9 mg/pessoa/dia de acordo com o Consumo Diário Aceitável (ADI) (U.S. Food and Drug Administration, 1988). É importante ressaltar que para queijos e produtos lácteos a quantidade de nisina permitida corresponde a 12,5 mg/Kg de peso do alimento, de acordo com a Portaria DETEN/MS nº 29, de 22 de janeiro de 1996.

A presença da nisina em filmes comestíveis à base de quitosana mostrou inibição de *L. monocytogenes* (PRANOTO et al. 2005). Os mesmos autores encontraram uma melhora no potencial antimicrobiano do filme de quitosana quando incorporado o óleo de alho como agente antimicrobiano. Este, além de melhorar a eficácia antimicrobiana, apresentou pouco efeito nas propriedades físicas e mecânicas do filme de quitosana, porém a aplicação deste agente em filmes interfere no sabor do alimento.

De acordo com Sanjurjo et al. (2006) a presença de nisina em filme comestível formulado com amido de tapioca e glicerol, reduziu o crescimento de *L. innocua*, produzindo um decréscimo de sua contagem e atuando como uma barreira contra a contaminação após o processamento. Estes autores também concluíram que a liberação gradual da substância é mais eficiente quanto à atividade antimicrobiana, do que a nisina empregada diretamente no meio.

2.2.2 - Aplicação de bacteriocinas em alimentos

Bacteriocinas são usadas para melhorar a segurança dos alimentos. Entre as bactérias Gram-positivas, as ácido lácticas tem sido amplamente exploradas por serem produtoras de peptídeos antimicrobianos com aplicação em alimentos. As bactérias ácido lácticas, em especial, o *Lactobacillus* spp. tem uma melhor perspectiva de utilização na fabricação de queijo de coalho, visando melhorar a qualidade sanitária do produto, pois apresentam melhor atividade antagonista frente a microrganismos patogênicos de relevância nesse alimento (NETO et al. 2005).

As bactérias ácido lácticas são as mais utilizadas em fermentações bacterianas de alimentos para consumo humano. Além de proporcionar sabor, textura e incremento no valor nutricional dos alimentos, são utilizadas na indústria como bioconservantes, podendo contribuir na prevenção da proliferação de microrganismos patogênicos e deteriorantes (REID, 1999; MARTÍNEZ-GONZÁLEZ et al. 2003).

A nisina foi a primeira bacteriocina a ser isolada e aprovada para uso em alimentos, especialmente para prevenir a germinação de esporos de *Clostridium botulinum* em queijos. Em 1988 teve seu uso aprovado para outros alimentos, sendo hoje um produto comercial amplamente aplicado (CHUNG et al. 1989).

As bacteriocinas têm sido adicionadas diretamente no queijo para a prevenção de *Clostridium* e *Listeria*. Nisina inibe os esporos de *C. botulinum* de queijo em pasta

(WESSELS et al. 1998). Quando nisina e pediocina foram fixadas em embalagem de celulose, elas inibiram totalmente *Listeria monocytogenes* em presunto, peito de peru e carne crua (QUINTAVALLA e VICINI, 2002).

DAVIES et al. (1999) examinaram a influência do conteúdo graxo e da emulsão de fosfato na efetividade da nisina em lingüiça e encontraram que o menor conteúdo graxo está relacionado com a maior atividade da nisina no sistema. Portanto, alguns pesquisadores concluem que a nisina não é efetiva na aplicação em carnes, devido seu pH elevado, baixa estabilidade, inabilidade para distribuir a nisina uniformemente e interferência por componentes da carne, tais como fosfolipídeos (DE VUYST e VANDAMME, 1994).

De acordo com BROMBERG et al. (2006) as bactérias lácticas, originalmente isoladas de produtos cárneos, são os microrganismos mais indicados para serem utilizados na intensificação da segurança microbiológica destes alimentos. Neste sentido, estes autores isolaram linhagens de bactérias lácticas produtoras de bacteriocinas em carne e seus derivados, resultando na detecção de *Lactococcus lactis* ssp. *hordniae* CTC 484, proveniente no frango. A bacteriocina inibiu não apenas outra bactéria láctica (*Lactobacillus helveticus*), mas também microrganismos patogênicos, tais como: *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens* e *Enterococcus faecalis*. Esta bacteriocina mostrou-se termoestável, mesmo à temperatura de autoclave, sendo produzida em condições de armazenamento sob refrigeração e permanecendo ativa dentro de uma faixa de pH de 2 a 10. Estes resultados indicaram que o tratamento da carne por meio da inoculação desta bactéria, contribuiu para o aumento da segurança e extensão da vida útil deste alimento.

Cenouras frescas minimamente processadas vendidas comercialmente, apresentam-se susceptíveis ao apodrecimento leve por ação de *Pseudomonas*. O uso de ácido etilenodiamino tetra-acético (EDTA), calor e nisina foram testados neste tecido, sendo que os resultados mostraram uma significativa redução atribuída ao tratamento com calor, mas não exclusivamente ao EDTA e a nisina. EDTA mais nisina a 37ºC reduziram as unidades formadoras de colônias (UFC/ml) de *E. carotovora, E. chrysanthemi, P. fluorescens* e *P. viridiflava* por 2 unidades logarítmicas (log), e a 49ºC por 3 unidades logarítmicas, quando comparadas com o tratamento a 25ºC (WELLS et al. 1998).

GRISI e LIRA (2006) avaliaram o potencial de inibição da nisina e o pH elevado em relação à multiplicação de *Staphylococcus aureus* e *Salmonella* sp. em culturas puras e inoculadas na carne de caranguejo-uça. Nas culturas puras, a multiplicação de *S. aureus*

foi fortemente inibida por nisina e a *Salmonella* sp. por nisina + EDTA (20mM). O pH elevado mostrou-se efetivo na inibição da multiplicação de *S. aureus* e *Salmonella* sp., porém nisina mais pH elevado empregados na carne contaminada, não obtiveram o mesmo efeito. Todavia o resultado encontrado sugere que o pH elevado apresenta um potencial como agente antibacteriano, podendo ser útil na preservação química da carne de caranguejo.

2.3 - Natamicina

A natamicina (Figura 3) é um macrolídeo antifúngico produzido por *Streptomyces natalensis* e outros *Streptomyces* spp relacionados. A natamicina mostra atividade contra um amplo espectro de fungos e leveduras, mas não apresenta efetividade contra bactéria e também não causa resistência **(**WELSCHER et al. 2007**)**. É usada como conservante em alimentos com fermentação bacteriana, tais como queijos e lingüiças, prevenindo o crescimento de bolores, mas não afetando a fermentação bacteriana ou a maturação destes alimentos (DELVES-BROUGHTON et al. 2006). É um dos poucos antifúngicos reconhecidos pelo FDA como um aditivo alimentar e classificado como componente GRAS (Generally Regarded As Safe). A natamicina é amplamente utilizada na indústria de alimentos como um conservante alimentar natural para prevenção da contaminação de bolores em bebidas, queijos, frutas e outros alimentos não estéreis (por exemplo, carnes curadas e lingüiças) (CHEN et al. 2008).

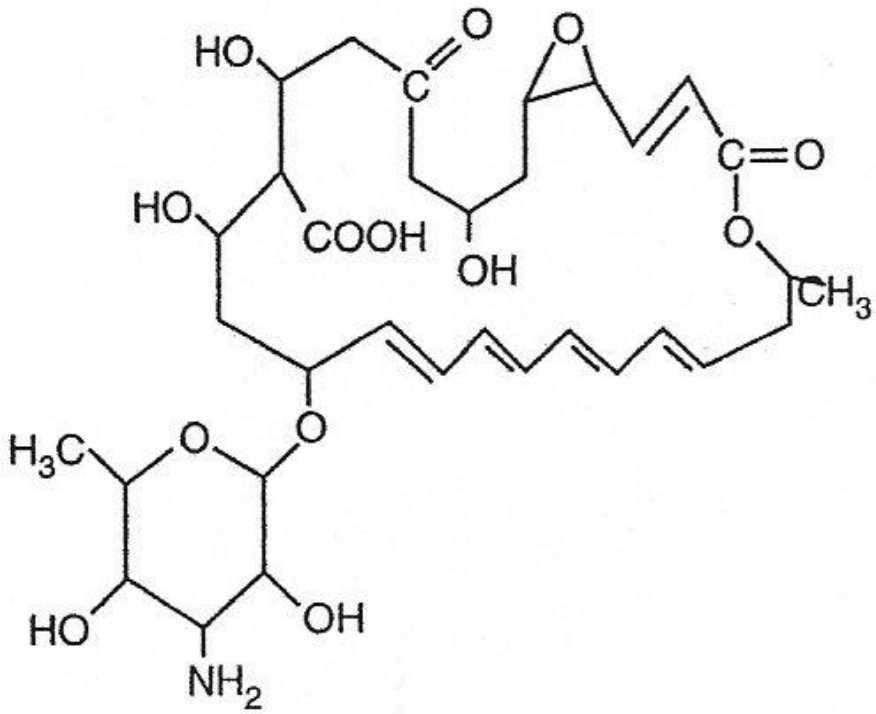


Figura 3. Estrutura química da natamicina

De acordo com o comitê de especialistas em aditivos alimentares da FAO/WHO a quantidade máxima diária permitida de natamicina é de 0,3 mg/Kg. O seu uso está permitido apenas em alimentos cárneos e queijos (VAR et al. 2006). Estes autores estudaram o efeito antimicrobiano da natamicina juntamente com o material de empacotamento (PVC - policloreto de vinila) nas propriedades microbiológicas de queijo kashar durante o período de maturação. Os autores relatam que a natamicina foi capaz de prevenir o crescimento de mofos em queijos maturados por 2 meses e por esta razão, recomendam que a reaplicação de natamicina seja feita após 6 semanas para estender a vida de queijos maturados.

OLIVEIRA et al. (2007) desenvolveram e avaliaram um filme com natamicina incorporada para conservação de queijo Gorgonzola. Os filmes com 2 e 4% apresentaram resultados satisfatórios para inibição de fungos (*Penicillium roquefortii*) na superfície do queijo e ainda relataram que a quantidade de natamicina liberada para o queijo foi abaixo do que o permitido pela legislação.

TURE et al. (2008), testaram a atividade antifúngica de biopolímeros contendo natamicina e extrato de alecrim contra *Aspergillus niger* e *Penicillium roquefortii* usando o teste de difusão em discos em agar. A concentração inibitória mínima foi de 2 e 1 mg de natamicina por 10 g de solução de filme contra *Aspergillus niger* e *Penicillium roquefortii*, respectivamente. O extrato de alecrim sozinho não mostrou ação inibitória antifúngica, mas quando combinado com a natamicina atuou sinergicamente para prevenir o crescimento de *Aspergillus niger*.

A maioria dos estudos relata o uso de natamicina em queijo e produtos cárneos, já que ela é permitida para esta classe de alimentos. Mas observam-se alguns poucos trabalhos onde o antifúngico é usado em frutas, como é o caso do estudo onde ela é utilizada em cobertura de superfície para melhorar o armazenamento de melão Hami à temperatura ambiente. Neste caso, observou-se que o tratamento com natamicina foi eficaz para controlar o principal patógeno causador da podridão, o *Fusarium*, no melão Hami após a colheita. Também a cobertura de quitosana juntamente com a natamicina, estendeu a vida de prateleira por diminuição da perda de peso, por reduzir a perda na concentração de ácido ascórbico e aumentar o pH durante o armazenamento a temperatura ambiente (CONG et al. 2007).

3 – RESULTADOS E DISCUSSÃO

Os resultados obtidos neste trabalho estão apresentados na forma de dois artigos científicos a serem submetidos à publicação em periódicos especializados na área.

Artigo 1 - Evaluation of minimally processed kiwi fruit covered with chitosan films incorporating nisin and natamycin. Artigo a ser submetido ao periódico Journal of Food Processing and Preservation.

Artigo 2 - Evaluation of minimally processed strawberry fruit covered with chitosan films incorporating nisin and natamycin. Artigo a ser submetido ao periódico Food Microbiology.

3. 1 - Artigo 1

Formatado para Journal of Food Processing and Preservation

EVALUATION OF MINIMALLY PROCESSED KIWIFRUIT COVERED WITH CHITOSAN FILMS INCORPORATING NISIN AND NATAMYCIN

Nísia Cé [a], Caciano P.Z. Noreña[a], Adriano Brandelli[a]*

[a]Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil

* Corresponding author: ICTA – UFRGS, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brazil; fax: +5551 3308 7048; e-mail: abrand@ufrgs.br

Running title: Edible chitosan films for minimally processed kiwis

ABSTRACT

Chitosan coatings were used aiming to extend the shelf-life of minimally processed kiwis (*Actinidia chinenesis* Planch). Fresh-cut fruits were covered with three types of chitosan-based films: 10 g/l chitosan, or 10 g/l chitosan incorporating either 12.5 mg/kg nisin or 0.1 g/l natamycin. Samples were stored for up to 14 days at 4 ± 2^{o}C. The microbiological analyses showed that the addition of nisin and natamycin were beneficial to increase the fruit quality, since a decrease in microbial counts was observed. The chitosan-based films do not induce significant changes in internal quality of minimally processed kiwi fruit during refrigerated storage. The physicochemical parameters soluble solids, vitamin C and pH were improved in samples receiving coverage with chitosan films.

PRACTICAL APPLICATIONS

The use of edible chitosan films incorporating antimicrobial agents represents an interesting alternative for preservation of minimally processed fruits. Products covered with these films would present enhanced physicochemical properties and microbiological quality, showing a potential to extend shelf-life.

INTRODUCTION

The kiwi fruit (*Actinidia chinenesis* Planch) is a climber plant from Chinese origin, which produces an oval fruit, with bittersweet pulp, bright green color, striking taste, rich in vitamins, minerals and fiber. The Hayward kiwi fruit is the most used cultivar in kiwi-producing countries, and its fruits are densely covered with fine and silky brown hair (Carvalho and Lima 2002). Although Brazil is the third world pole in fruit culture, with an annual production around 38 million tons, the cultivation of kiwi is relatively recent in temperate regions of this country (Heiffig *et al*. 2006).

The demand for healthy foods has increased every day. Minimally processed fruits and vegetables keep the quality of fresh product and facilitate consumption, which it is the biggest advantage. However, the operations for the preparation of minimally processed fruits produce a physiological impact as big as the processing level. This increases deterioration rates and decreases the shelf life for the whole product (Heiffig *et al.* 2006). Refrigeration is considered the most effective way to extend the shelf life of minimally processed fruits and vegetables (Ragaert *et al.* 2007). Cooling temperatures contribute to reduce the microbial activity and chemical changes. This maintains the quality of the product and increases the safety for the consumer (Brecht *et al*. 2003). In this context, edible films are an emerging application in minimally processed fruits, since they often work as natural preservatives, also maintaining the quality and increasing useful life of these foods.

Chitosan is a high molecular weight cationic polysaccharide, which has been used in preventive fruit coverage (Cong *et al*. 2007). Due to its harmless characteristics and easy formation of gels, chitosan has been considered for decades as a compound of great industrial interest (Campana Son and Desbrières 2000). Moreover, it has antibacterial and

antifungal activities, showing potential use in cut surfaces or in fruits that have high ripening rates after harvest (Ghaouth *et al.* 1991; No *et al.* 2002; Zhang and Quantick 1998). Particurly on kiwi, chitosan reduces the respiratory rates, decreasing the postharvest deterioration (Du *et al.* 1997).

Some authors have described the use of chitosan films incorporating antimicrobial agents in fruits (Park *et al*. 2004). Nisin, a bacteriocin produced by *Lactococcus lactis*, is considered GRAS (Generally Regarded as Safe) and its food use is approved by the Food and Drug Administration (FDA) (O'Sullivan *et al.* 2002). This bacteriocin has been used in many countries for diverse food application over the last 40 years. Nisin has bacteriostatic and/or bactericidal activity against a broad range of Gram positive bacteria, being used in the food industry as a safe and natural preservative (Deegan *et al.* 2006).

Natamycin is an antifungal agent produced by *Streptomyces natalensis* and other related *Streptomyces* species. This substance is used as a preservative in foods which undergoe bacterial fermentation, such as cheese and sausages, preventing the development of yeasts and molds (Delves-Broughton *et al.* 2006). Natamycin is among a few antifungal agents recognized by the FDA as a food additive with the GRAS status. Natamycin has been showed to prevent the growth of molds in cheese, beverages, fruits and other non-sterile foods (Chen *et al*. 2008), although its use is only allowed in cheese and meat products (Var *et al*. 2006).

Considering the properties of chitosan, nisin and natamycin as natural preservatives, their utilization in edible coatings or films may be advantageous to minimally processed fruits. This study intended to evaluate physicochemical and microbiological parameters of minimally processed kiwi fruits covered with chitosan films incorporating nisin and natamycin and stored under refrigeration temperature.

MATERIALS AND METHODS

Materials

Kiwi fruits, cv. Hayward, were acquired at the CEASA (Porto Alegre, Brazil). The fruits were transported to the laboratory of Engenharia de Processos em Alimentos of the Federal University of Rio Grande do Sul, where they were selected for maturity stage, similarity, and free from defects and presence of pathogens.

Chitosan films

The solutions for the different treatments were prepared by adding 1 g of chitosan (Sigma-Aldrich, minimum 85% deacetylated,) in 94 mL of distilled water with 5 mL of acetic acid (Merck) to entirely dissolve the chitosan. It was prepared a stock solution of nisin using 0.1 g Nisaplin (Danisco) dissolved in 1 mL 0.01 mol/L HCl. Nisin incorporation in the chitosan solution follows the allowed limit of 12.5 mg nisin per kg of food, according to the Brazilian legislation (ANVISA, 1996). The concentration of the natamycin (Danisco) was 0.1 g/L, dissolved in distilled water. The pH of the solutions was adjusted to pH 5 with 1 mol/L NaOH (Chien *et al.* 2007a,b).

Fruit Processing

Fruits were washed and peeled manually; each fruit was cut into approximately 1 cm thick slices. The pieces were divided into four different vessels, which received the following treatments: none (*in natura* – control), 10 g/L chitosan, 10 g/L chitosan + nisin,

and 10 g/L chitosan + natamycin. Fruit pieces were immersed for 1 min in each corresponding solution. The processing steps were carried out at room temperature and they were conducted under good manufacturer practices. Afterwards, the pieces were drained and stored under refrigeration temperature (4 ± 2ºC) in plastic pots covered with PVC films. Samples were submitted to physical, chemical and microbiological analysis at three different times: 1 (time 0), 7 and 14 days.

Microbiological analyses

The microbiological characteristics of 25g samples were determined from the homogenization in 225 mL of 1 g/L peptone water. Decimal dilutions were prepared from the initial homogenate. The total counts were determined on Plate Count Agar (PDA). The plates were incubated at 35^{o}C for 48 hours. Yeast and molds were determined on Potato Agar medium, after incubation at 28ºC for 4 days. Total and fecal coliforms were analyzed by the technique of multiple tubes, where the temperatures and times of incubation are respectively 37^{o}C, 45^{o}C and 48^{o}C for 24 hours. The results were expressed as the most probable number. Lactic acid bacteria were determined using the medium de Man Rogosa Sharpe (MRS), incubated at 35ºC for 48 hours (Pittia *et al*. 1999). Two samples of each storage time and treatment were analyzed.

Determination of weight loss and moisture content

Twenty grams of fruit were removed from each treatment at time 0, weighed and stored in individual packages. At the 7th and 14th days samples were retrieved from storage and weighed again to determine the fresh weight losses expressed as a percentage. The

moisture content was measured after constant weight in a stove at 65ºC (method No. 925.45, AOAC 1990).

Soluble solids, ascorbic acid, pH and water activity

The soluble solids were determined with a hand refractrometer (ATAGO, model NI, Japan), with results expressed in ^{o}Brix, according to ISO 2173 (ISO 1979). Ascorbic acid was measured using the dichloroindophenol method (method No. 967.21, AOAC 1990). The pH was determined using a pH-meter, according to ISO 1842 (ISO 1991). The water activity was measured in an AQUALAB analyzer, model 3TE (Method No. 978.18, AOAC 1990).

Statistical analysis

The experiment was planned as a 3x4 factorial and conducted in a completely randomized design. The levels of the first factor (time) were: 1, 7 and 14 days. The levels for the second factor were: chitosan, chitosan + nisin, chitosan + natamycin; and control. Each treatment, was performed in triplicate (n=3). The results were expressed as mean ± standard deviation. The statistical comparison was performed by two-way analysis of variance (ANOVA). The criterion for the decision of significance in each of the sources of variability was $P<0.05$. Averages were compared by Tukey's test. Data were analyzed by the software SAS 6.08 (SAS Institute).

RESULTS AND DISCUSSION

Microbiological analyses

The microbiological analyses indicate the absence of total and fecal coliforms during the storage period at refrigeration temperature, independently of treatments. This result is in agreement to that described for minimally processed kiwi treated with organic acids (Carvalho and Lima 2002).

For total counts, the values for control and chitosan coating at day 7 were 1.61 log CFU/g and 1.32 log CFU/g, respectively (Table 1). At this time, the addition of nisin or natamycin showed no synergistic effect with chitosan. However, after 14 days of storage both treatments chitosan + nisin and chitosan + natamycin showed significantly lower counts in comparison with the film with chitosan alone and the control (Table 1).

Chitosan can prevent the development of bacteria, yeast and molds when applied to some food (Park *et al.* 2004; Devlieghere *et al.* 2004). This polysaccharide has low solubility in water, being solubilized in acid media, and thus, it should be effective in acidic systems only (Han *et al.* 2005; Chi *et al.* 2006). This property is adequate in the case of kiwi, which has naturally an acid pH about 3.3, and the acid pH coating would constitute an excellent barrier against microbial development. Nisin is a broad range bacteriocin that has maximum solubility in acid media, and thus may show a synergistic effect with chitosan in fruits (Pranoto *et al.* 2005; Li *et al*. 2006).

Chitosan coatings were tested on sliced mango during 7 days at 6^{o}C (Chien *et al*. 2007b). The total counts for control and 10 g/L chitosan coating were 6.41 and 5.30 log CFU/g, respectively. Despite the difference in the storage temperature, those values were higher than found in the present work. This could be associated with the acidity of kiwi, which contribute to the efficacy of chitosan.

Reduced fungal growth was observed for the films of chitosan + nisin and chitosan + natamycin when compared with the control and chitosan treatment at the first day (Table 1). At the seventh day, it was noteworthy that the treatment with chitosan alone results the highest growth of yeasts and molds. At the end of the storage period, all coating treatments reduced fungal contamination, especially for chitosan + natamycin which showed the lowest value. The greater inhibitory effect observed with natamycin-containing coatings may be associated to a synergism with chitosan, which also contains antifungal properties. The mechanism of action of natamycin appears to be through binding of the molecule to the sterol moiety of the fungal cell membrane. Natamycin is an antifungal agent with maximum activity at pH 5-7, with established efficiency at lower quantity, may be an economical form to prevent the yeast and mold development on food surfaces (Welschier *et al*. 2008).

With respect to the counts of lactic acid bacteria none of the treatments showed significant efficiency in the first and seventh day of storage when compared to the control. The treatments were effective only at the end of the storage period (Table 1). The chitosan + natamycin was the treatment that showed the largest decrease in the growth of lactic bacteria when compared to the control. This result is surprising, because it is believed that chitosan + nisin would be the most effective treatment in this context, due to the functionality and stability of this substance in acidified systems.

Similar results with respect to lactic acid bacteria were found in the study of O`Connor-Shaw *et al*. (1994), with minimally processed kiwifruit stored at 4ºC. The authors reported that at the end of the storage period the loss of fruits was not consequence of microbiological growth.

In Brazil, regulation for microbiological quality of whole fresh, refrigerated or frozen fruits, indicate a limit of 2.30 log CFU/g for fecal coliforms (ANVISA 2001). For

other microbial groups there is no regulation in the current legislation. However, there is a French recommendation (Ministere de L'Economie des Finances et du Budget 1998), for shelf-life of minimally processed fresh vegetables which is usually calculated as the time needed to reach a total count of bacteria of 7.69 log CFU/g. Under the experimental conditions of the present work this cell load was not achieved, so that the kiwifruit might be considered without risk of being contaminated for consumption up to 14 days of refrigerated storage.

Minimally processed products are exposed to many kinds of contamination, since the peel acts as a barrier to the penetration of microorganisms (Heiffig *et al*. 2006). Thus, in fruits and vegetables subject to processing conditions, processing must be extremely hygienic, taking the proper care in each stage. Therefore, the aseptic conditions together with the use natural of additives are enough to reduce the microbial population in minimally processed products (Pittia *et al*. 1999).

Water activity

The results for water activity obtained in this work at the fourteenth day of storage showed similar values for the control group when compared to other treatments in the same period (Table 2). The coating treatments were not effective to reduce the activity of water in kiwifruits. Fruits and vegetables have a water activity around 0.98, allowing the growth of many microorganisms (Nguyenthe Carlin 1994; Tapia de Daza *et al.* 1996).

Weight loss, moisture and soluble solids

In this study, the maximum fresh weight losses during storage of kiwifruit were observed for chitosan and chitosan + nisin treatments at the day 7 of storage (Fig. 1). The final weight loss was higher in the fruits covered with chitosan + nisin, while no significant differences were observed among chitosan, chitosan + natamycin and control groups. This indicates that chitosan coatings were inefficient for retention of fresh weight when compared to kiwifruit without coverage. The fresh weight loss in fruits might occur by the run off of juice from the pulp. The loss of water can be a major cause of deterioration in minimally processed food, because it results in losses in appearance (wilting), texture (softening) and nutritional quality (Pereira *et al.* 2003).

Waimaleongora-Ek *et al.* (2008) showed that chitosan coating promoted the smallest fresh weight loss in their study with sweet potatoes stored for 17 days under refrigeration temperature. Cong *et al.* (2007) indicated that the chitosan has hydrophilic nature and the PVC film is hydrophobic, together they promote formation of a barrier to moisture on the surface of the fruit. The use of films with or without antimicrobial agents, together with plastic packaging, promote gas exchanges between the environment and fruit, creating a modified atmosphere favoring the maintenance of fresh weight (Heiffig *et al.* 2006, Chien *et al.* 2007a,b).

Regarding the moisture, all treatments showed a significant decrease at the end of the storage period, excepting for chitosan + natamycin. However, the differences among the treatments were not significant (Table 3). The food composition table of Universidade de São Paulo (TACO, 2008), shows that the standard for kiwifruit humidity is 83.06%. The values obtained in the present study after 14 days of storage with minimally Hayward

kiwis are slightly below that standard. The reduction in humidity is confirmed by the decrease in weight of the fruit, showing that the film does not retain moisture loss.

Soluble solids increased significantly after 14 days of storage in the control and the treatment with chitosan + nisin (Table 4). However, the treatments with chitosan and natamycin + chitosan, showed a decrease in soluble solids during incubation. Decreases in soluble solids with the use of chitosan in different concentrations (0.5%, 1% or 2%) was also described for red pitayas and sliced mango (Chen *et al.* 2007a,b). This reduction in the sugar concentration was associated with the increase in respiratory rate of the fruits.

Respiration is an oxidative process that increases in injured or sliced tissues, such as minimal processing, where the exposed surface of the fruit is larger. The peeling and cutting done in minimally processed kiwifruit increase the respiratory rate, because physiological and biochemical reactions become more active in response to stress (Watada *et al*. 1990). Therefore, the physical action of minimal processing may induce the production of ethylene and also the increase in respiration, which quickly use the reserve substrates, thus reducing the levels of soluble solids in fruits (Ragaert *et al*. 2007).

Ascorbic acid and pH

The amount of ascorbic acid tends to decrease with storage time in all treatments, including the controls (Fig. 2). A significant decrease was observed for the treatments with chitosan + nisin and chitosan + natamycin at the day 7 when compared to the other treatments. The treatment with chitosan alone showed a minor loss of vitamin C; it shows to be significantly different when compared to the control and other treatments.

This work shows that the film with chitosan decreases the loss of ascorbic acid in minimally processed kiwi. This may be due to the coverage of chitosan is dense (Han *et al.* 2005) together with the PVC, they form a barrier to O_2 entry and, consequently,

avoiding the acceleration on vitamin C oxidation. However, the reason why the coatings containing nisin and natamycin caused higher losses of vitamin C remains to be elucidated.

Vitamin C is one of the components that determine the nutritional quality of fruits. The amount of ascorbic acid in peeled kiwifruit is set at 75 mg% by the USDA (2008) or 70.8 mg% by USP (2008). The amount measured in the present work in the control group was of 55.4 mg% on the first day of storage. At the end of storage in the same group and the treatment with chitosan, the values were 41.3 mg% and 48.0 mg%, respectively. Perhaps this difference of values found when compared to the values of reference standard is explained by the difference in the initial concentration of ascorbic acid of samples, and also, as the variability of vitamin C among cultivars, the climate, and the place where the fruit is grown.

There was an increase in pH in the treatment with chitosan and chitosan + natamycin during 14 days of storage (Table 5). This result agrees with Ghaouth *et al.* (1991) and Garcia *et al.* (1998), which showed the increase of pH during storage of strawberries coated with edible films. Han *et al*. (2004) used of chitosan films (chitosan, chitosan + 5% Gluconal® and tocopherol acetate) to increase shelf life and nutritional value of strawberry and raspberry. The authors showed that the pH of the fruit increased significantly during cold storage for 14 days. These results agree with the tendency of organic acids to decrease during ripening, therefore the pH of the fruit should increase.

In controls and also in the treatment with chitosan + nisin, there was a reduction of pH values at the end of storage. Control fruits presented highest counts of lactic acid bacteria, which can explain this decrease in pH, while the decrease in fruits covered with chitosan + nisin remains to be elucidated. Nisin is more effective in acidic pH, either in solution or incorporated in edible films (Sanjurjo *et al*. 2006). Thus, the maintenance of a

more acidic pH would be favorable to enhanced nisin activity. Nisin targets cell membranes and has been associated with downregulation of the proton-translocating subunit of the F_0F_1 ATPase during acid tolerance response in prokaryotes (Bonnet *et al*. 2007). Although higher eukaryotic cells are largely resistant to antimicrobial peptides, vegetable cell membranes are altered during senescence and cold storage (Marangoni et al. 1996; Lurie and Crisosto 2005). Nisin could interact with such altered membranes resulting in unknown physiological effects.

CONCLUSIONS

Minimally processed kiwi showed low or absent microbial contamination under adequate hygienic conditions and refrigeration temperature. The coating films based on the chitosan with the addition of nisin and natamycin were useful for protection of minimally processed kiwi against bacterial and fungal infections. For total counts, the chitosan and chitosan + nisin films are the most effective, and chitosan + natamycin produces significant reduction in yeasts and molds, and lactic acid bacteria for up to 14 days storage.

The chitosan-based films caused some changes in physicochemical quality of kiwifruit during cold storage. Decreased values for soluble solids and increased pH were observed when coatings were applied to the kiwi slices. A film composed of chitosan + natamycin + nisin could maintain the microbiological standards and nutritional value of minimally processed kiwifruit during 14 days at refrigeration temperatures.

ACKNOWLEDGMENTS

Authors thank the technical support of Roberval Bittencourt de Souza in physicochemical analyses. This work received financial support of CNPq, Brazil.

REFERENCES

ANVISA. 1996. *Portaria DETEN/MS nº 29*. Agência Nacional de Vigilância Sanitária, Brasília, Brazil. Available at: http://www.anvisa.gov.br/legis/portarias/29_96.htm.

ANVISA. 2001. *Resolução RDC nº 12*. Agência Nacional de Vigilância Sanitária, Brasília, Brazil. Available at: http://e-legis.anvisa.gov.br/leisref/public/coliformes.

AOAC. 1990. *Official methods of analysis*, 15th Ed. Association of Official Analytical Chemists, Washington, DC.

BONNET, M., RAFI, M.M., CHIKINDAS, M.L. and MONTVILLE, T.J. 2006. Bioenergetic mechanism for nisin resistance, induced by the acid tolerance response of *Listeria monocytogenes*. Appl. Environ. Microbiol. *72*, 2556-2563.

BRECHT, J.K., CHAU, K.V., FONSECA, S.C. 2003. Maintaining optimal atmosphere conditions for fruit and vegetables throughout the postharvest handling chain. Postharvest Biol. Technol. *27*, 87-101.

CAMPANA SON, S.P. and DESBRIÈRES, J. 1998. Chitin, chitosan and derivatives. Crit. Rev. Food Sci. Nutr. *38*, 299-313.

CARVALHO, A.V. and LIMA, L.C.O. 2002. Qualidade de kiwis minimamente processados e submetidos a tratamento com ácido ascórbico, ácido cítrico e cloreto de cálcio. Pesq. Agrop. Bras. *37*, 679-685.

CHI, S., ZIVANOVIC, S. and PENFIELD, M.P. 2006. Application of chitosan films enriched with oregano essential oil on bologna – active compounds and sensory attributes. Food Sci. Technol. Int. *12*, 111-117.

CHEN, G.Q., LU, F.P. and DU, L.X. 2008. Natamycin production by *Streptomyces gilvosporeus* based on statistical optimization. J. Agric. Food Chem. *13*, 5057–5061.

CHIEN, P.J., SHEU, F. and LIN, H.R. 2007a. Quality assessment of low molecular weight chitosan coating on sliced red pitayas. J. Food Eng. *79*, 736-740.

CHIEN, P.J., SHEU, F. and YANG, F.H. 2007b. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. J. Food Eng. *78*, 225-229.

CONG, F., ZHANG, Y. and DONG, W. 2007. Use of surface coating with natamycin to improve the storability of Hami melon at ambient temperature. Postharvest Biol. Technol. *46*, 71-75.

DEEGAN, L.H., COTTER, P.D., HILL, C. and ROSS, P. 2006. Bacteriocins: useful tools for bio-preservation and shelf-life extension. Int. Dairy J. *16*, 1058-1071.

DEVLIEGHERE, F., VERMEULEN, A. and DEBEVERE, J. 2004. Chitosan: antimicrobial activity, interaction with food components and applicability as a coating on fruit and vegetables. Food Microbiol. *21*, 703-714.

DELVES-BROUGHTON, J., THOMAS, L.V. and WILLIAMS, G. 2006. Natamycin as an antimycotic preservative on cheese and fermented sausages. Food Australia *58*, 19-21.

DU, J., GEMMA, H. and IWAHORI, S. 1997. Effects of chitosan coating on the storage of peach, Japanese pear, and kiwifruit**.** J. Jap. Soc. Hort. Sci. *66*, 15-22.

GARCIA, M.A., MARTINO, M.N. and ZARITZKY, N.E. 1998. Plasticized starch-based coatings to improve strawberry (*Fragaria ananassa*) quality and stability. J. Agric. Food Chem. *46*, 3758-3767.

GHAOUTH, A.E., ARUL, J., PONNAMPALAM, R. and BOULET, M. 1991. Chitosan coating effect on storability and quality of fresh strawberries. J. Food Sci. *56*, 1618-1620.

HAN, C., ZHAO, Y., LEONARD, S.W. and TRABER, M.G. 2004. Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries

(*Fragaria* x *ananassa*) and raspberries (*Rubus ideaus*). Postharvest Biol. Technol. *33*, 67-78.

HAN, C., LEDERER, C., MCDANIEL, M. and ZHAO, Y. 2005. Sensory evaluation of fresh strawberries (*Fragaria ananassa*) coated with chitosan-based edible coatings. J. Food Sci. *70*, S172-S178.

HEIFFIG, L.S., AGUILA, J.S. and KLUGE, R.A. 2006. Caracterização físico-química e sensorial de frutos de kiwi minimamente processado armazenado sob refrigeração. Rev. Iber. Tecnol. Postcosecha *8*, 26-32.

ISO. 1979. *ISO 2173: fruit and vegetable products: determination of soluble solids content: refractometric method*. International Organization for Standardization, Gèneve, Switzerland.

ISO. 1991. *ISO 1842: fruit and vegetable products: determination of pH*, 2nd Ed. International Organization for Standardization, Gèneve, Switzerland.

LI, B., KENNEDY, J.F., PENG, J.L., YIE, X. and XIE, B.J. 2006. Preparation and performance evatuation of glucomannan-chitosan-nisin ternary antimicrobial blend film. Carbohydr. Polym. *65*, 488-494.

LURIE, S. and CRISOSTO, C.H. 2005. Chilling injury in peach and nectarine. Postharvest Biol. Technol. *37*, 195-208.

MARANGONI, A.G., PALMA, T. and STANLEY, D.W. 1996. Membrane effects in postharvest physiology. Postharvest Biol. Technol. *7*, 193-217.

MINISTERE DE l'ECONOMIE DES FINANCES ET DU BUDGET. 1998. Marché consommation, Produits vegetaux prets a lémploi dts de la "Ivemme Gamme": Guide de bonnes pratique hygieniques. J. Off. Rép. Française *1621*, 1-29.

NGUYENTHE, C. and CARLIN, F. 1994. Microbiology of minimally processed fruits and vegetables. Crit. Rev. Food Sci. Nutr. *34*, 371-401.

NO, H.K., PARK, N.Y., LEE, S.H. and MEYERS, S.P. 2002. Antibacterial activity of chitosan and chitosan oligomers with different molecular weights. Int. J. Food Microbiol. *74*, 65-72.

O'CONNOR-SHAW, R.E., ROBERTS, R., FORD, A.L. and NOTTINGHAM, S.M. 1994. Shelf life of minimally processed honeydew, kiwis, papaya, pineapple and cantaloupe. J. Food Sci. *59*, 1202-1206.

O'SULLIVAN, L., ROSS, R.P. and HILL, C. 2002. Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. Biochimie *84*, 593-604.

PARK, S.I., DAESCHEL, M.A., ZHAO, O.Y. 2004. Functional properties of antimicrobial lysozyme-chitosan composite films. J. Food Sci. *69*, 215-221.

PEREIRA, L.M., RODRIGUES, A.C.C., SARANTÓPOULOS, C.I.G.L., JUNQUEIRA, V.C.A, CARDELLO, H.M.A.B. and HUBINGER, M.D. 2003. Shelf life of minimally processed guavas stored in modified atmosphere packages. Ciênc. Tecnol. Aliment. *23*, 427-433.

PITTIA, P., NICOLI, M.C., COMI, G. and MASSINI, R. 1999. Shelf-life extension of fresh-like ready-to-use pear cubes**.** J. Sci. Food Agric. *79*, 955-960.

PRANOTO, Y., RAKSHIT, S.K. and SALOKHE, V.M. 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT Food Sci. Technol. *38*, 859-865.

RAGAERT, P., DEVLIEGHERE, F. and DEBEVERE, J. 2007. Role of microbiological and physiological spoilare mechanisms during storage of minimally processed vegetables. Postharvest Biol. Technol. 44, 185-194.

SANJURJO, K., FLORES, S., GERSCHENSON, L. and JAGUS, R. 2006. Study of the performance of nisin supported in edible films. Food Res. Int. *39*, 749-754.

USP. 2008. *Tabela Brasileira de Composição dos Alimentos*, Versão 5. Universidade de São Paulo, São Paulo, Brazil. Available at: http://www.fcf.usp.br/tabela/.

TAPIA DE DAZA, M.S., ALZAMORA, S.M. and WELTI-CHANES, J. 1996. Combination of preservation factors applied to minimal processing of food. Crit. Rev. Food Sci. Nutr. *36*, 629-659.

USDA. 2008. *USDA National nutrient database for standard reference*. United States Department of Agriculture, Washington, DC. Available at: http://www.nal.usda.gov/ fnic/foodcomp/.

VAR, I., ERGINKAYA, Z., GUVEN, M. and KABAK, B. 2006. Effect of antifungal agent and packaging material on microflora of Kashar cheese during storage period. Food Control *17*, 132-136.

WAIMALEONGORA-EK, P., CORREDOR, A.J.H., NO, H.K., PRINYAWIWATKUL, W., KING, J.M., JANES, M.E. and SATHIVEL, S. 2008. Selected quality characteristics of fresh out sweet potatoes coated with chitosan during 17 day refrigerated storage. J. Food Sci. *73*, S418-S423.

WATADA, A.E., ABE, K. and YAMUCHI, N. 1990. Physiological activities of partially processed fruits and vegetables. Food Technol. *44*, 116-122.

WELSCHER Y.M., NAPEL H.H., BALAGUÉ, M.M., SOUZA, C., RIEZMAN H., KRUIJFF, B. and BREUKINK, E. 2008. Natamycin blocks fungal growth by binding specifically to ergosterol without permeabilizing the membrane. J. Biol. Chem. *283*, 6393-6401.

ZHANG, D. and QUANTICK, P., 1998. Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. J. Hortic. Sci. Biotechnol. *73*, 763-767.

TABLE 1.

MICROBIOLOGICAL ANALYSIS OF MINIMALLY PROCESSED KIWIFRUIT TREATED WITH CHITOSAN FILMS.*

	Log CFU/g			
Days	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
Total	*counts*			
1	$0 \pm 0^{a(C)}$	$0 \pm 0^{a(C)}$	$0 \pm 0^{a(C)}$	$0 \pm 0^{a(B)}$
7	$1.61 \pm 0^{c(B)}$	$1.32 \pm 0^{d(B)}$	$2.17 \pm 0^{a(A)}$	$1.70 \pm 0^{b(A)}$
14	$2.49 \pm 0{,}01^{a(A)}$	$2.46 \pm 0^{b(A)}$	$1.70 \pm 0^{c(B)}$	$1.70 \pm 0^{c(A)}$
Yeast	*and molds*			
1	$4.56 \pm 0.003^{a(B)}$	$4.54 \pm 0.003^{b(C)}$	$3.39 \pm 0.002^{c(B)}$	$3.39 \pm 0{,}002^{c(B)}$
7	$2.70 \pm 0.001^{b(C)}$	$5.17 \pm 0.004^{a(A)}$	$1.70 \pm 0^{b(C)}$	$2.32 \pm 0.62^{b(C)}$
14	$5.43 \pm 0.003^{a(A)}$	$4.92 \pm 0.002^{b(B)}$	$4.91 \pm 0.03^{b(A)}$	$4.81 \pm 0.002^{c(A)}$
Lactic	*acid bacteria*			
1	$1.70 \pm 0^{c(B)}$	$2.17 \pm 0.01^{b(C)}$	$2.39 \pm 0{,}01^{a(B)}$	$1.70 \pm 0^{c(C)}$
7	$1.70 \pm 0^{c(B)}$	$4.13 \pm 0.02^{a(A)}$	$1.70 \pm 0^{c(C)}$	$2.32 \pm 0.01^{b(B)}$
14	$4.38 \pm 0.02^{a(A)}$	$3.93 \pm 0.02^{b(B)}$	$3.85 \pm 0.02^{c(A)}$	$3.50 \pm 0.02^{d(A)}$

*Total and fecal coliforms were not detected during the storage period. $^{(A)}$ Different letters indicate significant differences within the same column. ^{a} Different letters indicate significant differences within the same row ($P < 0.05$).

TABLE 2.

WATER ACTIVITY OF MINIMALLY PROCESSED KIWIFRUIT TREATED WITH CHITOSAN FILMS.

	a_w			
Days	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
1	0.987 ± 0.001 $^{a(A)}$	0.981 ± 0.001 $^{c(A)}$	0.980 ± 0.001 $^{c(B)}$	0.984 ± 0.001 $^{b(B)}$
7	0.976 ± 0.001 $^{b(B)}$	0.979 ± 0.001 $^{a(A)}$	0.978 ± 0.001 $^{a,b(B)}$	0.979 ± 0.001 $^{a(C)}$
14	0.988 ± 0.001 $^{a(A)}$	0.981 ± 0.001 $^{c(A)}$	0.985 ± 0.001 $^{b(A)}$	0.987 ± 0.001 $^{a,b(A)}$

$^{(A)}$ Different letters indicate significant differences within the same column. ^{a} Different letters indicate significant differences within the same row. ($P< 0.05$).

TABLE 3.

MOISTURE LOSS OF MINIMALLY PROCESSED KIWIFRUIT TREATED WITH CHITOSAN FILMS.

Days	**Moisture (%, wb)**			
	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
1	84.0 ± 0.4 $^{a(A,B)}$	83.7 ± 0.3$^{a,b,c(A)}$	84.1 ± 0.4 $^{a,b(A)}$	82.3 ± 1.2 $^{c(A,B)}$
7	83.4 ± 0.03 $^{a(B)}$	83.6 ± 0.4 $^{a,b(A)}$	83.7 ± 0.3 $^{b(A)}$	82.6 ± 0.5 $^{c(A)}$
14	81.3 ± 1.7 $^{a(C)}$	80.9 ± 1.7 $^{a(B)}$	81.4 ± 0.3 $^{a(B)}$	80.8 ± 0.6 $^{a(B)}$

$^{(A)}$ Different letters indicate significant differences within the same column. ^{a} Different letters indicate significant differences within the same row. ($P< 0.05$).

TABLE 4.

SOLUBLE SOLIDS OF MINIMALLY PROCESSED KIWIFRUIT TREATED WITH CHITOSAN FILMS.

Days	**Soluble solids (°Brix)**			
	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
1	15.6 ± 0.01 $^{b(B)}$	14.6 ± 0.14 $^{c(A)}$	14.0 ± 0.14 $^{d(B)}$	17.8 ± 0.14 $^{a(A)}$
7	14.0 ± 0.14 $^{b(C)}$	14.2 ± 0.14 $^{b(A,B)}$	14.1 ± 0.14 $^{b(B)}$	15.9 ± 0.14 $^{a(B)}$
14	17.2 ± 0.14 $^{a(A)}$	14.1 ± 0.14 $^{d(B)}$	16.0 ± 0.14 $^{b(A)}$	15.0 ± 0.14 $^{c(C)}$

$^{(A)}$ Different letters indicate significant differences within the same column. ^{a} Different letters indicate significant differences within the same row. ($P< 0.05$).

TABLE 5.

pH VALUES OF MINIMALLY PROCESSED KIWIFRUIT TREATED WITH CHITOSAN FILMS.

Days	pH			
	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
1	3.34 ± 0.02 $^{b(A)}$	3.45 ± 0.01 $^{a(C)}$	3.50 ± 0.01 $^{a(A)}$	2.60 ± 0.01 $^{c(B)}$
7	3.01 ± 0.02 $^{d(B)}$	3.55 ± 0.01 $^{a(A)}$	3.27 ± 0.01 $^{b(B)}$	3.24 ± 0.01 $^{c(A)}$
14	3.01 ± 0.01 $^{b(B)}$	3.50 ± 0.01 $^{a(B)}$	3.25 ± 0.01 $^{d(C)}$	3.26 ± 0.01 $^{c(A)}$

$^{(A)}$ Different letters indicate significant differences within the same column. ^{a} Different letters indicate significant differences within the same row. ($P< 0.05$).

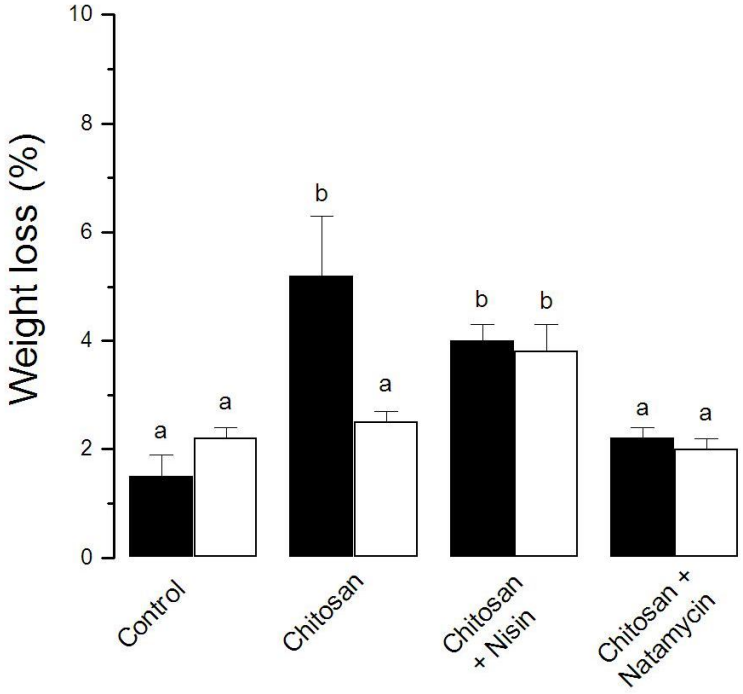
FIGURE LEGENDS

FIGURE 1.

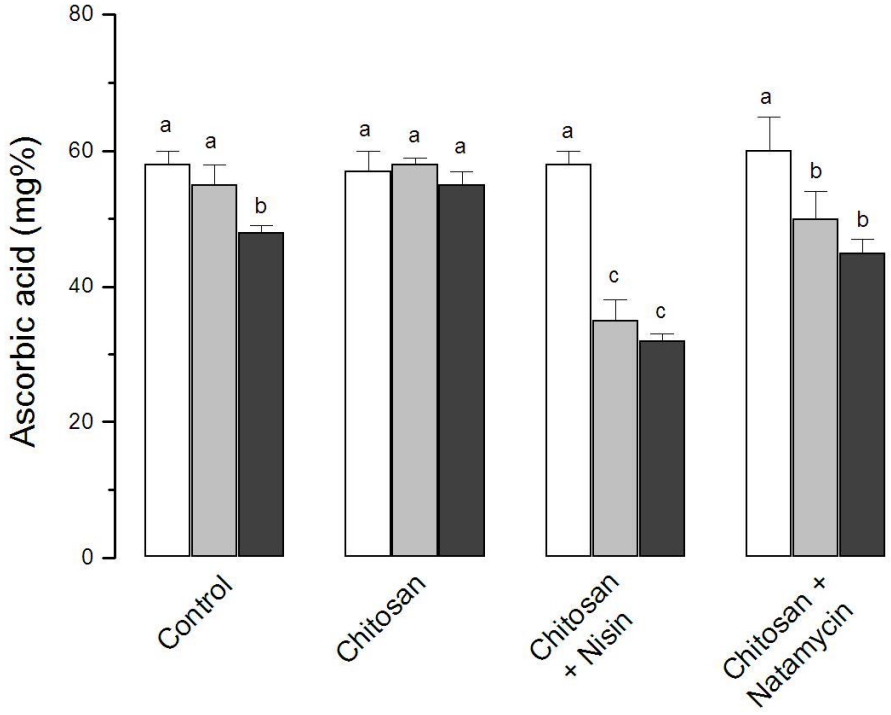
FRESH WEIGHT LOSS IN KIWIFRUIT TREATED WITH CHITOSAN FILMS. Fruits were incubated at 4^{o}C and weight loss was measured at days 7 (black bars) and 14 (white bars). Bars are the means ± standard deviations of three independent determinations. Different letters indicate significant differences (P<0.05).

FIGURE 2.

VITAMIN C IN KIWIFRUIT TREATED WITH CHITOSAN FILMS. Fruits were incubated at 4^{o}C and ascorbic acid concentration was determined at days 1 (white bars), 7 (grey bars) and 14 (black bars). Bars are the means ± standard deviations of three independent determinations. Different letters indicate significant differences (P<0.05).


Weight loss (%)
10
8
6
4
2
0
a
a
b
a
b
b
a
a
Control
Chitosan
Chitosan + Nisin
Chitosan + Natamycin

Cé et al., Fig. 1


Ascorbic acid (mg%)
80
60
40
20
0
a
a
b
a
a
a
a
c
c
a
b
b
Control
Chitosan
Chitosan + Nisin
Chitosan + Natamycin

Cé et al., Fig. 2

3.2 - Artigo 2

Formatação para Food Microbiology

Evaluation of minimally processed strawberry fruit covered with chitosan films incorporating nisin and natamycin

Nísia Cé [a], Caciano P. Z. Noreña[a], Adriano Brandelli[a]*,

[a]Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil

* Corresponding author: ICTA – UFRGS, Av. Bento Gonçalves 9500, 91501- 970 Porto Alegre, Brazil; fax: +55 51 33087048; e-mail: abrand@ufrgs.br

ABSTRACT

Camarosa strawberries were minimally processed and kept under refrigeration temperature at 4 ± 2°C. Chitosan films were used to extend shelf life, maintain the nutritional value and inhibit grow of microbiological pathogens of stored strawberries for 14 days. Three chitosan-based films: 10 g/L chitosan, or 10 g/L chitosan incorporating either 12.5 mg/kg nisin or 0.1 g/L natamycin. The physical-chemical and microbiological analysis were conducted in interval of seven days, and the time 0 represents the beginning of analysis. The results indicate that the use of chitosan-based biofilms plus nisin and natamycin is efficient to maintain the microbiological quality and physico-chemical analysis of vitamin C and moisture. However, in water activity, on weight loss in soluble solids and pH, the films did not result in significant changes. The edible films based on chitosan only guarantee the microbiological quality of minimally processed strawberries kept under refrigeration.

Key words: fruit, shelf life, refrigeration temperature, nutritional value.

INTRODUCTION

Brazil is the third fruitculture world pole with annual production of 38 million tons (Emater, 2007). The strawberry is cultured in temperate and subtropical climates regions, and it is produced to both *in nature* consumption and industrialization (Ibraf, 2005). Strawberry is a horticultural product of extreme importance to national and world economy. The world production reaches 3.1. million tons per year and the Brazilian production is about 37,600 tons, which is highlighted in Minas Gerais state (41.4%), Rio Grande do Sul (25.6%) and São Paulo (15.4%) (Cenci, 2008).

Strawberry is a small fruit, with soft texture, bright red color and slightly sour taste, containing considerable amount of vitamin C and in a lesser degree, iron and vitamin B5. It is highly perishable and with intense post-harvest physiological activity. Belonging to the genus *Fragaria,* the strawberry has a wide range of varieties and wild species (Moraes et al. 2008). The most used cultivars in Southern Brazil came from the United States, especially the Aromas, Camarosa, Diamante, Ventana and Oso Grande, originated from University of California, Dover and Sweet Charlie from University of Florida (Oliveira et al. 2005). Camarosa, a cultivar of short days, was launched in 1993 in Rio Grande do Sul, and it has vigorous plants and with large dark-green leaves in color. The cycle is premature with high production capacity; it is compared to Aromas and Diamond. The fruits are large, uniform, dark-red in color; firm flesh and slightly sour flavor (Santos, 2003).

The strawberry has a restricted lifetime of two or three days along the handling chain. Strawberries are very susceptible to fungi and bacteria and may register losses in post-harvest stage of more than 50% (Cenci, 2008). When strawberries are stored at temperatures of 0-4^{o}C, the shelf life of the fruit is less than five days (Han et al. 2004).

Thus, the minimum processing emerges as a factor to eliminate or reduce post-harvest losses and to prolong shelf life and furthermore, add value to the product. The minimum processing includes the selecting, cleaning, washing, peeling, cutting, sanitary procedure and packaging. These operations result in natural products and practical, whose preparation and consumption requires less time (Damasceno et al. 2001).

The chitosan is a cationic polysaccharide with high molecular weight, it is soluble in organic acids and it is used as material in preventive fruit coatings (Cong et al. 2007). Due to its characteristics of non toxic and easy formation of gels, it has been considered for decades as a compound of industrial interest (Campana Filho and Desbrières 2000). Moreover, it has antibacterial and antifungal activity, showing its potential use on land or in cut fruits with high metabolic rates of maturation after harvest (Kendra et al. 1989, No et al. 2002, Park et al. 2004).

Nisin is a bacteriocin produced from a strain of *Lactococcus lactis,* it is considered GRAS (Generally Regarded as Safe) and its use is approved by the Food and Drugs Administration (FDA), (Apha, 1992 and Food Codex, 1995). The bacteriocin is only allowed for use in food. It has antimicrobial activity against a broad spectrum of gram-positive bacteria and it is being used in the food industry as a safe and natural preservative (O'sullivan et al. 2002). A study of Pranoto et al. (2005) shows that the presence of nisin in edible films based on chitosan inhibited the *L. monocytogenes*.

Natamycin is an antifungal component produced by *Streptomyces natalensis* and other *Streptomyces* spp. It is used as a preservative in foods with bacterial fermentation such as cheese and sausages. Prevents the growth of molds, but it affects the bacterial fermentation and maturation of these foods (Delves-Broughton et al. 2006). Its use is only permitted in meat and cheese foods (Var et al. 2006). However, its potential as a natural preservative, as well as the chitosan and nisin, it is used in the application of minimally

processed fruits as a challenge to contribute to discovery of new technologies for this raw material.

This study aimed to evaluate, through physical, chemical and microbiological tests, the quality of minimally processed strawberries covered with chitosan biofilms, embedded with nisin and natamycin under refrigeration temperature.

MATERIALS AND METHODS

Materials

Strawberries (*Fragaria ananassa*) cv. Camarosa, acquired at the Porto Alegre CEASA, were transported to the laboratory of Engenharia de Processos em Alimentos of the Departamento de Ciência e Tecnologia dos Alimentos, from Federal University of Rio Grande do Sul. At the laboratory the fruit were selected for ripening stage, freedom from defects and sanitary condictions. Approximately 48 hours from harvest.

Chitosan films

The solutions of the treatments were prepared adding 1 g of chitosan (food grade, minimum 85% deacetylated, Sigma-Aldrich) in 94 mL of distilled water with 5 mL of acetic acid (Merck) to dissolve entirely the chitosan. A stock solution of nisin was prepared using 0.1 g Nisaplin (Danisco) dissolved in 1 mL 0.01 mol/L HCl. Nisin incorporation in the chitosan solution follows the allowed limit of 12.5 mg nisin per kg weight of food, according to Brazilian legislation (Ordinance DETENIDOS / MS No. 29, 22th January, 1996). The concentration of the natamycin (Danisco) was 0.1 g/L, dissolved in distilled water. The pH of the solutions was adjusted to pH 5 with 1 mol/L NaOH (Chien et al. 2007a,b).

Fruit Processing

The fruits were washed and peeled manually. The pieces were divided into four different vessels to which following the treatments were applied: control, 10 g/L chitosan, 10 g/L chitosan + nisin, and 10 g/L chitosan + natamycin. Fruit pieces were immersed for 1 min in each corresponding solution. The processing steps were carried out at room temperature and they were conducted under good manufacturer practices. After, the pieces were drained and they were stored under refrigeration temperature ($4 \pm 2^{o}C$) in plastic pots covered with PVC films. Samples were submitted to physical, chemical and microbiological analysis at three different times: 1 (time 0), 7 and 14 days.

Microbial analysis

The microbiological characteristics of 25 g samples were obtained from the homogenization in 225 mL of 1 g/L peptone water. Decimal dilutions were prepared from the 10^{-1} dilution. The total counts were determined on Plate Count Agar (PDA). The plates were incubated at $35^{o}C$ for 48 hours. Yeast and molds were determined on Potato Agar medium, after a 4 days incubation at 28ºC. Total and fecal coliforms were analyzed by the technique of multiple tubes, where the temperatures and times of incubation are respectively $37^{o}C$, $45^{o}C$ and $48^{o}C$ for 24 hours. To express the results the most probable number index's table was used (Instrução Normativa número 62, MAPA, August 26, 2003). For lactic acid bacteria the medium Man Rogosa Sharpe (MRS), incubated at 35ºC for 48 hours was used (Pittia et al. 1999). Two samples of each time and treatment were analyzed.

Weight loss and moisture contents determination

Twenty grams of fruit were removed from each treatment at time 0, weighed and stored in individual packages. After 7 and 14 days the samples were weighed again to determine the fresh weight loss. The calculation of the weight loss was done and expressed as a percentage. The moisture content was measured after constant weight in a stove at 65ºC (method No. 925.45 Aoac, 1990).

Soluble solids, ascorbic acid, pH and water activity measurements

The soluble solids were determined with a hand refractrometer (model N1, Atago, Japan), with results expressed in ^{0}Brix, according to Iso 2173 (Iso, 1978). Vitamin C was measured using the colorimetric method with dichloroindophenol (method No. 967.21 Aoac, 1990). The pH was determined by potentiometer in pH-meter (Tecnal), according Iso 1842 (Iso, 1991). The water activity was measured in an AQUALAB analyzer, model 3TE (Method No. 978.18 Aoac, 1990).

Statistical analysis

The experiment was planned as a 3x4 factorial experiment and conducted in a completely randomized design. The levels of the first factor (time) were: 1, 7 and 14 days. The levels for the second factor were: chitosan, chitosan + nisin, chitosan + natamycin; and control. Total of twelve treatments, each one was performed in triplicate (n=3). The results were expressed as mean ± standard deviation. The statistical comparison was performed by two-way analysis of variance (ANOVA). The criterion for the decision of significance in each of the sources of variability was $p<0.05$. For the comparison among treatments the Tukey test was employed. Data were analyzed by the software SAS 6.08 (SAS Institute).

RESULTS AND DISCUSSION

Microbiological analyses

No total on fecal coliforms were found on the strawberry samples during storage period. In total score in 7 days of storage the chitosan showed that it is most efficient coating with respect to the fruit without coverage (control) and other treatments. In the fourteenth day the treatment of chitosan + natamycin was also more efficient with the control group together with the chitosan treatment.

Chien et al. (2007b), used coating films of different concentrations of chitosan on sliced mango for 7 days of storage at 6ºC and at the end of the storage period determined total counts of 6.41 log CFU/g in the control treatment without coverage. When the authors applied 1% of chitosan determined a count of 5.30 log CFU/g. In total count of the present work, the numbers were lower than those above after the same period of treatment and storage, but the initial load of contamination in fruit is different. Mangoes presented initial load of 3.82 log CFU/g with 1% of chitosan while strawberry 0 log CFU/g.

The effectiveness of chitosan as an antimicrobial agent is shown also in minimally processed strawberries stored for 12 days in the work of Devlieghere et al. (2004). However, when chitosan is added to natamycin, it is more effective in reducing the total count of bacteria towards the end of storage period as observed in the present study.

Regarding to the group of yeasts and molds, it was observed that on the seventh day of storage there was a considerable increase when compared to the first day of testing in the control values (Table 1). The lowest value among at statistically significant was observed for strawberries treated with natamycin + chitosan. At the fourteenth day of storage, none of the treatments had effectively reduced of yeasts and molds in comparison to the fruit without coatings.

Natamycin is a substance unique to act against yeasts and molds, so when it is added to a medium containing the same property, such as chitosan, it tends to increase the effect, as shown in this work on the seventh day of storage.

In the study by Han et al. (2004), who used films of chitosan containing high concentrations of calcium and vitamin E to prolong the shelf life and improve the nutritional quality of different strawberry varieties of fresh and cooled conditions, the signs of development of mold in strawberry varieties, like Puget Reliance and Driscoll uncovered, it appeared after 5 days of storage and the end of 14 days, 65 to 83% of the strawberries without coverage, it depending on the variety, they were affected by the growth of mold. The incidences of mold in the fruits with chitosan coverage were lower than 25 and 50% according to the varieties Puget Reliance and Driscoll.

Chitosan induces the production of chitinases, a defense enzyme, which catalyzes the hydrolysis of chitin, it is a common component of the cell wall of fungi (Hou et al. 1998), thus diminishing its growth in the fruit (Han et al. 2004).

Var et al. (2006) to assess the efficiency of chitosan films when oleic acid is added, it suggested to increase the antimicrobial activity of chitosan to preserve the quality of strawberry cv. Camarosa stored at a temperature of 4 ± 1ºC, it found that in all fruit with coverage, it decreases the occurrence of fungal infection compared to non-covered strawberries. At the end of 10 days of storage, the percentage of infected coated strawberries was below 50%, however, the not covered samples showed highly visible signs of damage by fungi. These results are in agreement with other authors who have examined the use of chitosan for coating of strawberries (El Gaouth et al. 1991, Zhang and Quantick, 1998, Han et al. 2004) and other fruits such as japanese pear, peach, kiwifruit and grapes (Du et al. 1997, Romanazzi et al. 2002).

The count of lactic acid bacteria on the first day of testing, the chitosan shows efficiency because it hinders of bacterial growth in the samples, when compared to the control. With the addition to nisin and natamycin chitosan loses its effectiveness. On the seventh day the fruit showed a lower growth of lactic acid bacteria in all treatments when compared to the control, but the reduction is significantly lower using a coating of chitosan + nisin. And at the end of storage, in addition to chitosan + nisin, natamycin + chitosan treatment shows effectiveness, but it is not statistically different (Table 1).

The lactic acid bacteria develop easily in an acidic medium, so the chitosan + nisin is the most effective treatment in this context, because its functionality and stability of the substances in acidified systems.

Similar results were found in the work of Devlieghere et al. (2004), which it had lower values (<3 log CFU/g) for lactic acid bacteria during the 12 days of storage at 7ºC of minimally processed strawberries covered with a solution of chitosan-lactic acid/Na-lactate and it packed in a balanced modified atmosphere, it was concluded that the lactic acid bacteria shows no importance in the deterioration of strawberries.

Campaniello et al. (2008) evaluated the potential use of chitosan as a food preservative in minimally processed strawberries packaged with high and low percentage of O_2. The results found on growth of lactic acid bacteria are only in samples without chitosan coating and stored at high temperatures (15ºC). No observed growth was at 4 and 8ºC with coverage of chitosan.

In Brazil there are regulations only for fresh fruits, whole fruits, refrigerated or frozen, consumed directly, which provide the only limit for fecal coliform, which is 2.30 log CFU/g (RDC Resolution No. 12, of January 2th 2001). To the other microbial groups there is no reference in the actual legislation (Carvalho and Lima 2002). But there is a French recommendation (Ministere de l'Economie des Finances et Du Budget 1998), it is

for shelf life of minimally processed fresh vegetables which is usually calculated as the time needed to have a total count of bacteria of 7.69 log CFU/g. Undes the experimental conditions of our work this load cell was not achieved, so the strawberry can be considered without a risk of being contaminated fruit for consumption up to 14 days of refrigerated storage.

Minimally processed products are exposed to many kinds of contamination since the peel or the stem that acts as a barrier to the penetration of microorganisms is discarded. Thus, in fruits and vegetables subjected to minimum processing conditions, processing must be extremely hygienic, taking the appropriate care at each stage. And the aseptic conditions with the additives used are natural enough to reduce the microbial population in minimally processed products according to Pittia et al. (1999).

Determination of weight loss, humid content and soluble solids

The weight loss due to transpiration during storage showed significant difference sbetween treatments ($p<0.05$), but unlike the other works with strawberries presented in the literature (Han et al. 2004, Moraes et al. 2008), the weight loss during the intervals and the total storage period in this study was higher in treatments with chitosan, chitosan + nisin, and chitosan + natamycin (Figure 1).

Water is the largest component of fruits; it acts as a medium and a chemical reagent during growth and postharvest storage. It is also important for the fruit texture. The loss of water can be a major cause of deterioration in minimally processed food, because it may result in quantitative losses and there are, as well, losses in appearance (wilting), texture (softening) and nutritional quality (Pereira et al. 2003).

The highest percentage of water loss of strawberry before becoming commercially unacceptable is 6% of their fresh weight (Ronque, 1998). The strawberry, for its size, has

a large surface exposed to transpiration in comparison to its volume. Moreover, strawberries do not have a protective epidermal layer to impede the water loss, and besides it is a fruit with high water content (89.9%) (Cantillano, 2006).

So, for the marketing and consumption of strawberries cv. Camarosa of this work would be acceptable, when the maximum percentage of loss is 6%, the viable treatment beyond the control would be only with chitosan.

These results indicate that the coating films increase significantly the weight loss in strawberries. The weight loss in fruit can be attributed to the loss of juice from the pulp, because the use of films with or without added antimicrobial agents, with plastic packaging, promote the gas exchanges with the environment and fruit creating a modified atmosphere in its interior, thus unfavorable the maintenance of weight (Heiffig et al. 2006, Chien et al. 2007b).

Waimaleongora-Ek et al. (2008) showed the opposite in their study with sweet potatoes stored for 17 days under refrigeration temperatures coated with chitosan promote sweet potatoes lost less weight explained. Cong et al. (2007), explained that chitosan has a hydrophilic nature and the PVC film (poly vinyl chloride) is of hydrophobic nature, and together they promote a formation of a barrier to moisture on the surface of the fruit that results in the delay of the migration of water from fruit to the environment.

The moisture decreased significantly toward the end of the storage period compared to the control and to chitosan + natamycin treatments, and the loss of moisture was higher in the control treatment compared to the beginning of the experiment (Table 2).

The table of food composition of Universidade de São Paulo (Tbcausp, 2008) shows that the moisture standard for strawberry is 90.76%, so the values obtained in this

study after fourteen days of storage with minimally processed fruit is in agreement with that standard, except that the control is slightly below that reference.

The soluble solids decreased significantly after fourteen days of storage in all treatments, including the control. At the end of storage the amount of soluble solids of the control and of the treatment with chitosan was 7^0Brix, showing that there is no positive statistical significance in using that coating (Table 3). These results were not expected, because usually when we have natural loss of moisture in the fruit, Brix degrees tend to increase, i.e. when the loss of water will be high in the time, the soluble solids content will be high, too (Mesquita, 1999 and Pina, 1999).

Chien et al. (2007a,b), obtained red pitaias and sliced mango, they decrease in soluble solids with the use of different concentrations of chitosan (0.5%, 1% and 2%), stressing that the variation between them was very low. This reduction in sugar levels may be explained by the increase in respiratory rate of fruit. The physical action of minimal processing induces the production of ethylene, it is called injury ethylene and also induces an increase in respiration, which uses quickly the reserve substrates (Watada et al. 1990).

In the work of Vargas et al. (2006), the soluble solids content (around 6ºBrix) of cultivar Camarosa strawberries did not vary significantly during storage and the values were not affected by the application of chitosan-based coatings Vargas et al. (2004), plunged strawberries in a film of chitosan and 2%, pH 4.1 and stored them in the cooling temperature to evaluate the physical-chemical and microbiology quality of fruit.

Measurement of ascorbic acid, pH and water activity

Ascorbic acid decreases with storage time in all treatments. The treatment with chitosan + nisin was the lowest loss at the end of fourteen days of storage (Figure 2).

Vitamin C is a micronutrient that determines the nutritional quality of fruit. The amount of ascorbic acid in strawberries is set at 37 mg% by the USDA (2008) and 63.6

mg% by TACO (2006). Yurdugul (2008) assessed the characteristics of quality in fresh strawberries and dehydrated strawberry, obtained the values of vitamin C for both around 51 mg%, showing no differences between fresh and dried fruit. Possibly, there is difference regarding the amount of ascorbic acid between cultivars of strawberry, the climate and where the fruit is grown, and therefore, these changes occur regarding the standard quantity as the information listed above.

The addition of antimicrobials at time 0 increases the pH of the medium (Table 4). Furthermore, a significant increase in pH control in the fourteenth day of storage was obtained. But with the addition of antimicrobial treatments the decrease in pH on the fourteenth day of storage was significant.

The coverage with chitosan-based coatings delayed the senescence of strawberries, probably because of the semi-permeable film formed on the surface of the fruit can modify the internal atmosphere, i.e. alter the endogenous CO_2 of and the O_2 concentration in fruit (Bai et al., 1988, Lowings and Cutts, 1982). Furthermore, the low pH value restricts the microflora to acid tolerant microorganisms such as fungi and lactic bacteria (Pittia et al. 1999).

The strawberries have high respiratory rate (approximately 15 mg CO_2/Kg/hr at 0ºC), which increases between four to five times when the temperature increases to 10ºC, and it increases to ten times if the temperature increases to 20ºC (Cantillano et al., 2003). The respiratory rate increases by 50% when the fruit is unripe and move on to ripe (Ronque, 1998). The increase in respiratory rate occurs when the strawberries also suffer mechanical damage (Kader, 1991). So, there is the hypothesis that atmospheres containing high concentrations of CO_2, can interfere with intracellular pH of fruit and vegetables, increasing acidity and reducing the pH through the hydration of CO_2 produced by HCO^-_3 and H^+, and, or his effect on the metabolism of organic acids (Moraes et al., 2008).

The result of increasing on pH agrees with Ghaouth et al. (1991), Garcia et al. (1998) and Han et al. (2004). The decrease in acidity during storage shows that the fruit has ripped, because the organic acids tend to decrease during ripening, since they are used as respiration substrate.

Fruits and vegetables have a water activity about 0.98, allowing the growth of many microorganisms (Nguyenthe and Carlin, 1994 and Tapia de Daza et al. 1996, and Brackett, 1997). The results of this study show that the coatings began with a high water activity and statistically different from control at time 0. They were able to significantly decrease the activity of water at the fourteenth day of storage. The control samples showed statistically significant lower values for water activity at times 0 and on the end of 14 days of storage (Table 5).

CONCLUSIONS

In satisfactory conditions of cleanliness and refrigerate temperature, minimally processed strawberries present low or absent microbial contamination. Depending on the microorganism analyzed and storage time, we have better specific efficiency for each film. For the total count the chitosan films and chitosan + natamycin are the best. In the yeasts and molds with a week of storage it is efficient nisin + chitosan, and to lactic acid bacteria, the chitosan and chitosan + nisin also induce significant changes in the microbiological control on samples. So the edible films based on chitosan protect the strawberries from bacterial and fungal infections.

The chitosan-based films do not induce significant changes in physical-chemical parameters of “Camarosa” strawberries during cold storage. Only moisture and vitamin C was observed improvement in rates of humid loss when it was addition to coverage of

chitosan + natamycin and chitosan + nisin, respectively. According to these results, we suggest the use of a film composed of chitosan + natamycin + nisin to maintain the microbiological standards, some physical-chemical and nutritional value desired in the strawberry cv. Camarosa during refrigerate storage.

References

Anvisa – Agência Nacional de Vigilância Sanitária. Resolução RDC nº 12, de 02 de janeiro de 2001. Disponível em: http://e-legis.anvisa.gov.br/leisref/public/ Acesso em: 18 de janeiro de 2009.

Anvisa – Agência Nacional de Vigilância Sanitária. Portaria DETEN/MS nº 29, de 22 de janeiro de 1996. Disponível em: http://www.anvisa.gov.br/legis/portarias/29_96.htm. Acesso em: 23 de junho 2007

AOAC, 1990. *Official methods of analysis of the association of official analytical chemists*. Washington, DC.

APHA, 1992. Compendium of Methods for the Microbiological Examination of Food. Cap. 24, EUA, Washington.

Bai, R. K., Huang, M. Y. and Jiang, Y. Y., 1988. Selective permeabilities of chitosan-acetica acid complex membrane and chitosan-polymer complex membrane for oxygen and carbon dioxide. Polym. Bull. 20, 83.

Brackett, R. E., 1997. Alteración microbiológicas y microorganismos patógenos de frutas y hortalizas refrigeradas mínimamente procesadas. In: Wiley, R.C. Frutas y hortalizas minimamente procesadas y refrigeradas. Zaragoza: Acribia, pp. 263304.

401 Campana Son, S.P. and Desbrières, J., 2000. Chitin, chitosan and derivatives. In:
402 Debeaufort, F., Quezada-Gallo, J. A., Voilley A. Edible Films and Coatings:
403 Tomorrow's Packagings: A Review. Crit Rev Food Sci Nutr pp. 299-313.
404 Campaniello, D., Bevilacqua, M., Sinigaglia, M.R. and Corbo, 2008. Chitosan:
405 antimicrobial activity and potential applications for preserving minimally processed
406 strawberries. Food Microbiol. 25, 992-1000.
407 Cantillano, F., Bender, J. R. and Luchsinger, L. L., 2003. Fisiologia e manejo pós-
408 colheita. In: Cantillano, F. Morango: pós-colheita. Brasília: Embrapa Informação
409 Tecnológica, pp. 14-24.
410 Cantillano, F., 2006. Fisiologia e manejo na colheita e pós-colheita de morangos. In:
411 Carvalho, S. P. Boletim do morango: cultivo convencional, segurança alimentar,
412 cultivo orgânico. Belo Horizonte, FAEMG, pp. 160.
413 Carvalho, A. V. and Lima, L. C. O., 2002. Qualidade de kiwis minimamente
414 processados e submetidos a tratamento com ácido ascórbico, ácido cítrico e cloreto
415 de cálcio. Pesq. Agropec. Bras. 37, 679-685.
416 Cenci, A., 2008. Processamento mínimo do morango prolonga vida útil de
417 comercialização, mas a técnica simples é pouco aplicada. Revista Frutas e Derivados
418 - Instituto Brasileiro de Frutas – IBRAF, 3, 41-43.
419 Chien, P. J., Sheu, F. and Lin, H. R., 2007a. Quality assessment of low molecular
420 weight chitosan coating on sliced red pitayas. J. Food Eng. 79, 736-740.
421 Chien, P. J., Sheu, F., Yang, F. H., 2007b. Effects of edible chitosan coating on quality
422 and shelf life of sliced mango fruit. J. Food Eng. 78, 225-229.
423 Codex Alimentarius, 1995. Princípio de transferência dos aditivos alimentares. Seção
424 5.3, Vol 1A.

425 Cong, F., Zhang, Y. and Dong, W., 2007. Use of superface coating with natamycin to
426 improve the storability of Hami melon at ambient temperature. Postharvest Biol.
427 Technol. 46, 71-75.

428 Damasceno, K. S. F., Stamford, T. L. M. and Alves, M. A., 2001. Vegetais
429 minimamente processados: uma revisão. Higiene Alimentar 15, 20-25.

430 Delves-Broughton, J., Thomas, L. V. and Williams, G., 2006. Natamycin as an
431 antimycotic preservative on cheese and fermented sausages. Food Australia 58, 19-
432 21.

433 Devlieghere, F., Vermeulen, A. and Debevere, J., 2004. Chitosan: antimicrobial
434 activity, interaction with food components and applicability as a coating on fruit and
435 vegetables. Food Microbiol. 21, 703-714.

436 Du, J. M., Gemma, H. and Iwahori, S., 1997. Effects of chitosan coating on the storage
437 of peach, Japanese pear, and kiwi fruit. J. Jpn. Soc. Hort. Sci. 66, 15-22.

438 El Ghaouth, A, Arul, J., Ponnampalam, R. and Boulet, M., 1991. Chitosan coating
439 effect on storability and quality of fresh strawberries. J. Food Sci. 56, 1618-1631.

440 Emater (Rio Grande do Sul). Fruticultura. Disponível em:
441 http://www.emater.tche.br/site/inicial/ptbr/php/index.php. Acesso em: 30 abril 2007.

442 Garcia, M. A., Martino, M. N. and Zaritzky, N. E., 1998. Plasticized starch-based
443 coatings to improve strawberry (*Fragaria x ananassa*) quality and stability. J. Agric.
444 Food Chem. 46, 3758-3767.

445 Ghaouth, A. E., Arul, J., Ponnampalam, R., Boulet, M., 1991. Chitosan coating effect
446 on storability and quality of fresh strawberries. J. Food Sci. 56, 1618-1620.

447 Han, C., Zhao, Y., Leonard, S. W. and Traber, M. G., 2004. Edible coating to improve
448 storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria*
449 *x ananassa*) anda raspberries (*Rubus ideaus*). Postharvest Biol. Technol. 33, 67-78.

Heiffig, L. S., Aguila, J. S. and Kluge, R. A., 2006. Caracterização físico-química e sensorial de frutos de kiwi minimamente processado armazenado sob refrigeração. Rev. Iber. Tecnología Postcosecha. 8, 26-32.

Hou, W. C., Chen, Y. C. and Lin, Y. H. 1998. Chitinase activity of sweet potato (*Ipomoea batatas* L. Lam var. Tainong 57). Bot Bull. Acad. Sínica. 39, 93-97.

Ibraf (Instituto Brasileiro de Frutas). Estatísticas. Disponível em: <http://www.ibraf.org.br/x-es/f-esta.html> Acesso em: 02 de maio de 2005.

Iso 2173, 1978. Fruit and vegetable products: determination of soluble solids content: refractometric method. 1 ed. Genève: International Organization for Standardization.

Iso 1842, 1991. Fruit and vegetable products: determination of pH 2. ed. Gèneve: International Organization for Standardization.

Kader, A. A., 1991. Postharvest and technology: an overview. In: Kader, A. A. Postharvest of horticultural crops. 2. ed. Davis: University of California, pp. 15-20.

Kendra, D. F., Christian, D. and Hadwiger, L. A., 1989. Chitosan oligomers from *Fusarium solani*/pea interactions, chitinase/β-glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance. Physiological and Molecular Plant Pathology 35, 215-230.

Lowings, P. H. and Cutts, D. F., 1982. The preservation of fresh fruits and vegetables. In: Proceedings of the Institute of the Food Science and Technology, Annual Symposium, Nottingham, UK.

Mapa - Ministério da Agricultura Pecuária e Abastecimento. Instrução normativa número 62, de 26 de agosto de 2003. Disponível em: <http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=2851> Acesso em: 25 de novembro de 2008.

475 Mesquita, P. C., 1999. Conservação de pedúnculo de caju (*Anacardium occidentale, L.*)
476 por processamento mínimo/ métodos combinado com emprego de tecnologia de
477 obstáculos. Dissertação (Mestrado em Tecnologia de Alimentos) – Universidade
478 Federal do Ceará, Fortaleza, pp. 125.
479 Ministere de l'Economie des Finances et Du Budget, 1998. Marché consommation,
480 Produits vegetaux prets a lémploi dts de la "Ivemme Gamme": Guide de bonnes
481 pratique hygieniques. J. Off. Rép. Française 1621, 1-29.
482 Moraes, I. V. M., Cenci, S. A., Benedetti, B. C., Mamede, A. M. G. N., Soares, A. G.,
483 and Barboza, H. T. G., 2008. Physical and chemical characteristics of minimally
484 processed strawberries stored under refrigeration and controled atmospheres. Ciênc.
485 Tecnol. Aliment. 28, 274-281.
486 Nguyenthe, C. and Carlin, F., 1994. Microbiology of minimally processed fruits and
487 vegetables. Crit Rev Food Sci Nutr 34, 371401.
488 No, H.K., Parj, N.Y., Lee, S.H. and Meyers, S.P., 2002. Antibacterial activity of
489 chitosan and chitosan oligomers with different molecular weights. Int. J. Food Micr
490 Oliveira, R.P., Nino, A.F.P. and Scivittaro, W.B., 2005. Mudas certificadas de
491 morangueiro: maior produção e melhor qualidade da fruta. A Lavoura, 108, 35-38.
492 O'sullivan, L., Ross, R. P. and Hill, C., 2002. Potential of bacteriocin-producing lactic
493 acid bacteria for improvements in food safety and quality. Bioch. 84, 593-604.
494 Park S. I., Daeschel M. A., Zhao, Y., 2004. Functional Properties of Antimicrobial
495 Lysozyme-Chitosan Composite Films. J. Food Sci. 69, 8.
496 Pereira, L. M., Rodrigues, A. C. C., Sarantópoulos, C. I. G. L., Junqueira, V. C. A,
497 Cardello, H. M. A. B. and Hubinger, M. D., 2003. Shelf life of minimally processed
498 guavas stored in modified atmosphere packages. Ciênc. Tecnol. Aliment. 23, 427-433.

Pina, M. M. G., 1999. Conservação de manga (*Mangifera indica*, *L.*) por processamento mínimo/ métodos combinados. Dissertação (Mestrado em Tecnologia de Alimentos) – Universidade Federal do Ceará, Fortaleza. pp.138.

Pittia, P., Nicoli, M. C., Comi, G., Massini, R., 1999. Shelf-life extension of fresh-like ready-to-use pear cubes**.** J. Sci. Food Agric. 79, 955-960.

Pranoto, Y., Rakshit, S. K. and Salokhe, V. M., 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT 38, 859-865.

Romanazzi, G., Nigro, F., Ippolito, A., Venere, D. D. and Salerno, M., 2002. Effect of Pre and Postharvest chitosan treatments to control storage grey mold of table grapes. J. Food Sci. 67, 1862-1867.

Ronque, E. R. V., 1998. A cultura do morangueiro. Curitiba: EMATER-PR. 183-202.

Santos, A. M. Cultivares., 2003. In: Santos, A. M., Medeiros, A. R. M. (Ed.). Morango: produção. Brasília: Embrapa Informação Tecnológica, pp.24-30.

Tabela Brasileira de composição de alimentos - Tbcausp., 2008. Versão 5. São Paulo. Disponível [on line] em: <http://www.fcf.usp.br/tabela/> acesso em: 02 de janeiro de 2009.

Tabela Brasileira de Composição de Alimentos - Taco., 2006. 2º Ed. Campinas Disponível [on line] em: < http://www.unicamp.br/nepa/taco/ > acesso em 10 de dezembro de 2008.

Tapia de Daza, M. S., Alzamora, S. M., Welti-Chanes, J., 1996. Combination of preservation factors applied to minimal processing of food. Crit Rev Food Sci Nutr 36, 629-659.

USDA, 2008. Agricultural Research Service. Nutrient data laboratory. Usda National nutrient database for standard reference release 21. Disponível em:

http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl Acessado em: 08 de janeiro de 2009.

Var, I., Erginkaya, Z., Guven, M. and Kabak, B., 2006. Effect of antifungal agent and packaging material on microflora of Kashar cheese during storage period. Food Control 17, 132-136.

Vargas, M., Gillabert, M., Gonzálvez-Martínez, C., Albors, A. and Chiralt, A., 2004. Efecto de la aplicación de un film a base de quitosano en la calidad de fresas durante el almacenamiento. Actas del III Congreso Español de Ingeniería de Alimentos, [cd-room], 746-753.

Vargas, M., Albors, A., Chiralt, A. and Gonzálvez-Martínez, C., 2006. Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. Postharvest Biol. Technol. 41, 164-171.

Yurdugul, S., 2008. An evaluation of the retention of quality characteristics in fresh and freeze-dried alpine strawberries. Int. J. Food Sci. Technol. 43, 865-870.

Waimaleongora-Ek, P., Corredor, A. J. H., No, H. K., Prinyawiwatkul, W., King, J. M., Janes, M. E. and Sathivel, S., 2008. Selected quality characteristics of fresh out sweet potatoes coated with chitosan during 17 day refrigerated storage. J. Food Sci. 73, S418-S423.

Watada, A. E., Abe, K., Yamuchi, N., 1990. Physiological activities of partially processed fruits and vegetables. Food Technol., Chicago 44, 116-122.

Zhang, D. L. and Quantick, P. C., 1998. Antifungal effect of chitosan coating on fresh strawberries and raspberries during storage. J. Hortic. Sci. Biotechnol. 73, 763-767.

549 **Table 1**: Microbiological analysis of minimally processed cv. Camarosa strawberries
550 treated with chitosan films.*
551

Days	Log CFU/g			
	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
Total	*counts*			
1	$0 \pm 0^{a*(c)}$	$0 \pm 0^{a*(c)}$	$0 \pm 0^{a*(c)}$	$0 \pm 0^{a*(c)}$
7	$1{,}17 \pm 0^{b*(b)}$	$1{,}04 \pm 0^{c*(b)}$	$1{,}70 \pm 0^{a*(b)}$	$1{,}70 \pm 0^{a*(a)}$
14	$3{,}04 \pm 0{,}02^{a*(a)}$	$1{,}70 \pm 0^{b*(a)}$	$3{,}04 \pm 0{,}02^{a*(a)}$	$1{,}39 \pm 0^{c*(b)}$
Yeasts	*and molds*			
1	$1{,}32 \pm 0^{c*(c)}$	$1{,}70 \pm 0{,}38^{c*(c)}$	$4{,}38 \pm 0{,}03^{a*(a)}$	$4{,}14 \pm 0{,}03^{b*(a)}$
7	$3{,}90 \pm 0{,}02^{a*(a)}$	$2{,}81 \pm 0{,}01^{b*(b)}$	$2{,}70 \pm 0{,}01^{c*(c)}$	$2{,}55 \pm 0{,}01^{d*(c)}$
14	$3{,}35 \pm 0{,}01^{c*(b)}$	$3{,}49 \pm 0{,}02^{b*(a)}$	$4{,}24 \pm 0{,}2^{a*(b)}$	$3{,}49 \pm 0{,}02^{b*(b)}$
Lactic	*acid bacteria*			
1	$1{,}74 \pm 0^{a*(c)}$	$0 \pm 0^{b*(c)}$	$2{,}04 \pm 0{,}34^{a*(b)}$	$2{,}04 \pm 0{,}34^{a*(c)}$
7	$3{,}55 \pm 0{,}02^{a*(b)}$	$2{,}51 \pm 0^{c*(b)}$	$1{,}70 \pm 0^{d*(b)}$	$3{,}13 \pm 0{,}01^{b*(b)}$
14	$4{,}32 \pm 0{,}03^{a*(a)}$	$3{,}61 \pm 0{,}1^{c*(a)}$	$3{,}99 \pm 0{,}02^{b*(a)}$	$3{,}45 \pm 0{,}2^{c*(a)}$

552 * Total and fecal coliforms were not detected during the storage period. $^{(a)}$ Different
553 letters indicate significant differences within the same column. ^{a*} Different letters indicate
554 significant differences within the same row. ($P < 0.05$).
555
556
557
558
559
560

Table 2: Effect of films in the loss of moisture (wet basis) in strawberry minimally processed and stored under refrigeration temperature.

	Moisture (%, wb)			
Days	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
1	94,17 ± 0,22 $^{a*(a)}$	92,12 ± 0,83 $^{b,c*(a)}$	93,75 ± 0,29 $^{a*(a)}$	92,03 ± 0,06 $^{c*(a)}$
7	91,37 ± 0,40 $^{c*(b)}$	94,34 ± 0,74 $^{a*(b)}$	92,30 ± 0,36 $^{b*(b)}$	92,54 ± 0,35 $^{b*(b)}$
14	89,88 ± 0,21 $^{c*(c)}$	90,82 ± 0,70 $^{b*(a)}$	94,02 ± 0,20 $^{a*(a)}$	91,09 ± 0,75 $^{b*(c)}$

$^{(a)}$ Different letters indicate significant differences within the same column. ^{a*} Different letters indicate significant differences within the same row. ($P< 0.05$).

582 **Table 3:** Effect of coating films on soluble solid in cv. Camarosa strawberries minimally
583 processed and stored under refrigeration temperature.
584

	Soluble solids (°Brix)			
Days	Control (*in natura*)	Chitosan	Chistosan + Nisin	Chitosan + Natamycin
1	8,00 ± 0,14 $^{a*(a)}$	8,20 ± 0,14 $^{a*(a)}$	7,00 ± 0,14 $^{b*(a)}$	6,00 ± 0,14 $^{c*(a)}$
7	8,00 ± 0,14 $^{a*(a)}$	6,00 ± 0,14 $^{b*(b)}$	6,00 ± 0,14 $^{b*(b)}$	6,10 ± 0,14 $^{b*(a)}$
14	7,00 ± 0,14 $^{a*(b)}$	7,00 ± 0,14 $^{a*(c)}$	3,00 ± 0,14 $^{c*(c)}$	4,00 ± 0,14 $^{b*(b)}$

585 $^{(a)}$ Different letters indicate significant differences within the same column. ^{a*} Different
586 letters indicate significant differences within the same row. ($P< 0.05$).
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601

Table 4: Effect of films on the values of pH in cv. Camarosa strawberries minimally processed and stored under refrigeration temperature.

	pH			
Days	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
1	3,40 ± 0,01 $^{d*(b)}$	3,73 ± 0,03 $^{c*(a)}$	3,98 ± 0,01 $^{b*(a)}$	4,20 ± 0,01 $^{a*(a)}$
7	3,53 ± 0,02 $^{b*(a)}$	3,53 ± 0,04 $^{b*(b)}$	3,73 ± 0,04 $^{a*(b)}$	3,85 ± 0,01 $^{a*(b)}$
14	3,53 ± 0,04 $^{a*(a)}$	3,53 ± 0,02 $^{a*(b)}$	3,54 ± 0,01 $^{a*(c)}$	3,42 ± 0,01 $^{b*(c)}$

$^{(a)}$ Different letters indicate significant differences within the same column. ^{a*} Different letters indicate significant differences within the same row. ($P< 0.05$).

Table 5: Effect of films on the values of water activity in strawberry minimally processed and stored under refrigeration temperature.

	Aw			
Days	Control (*in natura*)	Chitosan	Chitosan + Nisin	Chitosan + Natamycin
1	0,988 ± 0,001 $^{c*(a)}$	0,993 ± 0,001 $^{b*(a)}$	0,994 ± 0,001 $^{a,b*(a)}$	0,996 ± 0,001 $^{a*(a)}$
7	0,990 ± 0,001 $^{a*(a)}$	0,989 ± 0,001 $^{a*(b)}$	0,984 ± 0,001 $^{b*(b)}$	0,986 ± 0,001 $^{b*(b)}$
14	0,981 ± 0,001 $^{c*(b)}$	0,990 ± 0,001 $^{a*(b)}$	0,985 ± 0,001 $^{b*(b)}$	0,982 ± 0,001 $^{c*(a)}$

$^{(a)}$ Different letters indicate significant differences within the same column. ^{a*} Different letters indicate significant differences within the same row. ($P< 0.05$).

Figure 1: Effect of films on the fresh weight loss in cv. Camarosa strawberry. Control (x), chitosan (1%) (■), chitosan (1%) + nisin (▲) and chitosan (1%) + natamycin (♦).

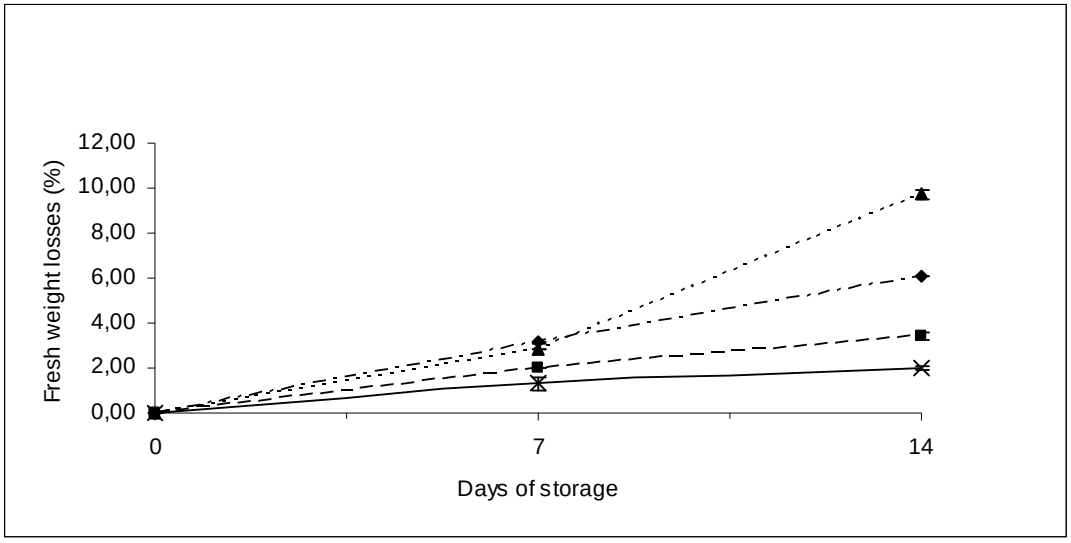
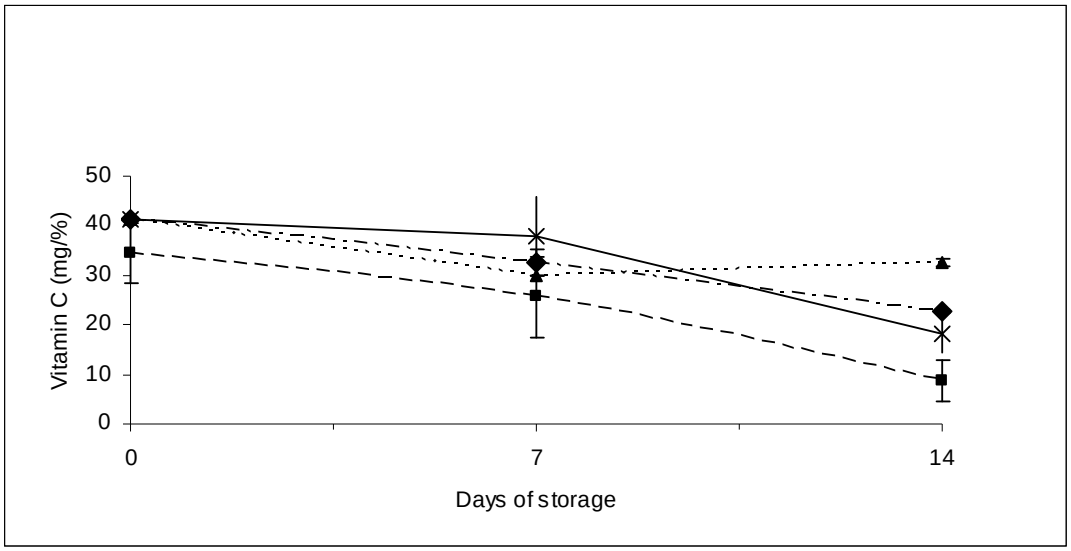


Figure 2: Effect of films in the loss of vitamin C in cv. Camarosa strawberries. Control (x), chitosan (1%) (■), chitosan (1%) + nisin (▲) and chitosan (1%) + natamycin (♦).



4 - CONCLUSÕES

Kiwis e morangos minimamente processados apresentaram ausência de contaminação microbiológica de coliformes fecais e coliformes totais em condições adequadas de higiene e temperatura de refrigeração quando adicionados os filmes de cobertura, mas apresentaram algumas poucas contaminações por bolores, leveduras e bactérias ácido lácticas.

As mudanças na qualidade físico-química de kiwis e morangos durante o armazenamento refrigerado foram significativas para algumas análises como: sólidos solúveis, vitamina C e pH em kiwis, umidade e vitamina C em morangos, observando melhoras nestes índices quando adicionado os filmes a base de quitosana juntamente com os antimicrobianos nisina e natamicina.

Os benefícios da incorporação dos agentes antimicrobianos nisina e natamicina nos filmes a base de quitosana são dependentes do tempo de armazenamento das frutas, portanto, sugere-se o uso do filme composto de quitosana + natamicina + nisina para ajudar a manter os padrões microbiológicos, algumas variáveis físico-químicos como sólidos solúveis e pH em kiwis e umidade em morangos, e também manter o valor nutricional de vitamina C desejado em kiwis e morangos minimamente processados durante os 14 dias de armazenamento refrigerado.

REFERÊNCIAS

ALTENA, K.; GUDER, A.; CRAMER, C. et al. Biosynthesis of the lantibiotic mersacidin: organization of a type B lantibiotic gene cluster. **Applied and Environmental Microbiology**, v. 66 p. 2565-2571, 2000.

ANVISA – Agência Nacional de Vigilância Sanitária. **Portaria DETEN/MS nº 29, de 22 de janeiro de 1996.** Disponível em: http://www.anvisa.gov.br/legis/portarias/29_96.htm>. Acesso em: 23 de junho 2007

APHA **Compendium of Methods for the Microbiological Examination of Food**. Cap. 24, EUA, Washington, 1992.

BOGUSLAWSKI, S.; BUNZEIT, M.; KNORR, D. Effect of chitosan treatment on clarity and microbial counts of apple juice. **Technology,** v. 41 p. 42-44, 1990.

BROMBERG, R.; MORENO, I.; DELBONI, R. R.; CINTRA, H. C. Characterisation of the bacteriocin produced by *Lactococcus lactis ssp*. Hordniae CTC 484 and the effect of this compound on *Listeria monocytogenes* in beef. **Ciência e Tecnologia de Alimentos,** v. 26 n. 1 p. 135-144, 2006.

CAMPANA FILHO, S.P.; DESBRIÈRES, J. Chitin, chitosan and derivatives. In: Debeaufort, F., Quezada-Gallo , J. A., Voilley A. Edible Films and Coatings: Tomorrow's Packagings: A Review. **Critical Reviews in Food Science and Nutrition**, v. 38 n. 4 p. 299-313, 2000.

CHEN, M. C.; YEB, GH-C.; CHIANG, B. H. Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. **Journal of Food Processing and Preservation**, v. 20 p. 379-390, 1996.

CHEN, G. Q.; LU, F. P.; DU, L. X. Natamycin Production by Streptomyces gilvosporeus Based on Statistical Optimization. **J. Agric. Food Chem**. v.13 p. 5057–5061, 2006.

CHIEN, C. S.; LIAU, W. Y.; TSAI, G. J. Antibacterial effects of N-sulfonated and N-sulfobenzoyl chitosan and application to oyster preservation. **J. Food Prot**., v. 61 p. 1124-1128, 1998.

CHIEN, P. J.; SHEU, F.; LIN, H. R.. Quality assessment of low molecular weight chitosan coating on sliced red pitayas. **Journal of Food Engineering**, v. 79 p. 736-740, 2007b.

CHIEN, P. J.; SHEU, F.; YANG, F.H.. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. **Journal of Food Engineering**, v. 78 p. 225-229, 2007a.

CHIEN, P. J.; SHEU, F.; LIN, H. R. Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life. **Food Chem.** v. 100 p. 1160-1164, 2007c.

CHUNG, K.; DICKSON, J.; CROUSE, J. Effects of nisin on growth of bacteri attached to meat. **Applied and Environmental Microbiology**, v. 55 p. 1329-1333, 1989.

CLEVELAND, J.; THOMAS, J. M.; NES, I. F.; CHIKINDAS, M. L. Bacteriocins: safe, natural antimicrobials for food preservation. **International Journal of Food Microbiology**, v. 71 p. 1-20, 2001.

CODEX ALIMENTARIUS. **Princípio de transferência dos aditivos alimentares**. 1995. Seção 5.3, Vol 1A.

COELHO, A. R.; HOFFMANN, F. L.; HIROOKA, E. Y. Biocontrole de doenças pós-colheita de frutas por leveduras: perspectivas de aplicação e segurança alimentar. **Ciências Agrárias**, v. 24 n. 2 p. 337-358, 2003.

COMA, V.; MARTIAL G.A.; GARREAU, S.; COPINET, A.; SALIN, F.; DESCHAMPS, A. Edible antimicrobial films based on chitosan matrix. **Journal of Food Science**, v. 67 n.3 p.1162-1169, 2002.

CONG, F.; ZHANG, Y.; DONG, W. Use of superface coating with natamycin to improve the storability of Hami melon at ambient temperature. **Postharvest Biology and Technology**, v. 46 p. 71-75, 2007.

DAVIES, E. A.; MILNE, C. F.; BEVIS, H. E.; POTTER, R. W.; HARRIS, J. M.; WILLIAMS, G. C.; THOMAS, L. V.; DEVES-BROUGHTON, J. Effective use of nisin to control lactic acid bacterial spoilage in vacuum-package bologna-type sausage. **J. Food Prot.** v. 62 p. 1004-1010, 1999.

DEBEAUFORT, F.; QUEZADA-GALLO, J. A.; VOILLEY, A. Edible films and coatings: Tomorrow's packagings: A review. **Critical Reviews in Food Science and Nutrition,** v. 38 p. 299-313, 1998.

DELVES-BROUGHTON, J.; THOMAS, L. V.; WILLIAMS, G. Natamycin as an antimycotic preservative on cheese and fermented sausages. **Food Australia**, v. 58 p. 19-21, 2006.

DEVLIEGHERE, F.; VERMEULEN, A.; DEBEVERE J. Chitosan: antimicrobial activity, interaction with food components and applicability as a coating on fruit and vegetables. **Food Microbiology**, v. 21 p. 703-714, 2004.

DE VUYST VANDAMME, E. J. Bacteriocins of lactic acid bacteria: microbiology, genetic and applications. London: blackie Academic & Professional, 540p., 1994.

DIAB, T.; BILIADERIS, C. G.; GERASOPOULOS, D.; SFAKIOTAKIS, E. Physicochemical properties and application of pullulan edible films and coatings in

fruit preservation. **Journal of the Science of Food and Agriculture,** v. 81 n. 10 p. 988-1000, 2001.

DONG, H.; CHENG, L.; TAN, J.; ZHENG, K.; JIANG, Y. Effects of chitosan coating on quality and shelf life of peeled litchi fruit. **Journal of Food Engineering**, v. 64 n. 3 p. 355-358, 2004.

DORNENBURG, H.; KNORR, D. Evaluation of elictor- and hight-pressure-induced enzymatic browning utilizing potato (*Solanumm tuberosum*) suspension cultures as a model system for plant tissues. **J. Agric. Food Chem.**, v. 45 p. 4173-4177, 1997.

DU, J. M.; GEMMA, H.; IWAHORI, S. Effects of chitosan coating on the storage of peach, Japanese pear, and kiwi fruit. **J. Jpn. Soc. Hort. Sci**, v. 66 p. 15-22, 1997.

EL GHAOUTH, A.; ARUL, J.; PONNAMPALAM, R.; BOULET, M. Chitosan coating effect on storability and quality of fresh strawberries. **Journal of Food Science,** v. 56 n. 6 p.1618-1631, 1991.

EL GHAOUTH, A.; PONNAMPALAM, R.; CASTAIGNE, F.; ARUL, J. Chitosan coating to extend the storage life of tomatoes. **Hort. Sci.** v. 27 p. 1016-1018, 1992.

EL GHAOUTH, A.; ARUL, J.; GRENIER, J.; ASSELIN, A. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. **Phytopathology**, v. 82 n. 4 p. 496-501, 1992.

EMATER (Rio Grande do Sul). Fruticultura. Disponível em: http://www.emater.tche.br/site/inicial/ptbr/php/index.php. Acesso em: 30 abril 2007.

FANG, S. W.; LI, C. F.; SHIH, D. D. C. Antifungal activity of chitosan and its preservative effect on low-sugar candied kumquat. **J. Food Prot**., v. 52 p. 136-140, 1994.

FREEPONS, D. Enhancing food production with chitosan seed-coating technology. In: Goosen MFA, editor. Applications of chitin and chitosan. 1st ed. **Technomic Publishing Co**., Lancaster, Pa. p 129–39, 1997.

GRISI, T. C. S. L.; GORLACH-LIRA, K. Action of nisin and hight pH on growth of *Staphylococcus aureus* and *Salmonella sp.* in pure culture and in the meat of land crab (Ucides cordatus). **Brazilian Journal Of Microbiology**, v. 2 n. 36 p.151-156, 2006.

HAN, C.; LEDERER, C.; MCDANIEL, M.; ZHAO, Y. Sensory Evaluation of Fresh Strawberries (*Fragaria ananassa*) Coated with Chitosan-based Edible Coatings. **Journal of Food Science,** v. 70 n. 3, 2005.

IPPOLITO, A.; EL GHAOUTH, A.; WILSON, C. L.; WISNIEWSKI, M. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. **Postharv. Biol. Technol**, v. 19 p. 265-272, 2000.

JIANG, Y.; LI, Y. Effects of chitosan coating on postharvest life and quality of longan fruit. **Food Chemistry,** v. 73 p. 143-159, 2001.

KENDRA, D. F.; CHRISTIAN, D.; HADWIGER, L. A.**.** Chitosan oligomers from *Fusarium solani*/pea interactions, chitinase/β-glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance. **Physiological and Molecular Plant Pathology**, v. 35 n. 3 p. 215-230, 1989.

KOIDE, S. S. Chitin-chitosan: properties, benefits and risks. **Nutrition Research.** v. 18 n. 6 p. 1091-1101, 1998.

LEE, C. H.; AN, D. S.; PARK, H. J.; LEE, D. S. Wide-spectrum antimicrobial packaging materials incorporating nisin and chitosan in coating. **Packaging Technology and Sciencethe,** v. 16 p. 99 – 106, 2003.

LI, P.; BARTH, M. M. Impact of edible coating on nutritional and physiological changes in lightly-processed carrot. **Postharvest Biology and Technology**, v.14 p. 51-60, 1998.

LI, B.; KENNEDY, J. F.; PENG, J. L.; YIE, X.; XIE, B. J. Preparation and performance evatuation of glucomannan-chitosan-nisin ternary antimicrobial blend film. **Carbohydrate Polymers**, v. 65 p. 488-494, 2006.

MARTÍNEZ-GONZÁLEZ, B. E.; TREVIÑO-GÓMEZ, M.; SALAS-JIMÉNEZ, Z. Bacteriocinas de probióticos. **Revista Salúd Pública y Nutrición**. v. 4 n. 2, 2003.

NES, I. F.; DIEP, D. B.; HAVARSTEIN, L. S.; BRURBERG, M.; EIJSINK, V.; HOLO, H. Biosynthesis of bacteriocins in lactic acid bacteria. **Antonie Van Leeuwenhoek.** v. 70 n. 2 p. 113-128, 1996.

NETO, L. G. G.; SOUZA, M. R.; NUNES, A. C.; NICOLI, J. R.; SANTOS, W. L. M. Atividade antimicrobiana de bactérias ácido-láticas de queijos de coalho artesanal e industrial frente a microrganismos indicadores. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia,** v. 57 n. 2 p. 245 - 250, 2005.

NO, H. K.; PARJ, N. Y.; LEE, S. H.; MEYERS, S. P. Antibacterial activity of chitosan and chitosan oligomers with different molecular weights. **International Journal of Food Microbiology,** v.74 p.65-72, 2002.

OLIVEIRA, T. M.; SOARES, N. D. F.; PEREIRA, R. M.; FRAGA, K. D. Development and evaluation of antimicrobial natamycin-incorporated film in Gorgonzola cheese conservation. **Packaging Technology and Science**, v. 20 p. 147-153, 2007.

O'SULLIVAN, L.; ROSS, R. P.; HILL, C. Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. **Biochimie**, v. 84 p. 593-604, 2002.

PAPINEAU, A. M.; HOOVER, D. G.; KNORR, D.; FARKAS, D. F. Antimicrobial effect of water-soluble chitosan with high hydrostatic pressure. **Food Biotechnol.**, v. 5 p. 45-57, 1991.

PARK S.I.; DAESCHEL M.A.; ZHAO, Y. Functional Properties of Antimicrobial Lysozyme-Chitosan Composite Films. **Journal of Food Science**, v. 69 n. 8, 2004.

PRANOTO, Y.; RAKSHIT, S. K.; SALOKHE, V. M. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. **LWT,** v. 38 p. 859-865, 2005.

QUINTAVALLA, S.; VICINI, L. Antimicrobial food packaging in meat industry. **Meat Science**, v. 62 p. 373-380, 2002.

REID, G. The Scientific Basis for Probiotic Strain of *Lactobacillus*. **Applied and Environmental Microbiology,** p. 3763-3766, 1999.

RIO GRANDE DO SUL. Secretaria de Agricultura e Abastecimento. Programa Estadual de Fruticultura (PROFRUTA). Disponível em: http://www.agricultura.rs.gov.br/. Acesso em: 23 jun. 2006.

ROMANAZZI, G.; NIGRO, F.; IPPOLITO, A. Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries. **Posthary. Biol. Technol**, v. 29 p. 73-80, 2003.

ROOT, T.; JOHNSON, E. L. A comparison of the use of chitosan and gelatin on the clarification of five blends of apple juice using both hot and cold treatment methods. In: MUZZARELLIi, M. M. A.; PARISER, E. R. (Eds.) MIT Sea Grant Program. Proceedings First International Conference on Chitin/Chitosan. **Massachusetts Institute of Technology**, Cambridge, MA, pp. 387-395, 1978.

SANJURJO, K.; FLORES, S.; GERSCHENSON, L.; JAGUS, R. Study of the performance of nisin supported in edible films. **Food Research International**, v. 39 p. 749-754, 2006.

SAPERS, G. M. Chitosan enhance control of enzymatic browning in apple and pear juice by filtration. **J. Food Sci.**, v. 57 p. 1192-1193, 1992.

SATHIVEL, S. Chitosan and Protein Coatings Affect Yield, Moisture Loss, and Lipid Oxidation of Pink Salmon (*Oncorhynchus gorbuscha*) Fillets During Frozen Storage. **Journal of Food Science**, v. 70 n. 8 p. 455-459, 2005.

SHAHIDI, F.; ARACHCHI, J. K. V.; JEON, Y. J. Food applications of chitin and chitosans. **Trends in Food Science & Technology**, v.10 p.37-51, 1999.

SOTO-PERALTA, N. V.; MULLER, H.; KNORR, D. Effects of chitosan treatments on the clarity and color of apple juice. **J. Food Sci**., v. 54 p. 495-496, 1989.

SUDARSHAN, N. R.; HOOVER, D. G.; KNORR, D. Antibacterial action of chitosan. **Food Biotechnol.**, v. 6 p. 257-272, 1992.

TAGG, J. R.; DAJANI, A. S.; WANNAMAKER, L. W. Bacteriocins of gram-positive bacteria. **Bacteriological Reviews**, Washington, v. 40 n. 3 p.722-756, 1976.

TURE, H.; EROGLU, E.; SOYER, F.; OZEN, B. Antifungal activity of biopolymers containing natamycin and rosemary extract against *Aspergillus niger* and *Penicillium roquefortii*. **International Journal of Food Science and Technology**, v. 43 p. 2026-2032, 2008.

U.S. FOOD AND DRUG ADMINISTRATION. Nisin Preparation: Affirmation of GRAS status as direct human food ingredient. Federal Register. 53, April 6, 1988.

VAR, I.; ERGINKAYA, Z.; GUVEN, M.; KABAK, B. Effect of antifungal agent and packaging material on microflora of Kashar cheese during storage period. **Food Control,** v.17 p. 132-136, 2006.

VARGAS, M.; GILLABERT, M.; GONZÁLVEZ-MARTÍNEZ, C.; ALBORS, A.; CHIRALT, A. Efecto de la aplicación de un film a base de quitosano en la calidad de fresas durante el almacenamiento. Actas del III Congreso Español de Ingeniería de Alimentos, [cd-room], 746-753, 2004.

WELLS, M. J.; LIAO, C. H.; HOTCHKISS, T. In vitro inhibition of soft-rotting bacteria by EDTA and nisin and in vivo response on inoculated fresh cut carrots. **The American Phytopathological Society**, p. 491-495, 1998.

WELSCHER Y. M.; NAPEL H. H.; BALAGUÉ, M. M.; SOUZA, C.; RIEZMAN H.; KRUIJFF, B.; BREUKINK, E. Natamycin Blocks Fungal Growth by Binding Specifically to Ergosterol without Permeabilizing the Membrane. **J. Biol. Chem**., v. 283 p. 6393-6401, 2007.

WESSELS, S.; JELLE, B.; NES, I. Bacteriocins of lactic acid bacteria. Report of the Danish Toxicology Centre, Denmark, 1998.

XIE, W. M.; XU, P. X.; LIU, Q. Antioxidant activity of water-soluble chitosan derivates. **Bioorg. Med. Chem. Lett.**, v. 11 p. 1699-1701, 2001.

YOUNG, D. H.; KOHLE, H.; KAUSS, H. Effect of chitosan on membrane permeability of suspension cultures *Glycine max* and *Phaseolus vulgaris* cells. **Plant Physiol.**, v. 70 p. 1449-1454, 1982.

ZHANG, D. L.; QUANTICK, P. C. Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Lichi chinensis* Sonn.) fruit. **Postharv. Biol. Technol**, v. 12 p. 195-202, 1997.

ZIVANOVIC, S.; CHI, S.; DRAUGHON, A. F. Antimicrobial Activity of Chitosan Films Enriched with Essential Oils. **Journal of Food Science**, v. 70 n.1, 2005.