VILMA BARBOSA DA SILVA ARAÚJO

OBTENÇÃO DE MANOPROTEINA E β-GLUCANA DE LEVEDURA DESCARTADA EM CERVEJARIA COM POTENCIAL PARA APLICAÇÃO EM ALIMENTOS

João Pessoa - PB

2014

VILMA BARBOSA DA SILVA ARAÚJO

OBTENÇÃO DE MANOPROTEINA E β-GLUCANA DE LEVEDURA DESCARTADA EM CERVEJARIA COM POTENCIAL PARA APLICAÇÃO EM ALIMENTOS

Dissertação apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal da Paraíba em cumprimento à exigências para obtenção do título de Mestre em Ciência e Tecnologia de Alimentos.

Orientadora: Dra. Marciane Magnani

João Pessoa - PB
2014

A663o Araújo, Vilma Barbosa da Silva.
Obtenção de manoproteína e β-glucana de levedura descartada em cervejaria com potencial para aplicação em alimentos / Vilma Barbosa da Silva Araújo.-- João Pessoa, 2014.
58f. : il.
Orientadora: Marciane Magnani
Dissertação (Mestrado) - UFPB/CT
1. Tecnologia de alimentos. 2. Cervejaria. 3. Leveduras. 4. Emulsificantes. 5. Formulações de maionese.

UFPB/BC CDU: 664(043)

VILMA BARBOSA DA SILVA ARAÚJO

OBTENÇÃO DE MANOPROTEINA E β-GLUCANA DE LEVEDURA DESCARTADA EM CERVEJARIA COM POTENCIAL PARA APLICAÇÃO EM ALIMENTOS

Aprovada em _______/_______/___________

Banca Examinadora

____

Prof. Dra. Marciane Magnani – PPGCTA/CT/UFPB
Orientadora

____

Prof. Dra. Marta Suely Madruga – PPGCTA/CT/UFPB
Examinadora interna

____

Profª. Dra. Thayza Christina Montenegro Stamford
Examinadora Externa

____

Profº. Dra. Lúcia Raquel Ramos Gerber
Examinadora Externa

A minha mãe (*in memorian*), Josefa Carneiro da Silva, pelo exemplo e dedicação. Sei que para ela valeu a pena tudo que viveu para ver suas filhas formadas. Deus esteve com ela durante toda sua vida (*27/11/1947 – †29/11/2011).

Dedico.

AGRADECIMENTOS

À Deus por me conhecer desde o ventre da minha mãe (Sl. 139:16) e por ter demonstrado Seu tão grande amor por mim (Jo. 3:16), sempre O adorarei.

Agradeço a minha mãe Josefa Carneiro da Silva (*in memorian*), pelo seu cuidado, amor, confiança, foram tantos momentos que não voltarão, mas que valeram a pena tudo que passamos juntas, gostaria mesmo que esse dia em que você partiu tivesse demorado mais a chegar, mas aprouve a Deus decidir isto, e sei que todas as cosias cooperam para o bem daqueles que O amam. Ao meu pai José Barbosa da Silva, por ter me dado uma educação segundo os conselhos de Deus, pelo incentivo, cuidado, amor, hoje já com a idade avançada somos nós que cuidamos do senhor. Muito obrigada!

Ao meu amado esposo Fabiano Juvino pelo companheirismo, amor, cuidado, palavras de incentivo e principalmente pela paciência, por tudo que temos vivido juntos, TE AMO MUITO!!!

Aos meus sogros, Luiz Juvino e Adelaide Araújo (*in memorian*), por terem me recebido com tanto carinho em sua família e pelo incentivo.

Aos meus irmãos: Vânia Barboza, por estar sempre presente em minha vida, sendo exemplo e me incentivando em tudo, desde a escolha do curso até hoje; a Verônica Barbosa, minha irmã caçula, sempre presente em minha vida; e Joaquim Barbosa (*In memorian*), pelas brincadeiras de criança que deixaram muita saudade.

Aos meus cunhados (Josinaldo Andrade, Geanderson Freitas), cunhada (Elaine Cristina) e sobrinhos (Lucas, Letícia, Beatriz, Baruc e João), pelo apoio sempre.

A Igreja Batista Esperança Viva (IBEV), na pessoa da Prª Maria do Socorro Tavares, pelo apoio e orações pela minha vida. Não Tenho palavras para a agradecer a IBEV, amo cada um que faz parte dela. Quero agradecer em especial a RJA (Rede de Jovens e Adolescentes) com quem tenho aprendido como ser uma líder melhor.

A minha orientadora Professora Dra. Marciane Magnani, saiba que és um exemplo de dedicação, seriedade, determinação, paciência, agradeço pela credibilidade depositada em mim desde o início deste trabalho. Muito obrigada!

Aos professores Dr. Evandro Leite e Dra. Tânia Stamford, por participarem da Qualificação e as professoras, Dra. Marta Suely Madruga, Dra. Thayza Stamford e Dra. Lúcia Raquel Berger, pela disponibilidade em participar da defesa, além das sugestões e contribuições neste trabalho.

À turma de Pós-Graduação do PPGCTA 2012 pelos momentos juntos, foi muito bom conhecer cada um (Mestrado: Daniel Farias, Marília Cícera, Thaisa Abrantes, Jéssica Ouriques, José Evangelista, Liliane Andrade, Aline Souza, Éricka Oliveira, Amanda Kelle, Claudiane Magliano; Doutorado: Katya Anaya, Cassiara Souza, Fábio Silva, Luciana Trigueiro).

As amigas Fátima Azevedo, Gisele Pequeno e Liliane Andrade que sempre me apoiaram e auxiliaram para que a caminhada se tornasse mais leve.

A aluna de Iniciação científica Adma Melo, por tudo que passamos durante as análises, foi bem mais leve com a sua alegria, rimos muito.

Ao colega Antônio Júnior pelo auxílio na empresa que disponibilizou a matéria-prima para este trabalho e ao Engenheiro Hélio.

À equipe de professores da Pós-graduação em Ciência e Tecnologia de Alimentos pelos ensinamentos doados, apoio e incentivo no curso das disciplinas.

À Universidade Federal da Paraíba, em especial ao Programa de Pós-graduação em Ciência e Tecnologia de Alimentos pela recepção e oportunidade concedida para a realização do Mestrado.

Aos funcionários que cuidam da limpeza e organização do bloco da Pós- graduação em Ciência e Tecnologia de Alimentos.

Aos Técnicos de laboratório do bloco da Pós- graduação em Ciência e Tecnologia de Alimentos (Gilvandro, Claudionor, June, Eunice) pelo apoio nesta pesquisa.

A CAPES (Coordenação de Aperfeiçoamento de Pessoal e Nível Superior), pelo suporte financeiro.

Ao CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) pelo financiamento do projeto.

Agradeço a todos que de uma forma direta ou indireta me abençoaram na realização do mestrado!

Tudo isso foi propósito de Deus na minha vida, a Ele toda honra e Glória (Rm. 8:37)!!!

Mas, buscai primeiro o Reino de Deus, e a sua justiça, e todas essas coisas vos serão acrescentadas" Mt. 6:33.

RESUMO

Leveduras do gênero *Saccharomyces* spp. são empregadas como ferramenta biotecnológica, na produção de cerveja e após em média cinco processos fermentativos de produção, são descartadas pela indústria cervejeira. A parede celular de leveduras é formada principalmente pelas β-glucana, que são homopolímeros de glicose capazes de alterar a resposta imune no hospedeiro, e pelas manoproteínas (MP) que possuem reconhecidas propriedades emulsificantes. No presente estudo foi proposto obter estas duas frações da parede celular de levedura descartada em cervejeira empregando uma etapa adicional para obtenção da MP em um processo aplicável a extração da β-glucana. As frações obtidas foram caracterizadas e a MP avaliada quanto ao potencial para aplicação como emulsificante em formulações de maionese. A partir da parede celular de levedura em base seca, o rendimento da MP e da β-glucana foi de 4,16% e 10%, respectivamente. A umidade residual da β-glucana foi de 4,9 ± 0,1% e da MP foi de 3,9 ± 0,9% e não foram observadas alterações neste parâmetro durante 28 dias de armazenamento em embalagens de polipropileno bi-orientado (BOPP) a 10 °C, 25 °C ou 40 °C. O perfil de SDS-PAGE da MP evidenciou proteínas de peso molecular de 58 kDa e 64 kD e o maior teor de aminoácidos constituintes foi observado para a valina (8,9%), ácido aspártico (8,2%) e leucina (6,9%). Cada 100 g de maionese preparada com MP nas concentrações de 0,6%; 0,8% e 1,0% continham, em média, 25 ± 0,27g de umidade, 1,6 ± 0,10 g de cinzas, 2,4 ± 0,04 g de proteína, 56 ± 0,41 g de lipídeos e 14 ± 0,12 g de carboidratos, e não foram observadas alterações nos atributos aroma, cor, sabor, textura no período analisado (28 dias). Os resultados obtidos evidenciam aplicabilidade do método proposto para obtenção de MP e β-glucana e sugerem que a MP possui potencial para aplicação como bioemulsificante em alimentos, em especial em formulações de maionese.

Palavras-chave: cervejaria, leveduras, emulsificantes, maionese.

ABSTRACT

Yeasts of the genus *Saccharomyces* spp. are used as biotechnological tool in brewery and after five fermentative production processes; they are discarded by the beer industry. The cell wall of yeasts is mainly composed of β - glucan, which is a glucose homopolymer capable of changing the immune response in the host, and by manoproteins (MP), which have recognized emulsifying properties. The aim of the present study was to obtain these two cell wall fractions of discarded yeast employing an additional step to obtain MP in a process applicable to the extraction of β - glucan. The fractions obtained were characterized and MP was evaluated for potential for application as an emulsifier in mayonnaise formulations. From the cell wall of yeasts on dry basis, the MP and β – glucan yield was 4.16% and 10 %, respectively. The residual moisture of β - glucan was 4.9 ± 0.1 % and MP was 3.9 ± 0.9 % and no changes in this parameter were observed during 28 days of storage in bi-oriented polypropylene packs (BOPP) at 10°C, 25°C or 40° C. The SDS-PAGE profile of MP showed proteins with molecular weight of 58 kDa and 64 kDa, and the highest content of constituent amino acids was observed for valine (8.9%), aspartic acid (8.2%) and leucine (6.9%). Each 100 g of mayonnaise prepared with MP at concentrations of 0.6%, 0.8 % and 1.0 % contained, on average, 25 ± 0.27 g moisture, 1.6 ± 0.10 g ash, 2.4 ± 0.04 g protein, 56 g ± 0.41 lipid and 14 ± 0.12 g carbohydrates, and no changes in attributes aroma, color, flavor and texture were observed in the study period (28 days). The results showed applicability of the method proposed for obtaining β -glucan and MP and suggest that MP has potential for application as bioemulsifier in foods, particularly in mayonnaise formulations.

Keywords: brewery, yeasts, emulsifiers, mayonnaise.

LISTA DE ILUSTRAÇÃO

Figura 1. Estrutura da β-glucana de levedura (adaptado de VOLMAN et al., 2008). 18

Figura 1. Estrutura da manoproteína de parede celular de *Saccharomyces* sp. (adaptado de CORBACHO et al., 2005). 20

Figure 1. Infrared spectrum of β-glucan extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes. 41

Figure 2. SDS-PAGE profile of mannoprotein extracted from cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes. 42

LISTA DE TABELAS

Table 1. Amino acid composition of themannoprotein extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes. 42

Table 2. Emulsifying activity (EA) and emulsion stabilizing activity (SA) of mannoprotein extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes. 42

Table 3. The observed stability ofmayonnaise formulations during 28 days of refrigerated storage that were prepared with mannoprotein extracted from the cell wall of brewer's yeast (Saccharomyces uvarum) after successive fermentation processes. 42

Table 4. Color analyses of different mayonnaise formulations during 28 days of refrigerated storage that were prepared with mannoprotein extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) after successive fermentation processes. 43

SUMÁRIO

1 INTRODUÇÃO 13
2 REVISÃO DA LITERATURA 14
2.1 PAREDE CELULAR DE LEVEDURAS 14
2.2 β-GLUCANAs DE LEVEDURAS 16
2.3 MANOPROTEÍNAS DE LEVEDURA 19
3 MATERIAIS E MÉTODOS 22
3.1 MATERIAL 22
3.2 ASPÉCTOS ÉTICOS 22
3.3 AUTÓLISE E TRATAMENTO DO CREME DE LEVEDURA 22
3.4 EXTRAÇÃO DA β-GLUCANA 23
3.5 EXTRAÇÃO DA MANOPROTEÍNA 23
3.6 SECAGEM E RENDIMENTO DA β-GLUCANA E DA MANOPROTEÍNA 23
3.7 CARACTERIZAÇÃO DA β-GLUCANA 24
3.8 CARACTERIZAÇÃO DA MANOPROTEÍNA 24
3.9 AVALIAÇÃO DA β-GLUCANA E DA MANOPROTEÍNA DURANTE ARMAZENAMENTO 25
3.10 AVALIAÇÃO DA ATIVIDADE EMULSIFICANTE DA MANOPROTEÍNA 25
3.11 AVALIAÇÃO DA MANOPROTEÍNA COMO SUBTITUTO DE GOMA XANTANA EM FORMULAÇÃO DE MAIONESE 25
3.12 CARACTERIZAÇÃO DA MAIONESE ELABORADA COM MANOPROETEINA 26
3.13 ANÁLISE SENSORIAL 26
3.14 ANÁLISES ESTATÍSTICAS 27
REFERÊNCIAS 28
4 RESULTADOS E DISCUSSÃO 37
ARTIGO: Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the obtained mannoprotein as a stabilizer in mayonnaise. 38

ANEXOS 45

1 INTRODUÇÃO

A progressiva demanda dos consumidores por alimentos mais naturais, caracterizados por serem livres ou com baixos níveis de aditivos químicos, porém apresentando longa e estável vida da prateleira, resulta na busca contínua de alternativas para suprir esta demanda. Neste contexto, é de suma importância estudar a obtenção de compostos naturais, ou biocompostos, que possam ser empregados na formulação dos alimentos em substituição aos sintéticos, normalmente utilizados na indústria de alimentos. Além disso, existe atualmente uma preocupação com os subprodutos gerados em processos produtivos industriais e seu aproveitamento tem sido alvo de inúmeras pesquisas. Na indústria cervejeira leveduras do gênero *Saccharomyces* são empregadas como ferramenta biotecnológica e após, em média, cinco processos fermentativos de produção, são descartadas. Assim, toneladas de biomassa de levedura são geradas e descartadas a cada ano, sendo que a maior parte deste subproduto é destinada a alimentação animal e vendida a baixo custo. Entretanto, devido à baixa digestibilidade dos componentes da parede celular de leveduras, inúmeros estudos vêm explorando o isolamento de seus constituintes celulares como enzimas, proteínas, polissacarídeos e lipídios.

A parede celular de *Saccharomyces* spp. é formada principalmente por β-glucana, um homopolímero de glicose que possui a capacidade de modular a resposta imune no organismo, classificado como modulador da resposta biológica. O segundo maior componente da parede celular é a manoproteína (MP), que vem recebendo atenção da comunidade científica especialmente devido a sua estrutura anfipática, que lhe confere propriedades emulsificantes. Assim sendo, estes dois principais constituintes da parede celular da levedura descartada em cervejarias, possuem potencial para ser utilizados em alimentos agregando valor a um subproduto industrial. No entanto, para que estas duas frações da parede celular mantenham suas propriedades é necessário que o processo de extração seja o menos agressivo possível a estrutura original dos polímeros, pois sua modificação pode comprometer não só o rendimento, como também a funcionalidade. Considerando estes aspectos, o presente estudo foi desenvolvido para investigar a possibilidade de obtenção de β-glucana e MP da parede celular de levedura descartada em cervejaria partindo do mesmo processo de extração, sem o emprego de lavagens ácidas e ou alcalinas, que sabidamente danificam sua estrutura original e que permitisse a obtenção da MP pela adição de uma etapa no processo de extração da β-glucana, e avaliar o potencial de aplicação da MP como emulsificante em formulação de maionese.

2 REVISÃO DA LITERATURA

2.1 PAREDE CELULAR DE LEVEDURAS

Leveduras são fungos unicelulares capazes de crescer em diversas fontes de carbonos gerando um grande volume de biomassa, com amplo potencial de exploração comercial. As leveduras são utilizadas em vários processos biotecnológicos, como produção de produtos de panificação, farmacêutica, cerveja, vinhos e outras bebidas alcoólicas (SCENI, et al., 2009).

Dentre as leveduras com potencial emprego biotecnológico, destaca-se o gênero *Saccharomyces* devido a sua ampla aplicação na indústria, sobretudo em cervejarias. Leveduras deste gênero caracterizam-se pelo rápido crescimento, boa capacidade de produção de etanol e uma elevada tolerância ao estresse ambiental, como alta concentração de etanol e baixos níveis de oxigênio (PISKUR, 2004).

Em cervejarias as leveduras do gênero *Saccharomyces* são empregadas de acordo com o tipo de fermentação desejada (baixa ou “larger”; alta ou “ale”). As leveduras utilizadas em fermentações do tipo “lager” (*S. uvarum*) tendem a formar uma massa no fundo dos tanques, já as do tipo “ale” (*S. cerevisae*) tendem a fermentar na superfície dos tanques fermentadores. Porém, após o processo fermentativo as leveduras são descartadas gerando grandes volumes de células excedentes (ZECHNER-KRPAN, 2010; COSTA et al., 2012). Esta realidade impõe a busca de alternativas para utilização deste subproduto para atenuar os impactos ambientais, e consequentemente, também diminuir os custos de tratamento de resíduo-efluentes das indústrias.

As células excedentes de levedura descartadas em cervejaria podem ser utilizadas diretamente ou processadas para obtenção de derivados, como extratos proteicos. A composição de aminoácidos de leveduras é estritamente balanceada com destaque para os elevados teores de ácido glutâmico, aspártico, leucina e alanina (SGARBIERI et al., 1999).

Entretanto, embora as células inativas de leveduras sejam destinadas à alimentação animal, como fonte de proteínas e minerais, sua utilização pelo organismo é limitada pela parede celular, que é resistente às enzimas digestivas (VILELA et al., 2000; COSTA et al., 2012). A biomassa de leveduras também pode ser utilizada pela indústria na produção de concentrados e hidrolisados, estes são encontrados no mercado em forma de pós, comprimidos ou na forma líquida, mantendo suas propriedades como elevado teor proteico, vitaminas do complexo B, minerais e fibra dietética (FERREIRA, 2010). Nas últimas décadas, tem sido

observada uma tendência crescente na separação dos constituintes das leveduras para obtenção de enzimas, MPs, glucanas e mananas, além de lipídios como ergosterol (COSTA et al., 2012).

Nos últimos anos, tem-se explorado a biomassa de levedura através do processamento das células e obtenção de extratos, os quais são obtidos após processos mecânicos ou autólise enzimática, uma vez que constituem uma valiosa fonte de proteínas, lipídeos e principalmente de polissacarídeos que apresentam diversas propriedades benéficas para a saúde humana (FUKUDA et al., 2009).

Em *S. cerevisiae* é composta principalmente por β-glucana, caracterizado como um homopolímero de glicose com ligações β(1-3)/β(1-6) e MPs, que são unidades de manose unidas a proteínas. A distribuição destes componentes na parede celular, está organizada em duas camadas principais, sendo a externa onde encontram-se os polissacarídeos de manose, que unem-se a proteínas para formar uma camada de MPs e a interna de β-glucana e quitina, em uma estrutura interconectada por ligações covalentes (KOLLAR et al., 1997; CABIB et al., 2001; KLIS et al., 2002; LIU et al., 2008).

A camada externa de leveduras, composta de MPs, representa de 20 a 23% do peso seco da parede celular. Estas são altamente glicosiladas, com uma fração de carboidratos que perfazem mais de 90% do total (ORLEAN, 1997). As cadeias de carboidratos das MPs contêm múltiplas pontes fosfodiéster, resultando em numerosas cargas negativas na superfície celular em valores fisiológicos de pH. Esta porção é responsável pelas propriedades hidrofílicas da parede e pode estar envolvida na proteção contra o ressecamento, devido a retenção de água (KLIS et al., 2002; KIM; YUN, 2006). As MPs presentes na camada externa são responsáveis por limitar a ação de enzimas produzidas por outros microrganismos, conferindo resistência as células vivas de leveduras. A presença de cadeias laterais de β(1-3)-glucana formada através de ligações de hidrogênio, representando uma rede tridimensional contínua, confere elasticidade a parede celular de leveduras em condições normais osmóticas. Porém quando expostas a meios hipertônicos tendem a encolher podendo perder até 60% do seu volume original (MORRIS, 1986; KLIS et al., 2006). Por sua vez, a β(1-6) glucana serve de apoio para a estrutura e integridade da camada de MPs (ROEMER et al., 1994), sendo sua síntese inculada a expressão de genes de proteínas especificas e características da parede celular (SIMONS; EBERSOLD; HELENIUS, 1998).

A parede celular das leveduras pode sofrer alterações quando submetida a condições ambientais inadequadas como variação na disponibilidade de nutrientes, variações no pH, temperatura, disponibilidade de hidrogênio e na composição do meio de cultivo. Estudos

demonstraram que esses fatores influenciam a quantidade de polissacáridos que a parede celular secreta para o meio de fermentação (GUILLOUX-BENATIER et al., 1995; ROSI, 2003).

Alguns estudos fornecem evidências de que a parede celular não é uma estrutura estática, que em reposta as adaptações, a parede celular de leveduras é reorganizada na tentativa de se regenerar. A liberação de polissacarídeos por células de leveduras viáveis poderia, portanto, ser uma consequência da remodelação da parede celular (KLIS et al., 2002; KLIS et al., 2006; LIU et al., 2008; ARROYO et al., 2009). Desta forma, condições de fermentação, como aquelas aplicadas na produção de cerveja, influenciam na composição da parede celular e consequentemente na quantidade de cada constituinte presente.

Diversos métodos têm sido propostos para extração eficiente da β-glucana e a MP da parede celular de leveduras (LIU et al., 2013). De modo geral, para extração da β-glucana e da MP da parede celular de *S. cerevisiae*, são descritos processos que utilizam lavagens ácidas e alcalinas. Esses métodos oferecem um bom rendimento, mas podem comprometer a estrutura original dos polímeros, alterarando sua estrutura e, consequentemente, suas propriedades específicas (AKLUJKAR, 2008). Tratamentos térmicos com água e enzimas aplicados com a finalidade de extrair MP e β-glucana de *Saccharomyces* spp. tem sido avaliados, visto que preservam a estrutura nativa dos polímeros, não afetando a composição e as suas propriedades. Adicionalmente, métodos que dispensam o uso de soluções ácidas e alcalinas resultam em efluentes menos nocivos ao meio ambiente (LIU et al., 2008).

Com o objetivo de otimizar a extração da β-glucana presente na parede de *S. cerevisiae,* Magnani et al. (2009) descreveram um método que permite obter o polímero puro, sem empregar tratamentos drásticos ou gerar efluentes nocivos ao meio ambiente, utilizando sonicação e tratamento enzimático após extração com água quente. Neste mesmo processo a MP é separada durante a etapa inicial de extração da β-glucana, onde o material autolizado é submetido a tratamento térmico (121ºC; 5h). Após a centrifugação do material tratado, as MPs ficam no sobrenadante que é descartado para dar sequência à extração da β-glucana (FREIMUND et al., 2003). Assim, uma etapa de precipitação com solução de etanol, permitiria separar a MP.

2.2 β-GLUCANA DE LEVEDURAS

As β-glucanas são homopolímeros de glicose formados por uma cadeia principal de glicose ligadas na posição β-(1-3) com ramificações laterais e glicose unidas em β-(1-6) de

tamanhos variados, os quais podem ocorrer em diferentes intervalos ao longo do esqueleto central (MAGNANI, 2009; PETRAVIĆ-TOMINAC, 2010).

A β-(1-3)-glucana encontrada na parede celular de *S. cerevisiae* possui uma porção solúvel e outra insolúvel. A porção insolúvel contem de 3 a 6% de ramificações unidas em β-(1-6) sendo o maior componente da parede celular. A parte solúvel, por sua vez, representa de 15 a 20% das β-glucanas presentes na parede celular e tem estrutura semelhante à porção insolúvel, mas com número maior de ramificações β-(1-6) (MANNERS et al., 1973; MAGNANI; CASTRO-GOMEZ, 2008). Quanto a solubilidade em água, depende principalmente do número de resíduos de glicose ligados em β-(1-6) das cadeias laterais (KLIS, et al., 2002).

As β-glucanas (Figura 1) pertencem a uma classe conhecida como MRB's (modificadores da resposta biológica), pois são capazes de alterar no hospedeiro a resposta biológica pelo estímulo do sistema imune. Esta atividade está relacionada às características do polímero como tipo de ligações glicosídicas, peso molecular, conformação espacial, grau de polimerização e de ramificação (DIETRICH-MUSZALSKA et al., 2011). A β-glucana é obtida a partir da parede de leveduras como um polímero insolúvel em água, e sabe-se que a derivatização da molécula pode, além de facilitar seu uso, potencializar sua bioatividade (CHEN; SEVIOUR, 2007). Dentre os derivados solúveis, sabe-se que a forma carboximetilada (CM-G), tem efeito modulador da resposta imune inata e adaptativa (BABINCOVÁ et al., 2002; MIADOKOVÁ et al., 2005; MAGNANI et al., 2011a, 2011b).

β-glucanas com elevado grau de ramificações são consideradas solúveis, sendo mais eficiente em sua bioatividade (CHORVATOVICOVA, et al., 1996; PETRAVIĆ-TOMINAC, et al., 2010). Assim, supostamente, β-glucanas com elevado grau de ramificações e, consequentemente solúveis, seriam mais eficientes na ativação da resposta imune do hospedeiro.

A relação entre o peso molecular da β-glucana e sua bioatividade não é totalmente conhecida, mas é sabido que glucanas de alto peso molecular ativam leucócitos, estimulando a atividade fagocítica e citotóxica, bem como a produção de mediadores pro-inflamatórios como citocinas e quimiocinas (WILLIAMS, et al., 1996; BROWN; GORDON, 2003). Glucanas de peso molecular intermediário ou baixo possuem bioatividade, porém seus efeitos são menos conhecidos *in vivo* (BROWN; GORDON, 2003).

A β-glucana ativa a resposta imune via sistema complemento, diretamente ou, com auxílio de anticorpos, e produzem fatores quimiotáticos que induzem a migração de leucócitos para o sítio da infecção (LIU et al., 2008). O reconhecimento da β-glucana pelo sistema imune

inato dos vertebrados ocorre através de receptores de superfície celular, já identificados em células imunes como macrófagos/monócitos, neutrófilos e *natural killer* (NK). Diversos receptores estão relacionados a este reconhecimento, incluindo dectin-1, Receptor do Sistema Complemento 3 (CR3), lactosil ceramida e *scavenger receptors*. Os mecanismos envolvidos na resposta imune desencadeada incluem estímulo da hematopoiese, ativação de macrófagos, neutrófilos e células NK. Além disso, envolvem a resposta imune específica pela indução da expressão de diversas citocinas (BROWN; GORDON, 2005).

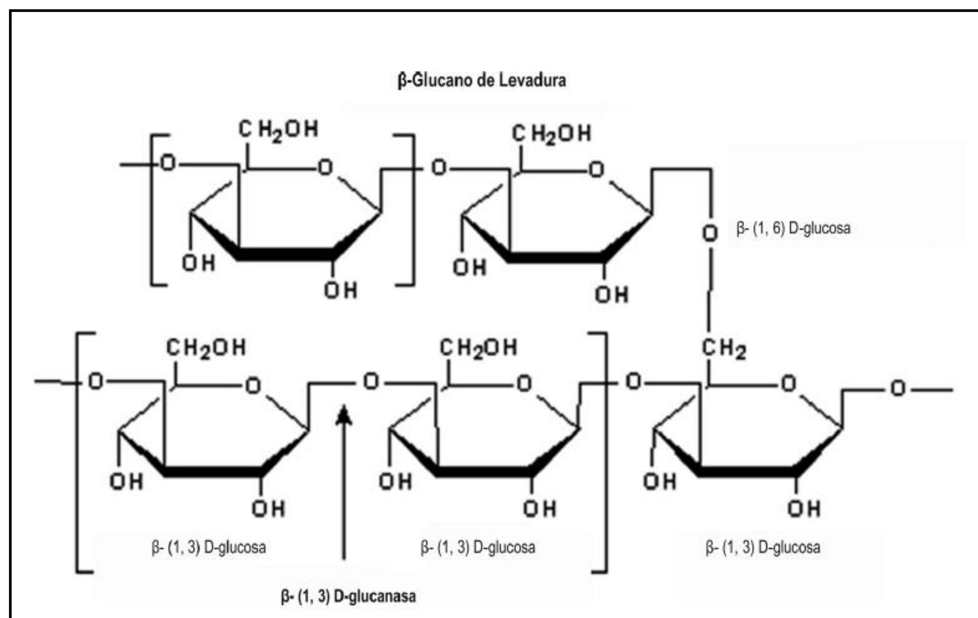

Figura 1. Estrutura da β-glucana de levedura (adaptado de VOLMAN et al., 2008).

Várias formas de administração de β-glucana vem sendo estudadas para verificar a atividade biológica deste composto, incluindo a administração intraperitoneal, subcutâneas, intravenosa e oral (VETVICKA; VETVICKOVA, 2008; MAGNANI et al., 2010). Vale destacar também que o uso oral de β-glucana, mesmo em pacientes com diversos tipos de câncer ou debilitados por outras doenças, não apresenta quaisquer efeitos adversos. Aparentemente, a β-glucana obtida da parede celular de leveduras parece ser mais efetiva do que aquela obtida de outras fontes como aveia e cogumelos (DEMIR et al., 2007; WEITBERG, 2008; MAGNANI et al., 2010). Administrada em cápsulas à pacientes com câncer de próstata em estado avançado e terminal, a β-glucana extraída da parede celular de levedura (*S. cerevisiae)*, mostrou propriedades imunomoduladoras, além de estímulo da hematopoiese (MAGNANI et al., 2010, 2011a).

Estudos têm demonstrado que a β-glucana possui capacidade para eliminação de radicais livres sendo capaz de melhorar a resposta antiinflamatória (PELIZON, et. al., 2005; DIETRICH-MUSZALSKA, et al., 2011; SALUK, et. al., 2013). Foi relatado que este polímero possui significativa propriedade de defesa contra os oxidantes fisiológicos ou radicais livres como o hidroperóxido da peroxidação lipídica induzida no sangue humano (SALUK et al., 2013). Também já foi verificado que a β(1-3)-glucana carboximetilada da parede celular de *S. cerevisiae* apresentaram atividade antioxidante atuando na eliminação de radicais livres. Ainda, é relatado que a administração oral de (1-3)/(1-6)-glucana solúvel extraída de *S. cerevisiae* também pode acelerar a recuperação de monócitos no sangue periférico de ratos com leucopenia (KOGAN et al., 2005; HARNACK, et. al., 2010).

A β-glucana de levedura também tem se destacado entre os ingredientes utilizados para produção de alimentos funcionais. Adicionada em suco de laranja reduziu significativamente o colesterol total e os níveis séricos de colesterol LDL em pacientes obesos hiperolesterolêmicos (NICOLOSI et al., 1999).

Assim, a β-glucana adicionada a alimentos poderia ampliar o consumo e facilitar a aceitação da população em geral, deixando de ser consumida somente por indivíduos debilitados por doenças. Devido às propriedades antigenotóxicas, o consumo da β-glucana de levedura pode inclusive prevenir danos ao DNA, que estão associados a inúmeras doenças relacionadas a danos oxidativos, incluindo o câncer (MAGNANI et al., 2011b).

2.3 MANOPROTEÍNAS DE LEVEDURA

Manoproteínas são glicoproteínas, compostas de proteínas ligadas covalentemente, contendo mais de 90% de manose, e estão localizados na camada mais externa da parede celular da leveduras, representando cerca de 35 e 40% do seu peso seco (QUIRÓS, 2010).

Na parede celular de *Saccharomyces spp.*, as MPs estão organizadas de acordo com sua estrutura química, são compostas por aproximadamente de 10% de proteínas e 90% de carboidratos, na maioria fosforilados, e conectados a proteínas por ligações tipo N unidos a resíduos de asparagina, ou compostas especialmente por cadeias curtas de manose, com até cinco unidades deste monossacarídeo, apresentando ligações do tipo O unidas a resíduos de serina ou treonina, conectando manana à proteína (BARRIGA et al., 1999). A figura 2 contempla a estrutura das MPs com ligações tipo N e tipo O.

Devido a sua estrutura química das Mp, que apresentam uma porção hidrofílica e outra hidrofóbica, que lhe conferem a capacidade de reduzir à tensão superficial e interfacial de

misturas entre compostos imiscíveis, e consequentemente propriedades emulsificantes (BERTON, et al., 2011).

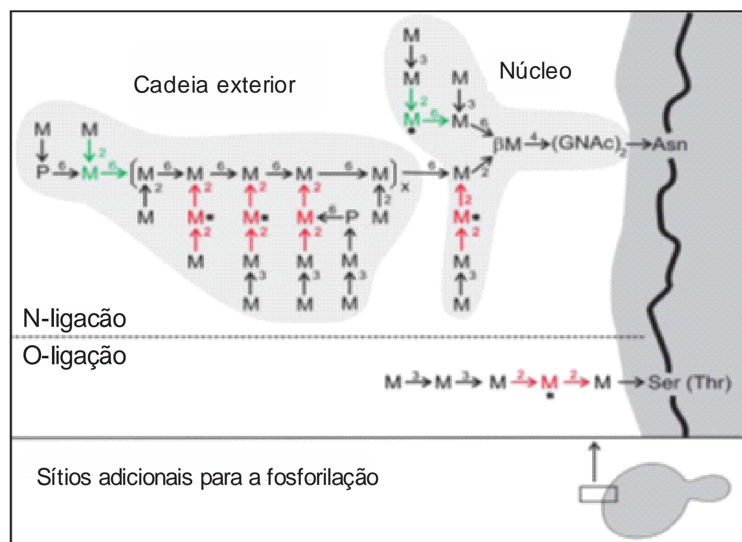

Figura 2. Estrutura da manoproteína da parede celular de *Saccharomyces* ssp. (adaptado de CORBACHO et al., 2005)

Os emulsificantes extraídos de microrganismo têm sido de grande importância para indústria de alimentos, devido as suas características como: tolerância ao pH e temperatura, força iônica, biodegradabilidade e baixa toxicidade (NITSCHKE; COSTA, 2007; DARPOSSOLO, et al., 2012). As vantagens das MPs de leveduras em relação aos emulsificantes sintéticos, é que além de biodegradáveis, evitando acúmulos ambientais e aumentando o potencial de aplicação industrial (AMARAL et al., 2006), não apresentam toxicidade e podem ser obtidas a partir de um subproduto industrial de baixo custo (DARPOSSOLO, et al., 2012).

Nos países industrializados, o consumo de emulsificante de origem petroquímica está entre 70 e 75%, entretanto em países em desenvolvimento o uso de compostos artificiais ou sintetizados quimicamente, está sendo substituídos por aditivos de origem natural. Em adição, a preocupação com os efeitos alérgicos que esses aditivos artificiais podem causar a população, direciona as pesquisas relacionados ao uso de emulsificantes de origem microbiana (CAMEOTRA; MAKKAR, 1998; CAMEOTRA; MAKKAR, 2004; BARROS et al., 2008, SHIM et al., 2011).

Em alimentos as principais funções de emulsificantes são, aeração, volume, viscosidade, e consistência do produto além de proporcionar estabilidade a espumas, além e melhorar a palatabilidade além de influenciar indiretamente o sabor dos produtos (SUMANA, 2009).

Diversos estudos relatam a atividade emulsificante (AE) das MPs extraídas da parede celular de *Saccharomyces* (TORABIZADEH et al., 1996; BARRIGA et al.,1999; COSTA et al., 2012; DARPOSSOLO et al., 2012). Nestas leveduras, a presença de polímeros hidrofílicos de manose ligados covalentemente a porção proteica confere as MPs uma estrutura anfipática, característica de emulsificantes. Na estrutura a AE é decorrente da fração proteica e potencializada pela manana, que sozinha não é capaz de formar emulsão (BARRIGA et al., 1999).

Torabizadeh et al. (1996) relataram que a atividade emulsificante da MP extraída de *S. cerevisiae* (AE 77%) é semelhante a do caseinato de sódio (AE 79%), amplamente empregado pela indústria de alimentos. Ainda, as emulsões de MP mostram boa estabilidade durante armazenamento de 30 dias a 4ºC. Em seus estudos, Costa et al. (2012) comprovaram que o etanol residual do processo fermentativo da produção de cerveja não interfere na obtenção da parede celular da levedura descartada, nem nas propriedades emulsificantes das MPs extraídas. Conforme Darpossolo et al., (2012), emulsões formadas com MPs extraídas de leveduras descartadas em cervejaria, além de boa estabilidade em condições físicas e químicas comuns em indústrias alimentícias, têm atividade emulsificante de 82,33% em concentração de apenas $5g.L^{-1}$. De acordo com Melo et al. (2012), a MP extraída de levedura cervejeira em diferentes concentrações (0,4; 0,5; 0,6 e 0,7 g), resulta na obtenção de emulsões de boa estabilidade, com índices de atividade emulsificante de 98,23 ± 2,0 %.

Em substituição a lecitina de soja em molho para salada tipo *frensh*, a MP permitiu a manutenção da estabilidade do molho durante 28 dias de armazenamento refrigerado, sendo que, a formulação elaborada com 0,8 % de MP diferiu significativamente ($p \leq 0,5$) dos valores daquelas contendo 0,8 % de Lecitina de soja após 1 e 28 dias de armazenamento refrigerado, apresentando melhor aceitação para todos os atributos sensoriais analisados sem afetar negativamente as características sensoriais do molho (ARAÚJO et. al., 2013)

Considerando esses aspectos, é necessário a busca por alternativas para o reaproveitamento dos excedentes de biomassa de *S. cerevisiae* gerados pela indústria cervejeira e a obtenção dos constituintes da parede celular de leveduras descartadas em cervejarias, particularmente a MP é uma alternativa promissora como emulsificante.

3 MATERIAIS E MÉTODOS

3.1 MATERIAL

Saccharomyces uvarum foi coletada após processo de baixa fermentação para produção de cerveja em suspensão denominada creme de levedura (± 20% sólidos totais), gentilmente cedida por uma indústria cervejeira com unidade em João Pessoa, Paraíba. A enzima Protemax (EC 3.4.21.62) foi fornecida por Prozyn® Bio soluções para a vida (São Paulo, Brasil).

3.2 ASPECTOS ÉTICOS

O projeto de pesquisa foi aprovado pelo Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba – CEP/CCS, em observância a resolução 196/96 do Conselho Nacional de Saúde (CNS) que regulamenta os aspectos éticos de pesquisas envolvendo seres humanos, sendo aprovado sob o protocolo 10734712.8.0000.5188 (ANEXO 1). Este projeto foi financiado conforme Edital: MCT/CNPq Nº 14/2011 – Universal – Faixa A.

3.3 AUTÓLISE E TRATAMENTO DO CREME DE LEVEDURA

A etapa inicial do processo para obtenção das frações de β-glucana e MP foi realizada conforme Magnani et al. (2009). O creme de levedura em suspensão aquosa 20 % (v/v) foi submetido à autólise, induzida pela adição de 3 % NaCl (p/v), em pH 5.0 a 55 ºC em banho-maria com agitação branda (120 rpm) durante 24 h. Em seguida o material foi aquecido até 85 ºC, mantido durante 15 min, resfriado até 25 ºC e centrifugado a 4500 *g*, 10 min. O precipitado foi ajustado para 30 % de sólidos totais em tampão fosfato de sódio 0.02 M, pH 7.5 e adicionado de esferas de vidro (diâmetro 0.2 - 0.4 mm). A suspensão foi aquecida até 121 ºC em autoclave, mantida durante 4 h e resfriada a 25 ºC. O material foi centrifugado a 5000*g* durante 10 min a 4 ºC. Feito isso, a β-glucana ficou no precipitado e as MP no sobrenadante.

3.4 EXTRAÇÃO DA β-GLUCANA

Após a centrifugação do material tratado em autoclave, a β-glucana (precipitado) foi extraída conforme Magnani et al. (2009). O precipitado foi ajustado para suspensão aquosa 15 % e sonicado a 20 KHz, 150 W, durante 6 min em banho de gelo. Após centrifugação a 4500*g* durante 15 min a 10 ºC, o precipitado foi submetido à extração de lipídeos em Soxlet durante 2 h sob refluxo, utilizando éter de petróleo como solvente. Em seguida, o material foi lavado por centrifugação com acetona 1:1 (p/v), a 4500 *g* durante 5 min. Posteriormente, foi realizado tratamento enzimático com a enzima Protemax ® N200 (5 h a 55 °C, pH 7,5 e 0,4 U por grama de parede celular de uma suspensão aquosa a 20%). Decorrido o tempo de reação, a enzima foi inativada a 85ºC, 20 min e o material resfriado. Em temperatura ambiente foi lavado 5 vezes por centrifugação a 4500 x *g* por 5 min para retirada total da protease. Posteriormente, foi obtida a forma carboximetilada da β-glucana (CM-G) utilizando ácido monocloroacético, sendo o controle da derivatização à CM-G realizado por titulação potenciométrica (SANDULA et al., 1999). A CM-G foi dializada 24 h contra água destilada sob agitação branda, com trocas frequentes da água.

3.5 EXTRAÇÃO DA MANOPROTEÍNA

O sobrenadante, que seria descartado para dar seqüência à extração da β-glucana, após o tratamento térmico em autoclave, foi coletado para obtenção das MPs através de precipitação com etanol utilizando 1:3 volumes de etanol absoluto a 4 ºC durante 12 h (Costa et al., 2012). Posteriormente, as MPs foram separadas por centrifugação a 5000 x *g* durante 5 min a 10 ºC.

3.6 SECAGEM E RENDIMENTO DA β-GLUCANA E DA MANOPROTEÍNA

A CM-G dializada e a MP foram congeladas a -20 ºC e então liofilizadas a vácuo, em temperatura -80 ºC. O rendimento de cada fração extraída foi calculado a partir das quantidades obtidas em base seca, em relação à quantidade inicial de creme de levedura, também em base seca (MAGNANI et al., 2009; COSTA et al., 2012).

3.7 CARACTERIZAÇÃO DA β-GLUCANA

A confirmação da identidade química da β-glucana foi realizada através de análises Infravermelho e RMN. Os espectros de infravermelho da β-glucana foram obtidos em um espectrofotômetro Shimadzu modelo FT-IR 3300. Pastilhas de KBr foram utilizados para a preparação de amostras, e as leituras foram considerados como tendo um desvio de ± 2 cm^{-1}. Os espectros do ^{13}C e ^{1}H foram obtidos a partir de 50 mg de (1-3)/(1-6)-β-glucana dissolvidas em *d6*-DMSO por um espectrómetro Varian Gemini equipado com um NMR300-OXFRORD operando a 75,449 MHz para ^{13}C e 300,059 MHz para ^{1}H. Os desvios químicos foram expressos em ppm em referência aos picos dos solventes (δ_H 02:49 e δ_C 39,50 ppm para o *d6*-DMSO).

3.8 CARACTERIZAÇÃO DA MANOPROTEÍNA

O teor de carboidratos da MP foi determinado pelo método fenol-ácido sulfúrico de acordo com Dubois et al. (1956), utilizando glicose para a curva padrão. A análise qualitativa de carboidratos presentes na estrutura da MP foi realizada por cromatografia em camada delgada de acordo com Costa et al. (2012). A proteína bruta foi determinada pelo método de Kjeldahl, utilizando 6,25 como fator de conversão, descrito por (AOAC, 2000).

O peso molecular da fração de proteína de PM foi determinada por meio de SDS-PAGE, a uma concentração de 50 μg/mL e visualizadas com nitrato de prata de acordo com um protocolo previamente descrito (Costa et al., 2012). O marcador 220-10 kDa (Invitrogen ®) foi usado como um padrão de peso molecular.

O perfil de aminoácidos da PM foram separados, identificados e quantificados seguindo a metodologia descrita por White, Hart e Fry (1986) adaptada. Foi utilizado um cromatógrafo líquido de alta eficiência (VARIAN, Waters 2690) equipado com sistema binário de solventes, válvula "Rheodyne" com alça de 20 μl; coluna C18 (PICO-TAG, 3,9 x 150 mm); vazão da fase móvel de 1,0 ml/min; detector por conjunto de diodos (VARIAN 330) a 35º C e 254 nm e Software de processamento GALAXIE Chromatography Data System. Para a fase móvel foram utilizados como eluentes o tampão acetato de sódio, pH 6,4 (eluente A) e acetonitrila 60% (eluente B).

3.9 AVALIAÇÃO DA β-GLUCANA E DA MANOPROTEÍNA DURANTE ARMAZENAMENTO

A estabilidade da β-glucana e da manoproteína a 10 ºC, 25 ºC e 40 ºC foi avaliada durante 90 dias. Porções secas de 2 g de cada fração (5 ± 1 % de umidade) foram embaladas em sachês de polipropileno bi-orientado (BOPP) metalizado, e hermeticamente seladas. Análises de umidade foram conduzidas em triplicata, a cada 7 dias para cálculo de média e posterior análise estatística por ANOVA, considerando $p \leq 0,05$ (GUERGOLETTO et al., 2010).

3.10 AVALIAÇÃO DA ATIVIDADE EMULSIFICANTE DA MANOPROTEÍNA

A atividade emulsificante (AE) e estabilidade química e física das emulsões formadas pelas MPs foram avaliadas conforme Martinez-Checa, et al., (2007). Misturas contendo 5 mL de água, 5 ml de óleo de soja comercial e MP, em concentrações de 0,8 g / 100 g (A), 0,6 g / 100 g de (B), 0,4 g / 100 g de (C) e de 0,2 g/100 g (D) foram agitados a 9500 xg durante 1 min (em temperatura ambiente), em seguida, a emulsão obtida foi centrifugada (3500 x *g*, 5 min, 25 °C). A atividade emulsificante (AE) foi determinada (em percentagem), considerando-se a solução total, e a emulsão formada. A estabilidade da emulsão (EE) obtida foi determinada por aquecimento da emulsão formada, a 80 °C durante 30 min e resfriada em água corrente durante 10 min, seguida de centrifugação (3500 x *g*, 25 °C, 30 min).

3.11 AVALIAÇÃO DA MANOPROTEÍNA COMO SUBTITUTO DE GOMA XANTANA EM FORMULAÇÃO DE MAIONESE

As formulações de maionese foram preparadas em relação às concentrações de MP que mostraram as melhores propriedades de emulsificação e de estabilização. Três formulações de maionese foram elaborados de acordo com o procedimento descrito por Dikit et al. (2010) com a MP, em concentrações de 0,6 g/100g (MP1), 0,8 g/100g (MP2) e 1,0 g/100g (MP3). Os ingredientes utilizados na preparação da maionese foram óleo de soja (65 g/100g), ovo em pó pasteurizado (45 g/100g), vinagre (4 g/100g), açúcar (2 g/100g), sal (1,5 g/100g) e água (2,5 g/100g). Em um vaso de liquidificador foram acrescentados a água, o ovo em pó e homogeneizou-se por 60 segundos, depois foram adicionados o açúcar, sal, emulsificante e o vinagre, por fim o óleo foi adicionado lentamente. Posteriormente as formulações foram submetidas as análises para verificar a estabilidade, cor, pH e composição nutricional, as quais

foram realizadas após 1 dia (produto após 24 horas de preparação), 14 e 28 dias de armazenamento refrigerado (7 °C). A formulação da maionese preparada com goma xantana (0,1 g/100g), em substituição à MP foi utilizada como controle.

3.12 CARACTERIZAÇÃO DA MAIONESE ELABORADA COM MANOPROTEINA

As análises de cor foram realizadas de acordo com Shen et al., (2011), lidas em colorímetro digital Minolta CR-300 nos parâmetros L* (Luminosidade), a* (intensidade de vermelho/ verde) e b* (intensidade de amarelo/azul); o pH e a composição centesimal (proteínas, lipídios, carboidratos, umidade e cinzas) foram verificados de acordo com o método descrito por AOAC (2000); a estabilidade por separação de fases da amostra por centrifugação (3500 x g/30min) de acordo com (FRANGE; GARCIA, 2009) e calculada conforme a Equação.

$$\%E.M = \frac{Vmf \times 100}{Vmi}$$

Onde:

E.M = Percentual de estabilidade da maionese

Vmf = Altura final da maionese (cm)

Vmi = Altura inicial da maionese (cm)

3.13 ANÁLISE SENSORIAL

A análises sensorial das formulações de maionese foram realizadas utilizando testes de aceitação e de intenção de compra com 60 provadores não treinados após 1 e 28 dias de armazenamento refrigerado. Todos os testes foram realizados após a aprovação pelo Comitê de Ética em Pesquisa Envolvendo Seres Humanos (Processo Número 10734712.8.0000.5188, Universidade Federal da Paraíba, João Pessoa, Brasil). A análise foi realizada sob temperatura controlada e as condições de iluminação em cabines individuais. As amostras foram servidas monadicamente imediatamente após a remoção do armazenamento refrigerado. Cada provador recebeu as amostras de maionese (50 mg) correspondentes aos diferentes tratamentos, que

foram servidos com torradas e com auxílio de colheres descartáveis em copos descartáveis codificados com número de três dígitos aleatórios. Os provadores foram instruídos a comer um biscoito salgado e beber água entre as amostras. Para a aceitação da aparência, cor, sabor, textura, avaliação global, foi utilizada uma escala hedônica estruturada de nove pontos, variando de um (gostei extremamente) a nove (desgostei extremamente). A intenção de compra foi avaliada com uma escala hedônica estruturada de cinco pontos, variando de um (compraria) a cinco (não compraria) (NIKZADE et al., 2012).

3.14 ANÁLISES ESTATÍSTICAS

As análises estatísticas dos resultados obtidos foram realizadas com auxílio do programa computacional Statistica 7.2. Os resultados obtidos foram submetidos à Análise de Variância (ANOVA) e as médias comparadas pelo teste de Tukey ($p \leq 0,05$).

REFERÊNCIAS

AKLUJKAR, P. P., SANKH, S. N., ARVINDEKAR, K. U. A. Simplified Method for the Isolation and Estimation of Cell Wall Bound Glycogen in *Saccharomyces* cerevisiae. **The Institute of Brewing & Distilling**, v. 114, n. 3, p. 205-208, 2008.

AMARAL, P.F.F.; SILVA, J.M.; LEHOCKY, M.; BARROS-TIMMONS, A.M.V.; COELHO, M.A.Z.; MARRUCHO, I.M.; COUTINHO, J.A.P. Productios and characterizatins of a bioemulsifier from Yarrowia lipolytica. **Process Biochemistry**, v. 41, p.1894-1898, 2006.

ARAÚJO, V. B. S., MELO, A. N. F., CASTRO-GOMEZ, R. J. H., SOUZA, E. L., MAGNANI, M. Sensory evaluation of french-type salad dressing prepared with mannoprotein from *saccharomyces uvarum* to replace soy lecithin. **Latin American Symposium of Food Science**, Unicamp, Campinas-SP, Brasil. November 3-6. 2013.

ARROYO, J., BERMEJO, C., GARCÍA, R., RODRÍGUEZ-PEÑA, J.M. Genomics in the detection of damage in microbial systems: cell wall stress in yeast. **Clinical Microbiology and Infection,** v. 15, p. 44-46. 2009.

AOAC – Official Methods of Analysis of AOAC International.17th ed., Gaithersburg, 2000.

BABINCOVÁ M, BACOVA Z, MACHOVA E., KOGAN G. Antioxidant activity of carboxymethyl glucan: Comparative analysis. **Journal of Medical Food**, v.5, p.79-83, 2002.

BAPTISTA, A. S., HORII, J., PIEDADE, S. M. S. Cells of Yeasts Adhered in Corn Grains and the Storage Perspective for Use as Probiotic. **Brazilian Archives of Biology and Technology**, v. 48, n. 2, p. 251-257, 2005.

BARRIGA, J. A. T.; COOPER, D. G.; IDZIAK, E. S.; CAMERON, D. R. Components of the emulsifier from *Saccharomyces cerevisiae*. **Enzyme and Microbial Technology**, v. 25, p. 96-102, 1999.

BARROS, F. F. C., QUADROS, C. P., PASTORE, G. M. Propriedades emulsificantes e estabilidade do biossurfactante produzido por *Bacillus subtilis* em manipueira. **Ciência e Tecnologia de Alimentos**, v. 28, n. 4, p. 979-985. 2008.

BERTON, C., GENOT, C., MARIE-HÉLÈNE ROPERS. Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. **Journal of Colloid and Interface Science,** v. 354, p. 739–748, 2011.

BROWN, G. D.; GORDON, S. Fungal β-glucans and mammalian immunity. **Immunity,** v. 19, n. 3, p. 311-315, 2003.

BROWN, G. D.; GORDON, S. Immune recognition of fungal. **Cellular Microbiology,** v. 7, n. 4, p. 471-479, 2005.

CABIB, E.; ROH, D.; SCHMIDT, M.; CROTTI, L.B; VARMA, A. The yeast cell wall and septum as paradigms of cell growth and morphogenesis. **The Journal of Biological Chemistry**, v. 276, p. 19679-19682, 2001.

CAMEOTRA, S. S., & MAKKAR, R. S. Synthesis of biosurfactants in extreme conditions. **Applied Microbiology and Biotechnology**, v. 50, p. 520-529, 1998.

CAMEOTRA S. S.; MAKKAR, R. S. Recent applications of biosurfactants as biological and immunological molecules. **Current Opinion in Microbiology**, v. 7, n. 3, p. 262-266, 2004.

CHEN, J., RAYMOND, K. Beta-glucans in the treatment of diabetes and associated cardiovascular risks. **Vascular Health and Risk Management**: v. 4, n. 6, p. 1265–1272, 2008.

CHORVATOVICOVÁ D, MACHOVA E AND SANDULA J. Effect of ultrasonicated carboxymethylglucan on cyclophosphamide induced mutagenicity. **Mutation Research,** v. 371, p. 115-120, 1996.

COSTA, A. G., MAGNANI, M., CASTRO-GOMEZ, R. J. H. Obtenção e caracterização de extrato contendo manoproteínas da parede celular de levedura descartada em cervejaria. **Acta Scientiarum - Biological Science**, v. 34, n. 1, p. 77-84, 2012.

DARPOSSOLO, F. P. B., ETO, S. F., ENANCIO, E. J., RAUL JORGE HERNAN CASTRO-GOMÉZ, R. J. H. Saccharomyces uvarum Mannoproteins Stimulate a Humoral Immune Response in Mice. **Brazilian Archives of Biology and Technology,** v.55, n.4, p. 597-602, 2012.

DEMIR, G.; KLEIN, H. O.; MANDEL-MOLINAS, N.; TUZUNER, N. β-glucan induces proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer. **International Immunopharmacology**, v. 7, n. 1, p. 113-116, 2007.

DIKIT, P. S. M., MUSIKASANG, H., H-KITTIKUN, A. Emulsifier properties of the mannoprotein extract from yeast isolated from sugar palm wine. **Science Asia**, v. 36, p. 312-318, 2010.

DIETRICH-MUSZALSKA, A., OLAS, B., KONTEK, B., RABE-JABŁONSKA, J. Beta-glucan from *Saccharomyces cerevisiae* reduces plasma lipid peroxidation induced by haloperidol. **International Journal of Biological Macromolecules,** v. 49, p. 113-116, 2011.

FRANGE, R.C.C.; GARCIA, M.T.J. Desenvolvimento de emulsões óleo de oliva/água: avaliação da estabilidade física. **Revista de Ciências Farmacêuticas Básicas e Aplicadas**, v. 30, n.3, p.263-271, 2009.

FREIMUND, S.; SAUTER, M.; KÄPPELI, O.; DUTLER, H. A new non-degrading isolation process for 1,3-β-D-glucan of high purity from baker's yeast *Saccharomyces cerevisiae*. **Carbohydrate Polymers,** v. 54, p. 159-171, 2003.

FUKUDA, E. K.; VASCONCELOS, A. F. D.; MATIAS, A. C.; BARBOSA, A. M.; DEKKER, R. F. H.; SILVA, M. L. C. Polissacarídeos de parede celular fúngica: purificação e caracterização. **Semina,** v. 30, n. 1, p. 117-134, 2009.

GUERGOLETTO, K. B., MAGNANI, M., SAN MARTIN, J., TARDELI DE JESUS, C. G., GARCIA, S. Survival of Lactobacillus casei (LC-1) adhered to prebiotic vegetal fibers. **Innovative Food Science and Emerging Technologies**, v.11, p.415-421, 2010.

GUILLOUX-BENATIER, M., GUERREAU, J., FEUILLAT, M. Influence of initial colloid content on yeast macromolecule production and on the metabolism of wine microorganisms. **American Journal of Enology and Viticulture,** v. 46, p. 486-492, 1995.

HARNACK, U., ECKERT, k., FICHTNER, I., PECHER. Comparison of the Effect of Orally Administered Soluble β-(1-3),(1-6)-D-Glucan and of G-CSF on the Recovery of Murine Hematopoiesis. **In vivo**, v. 24, p. 59-64, 2010.

KANENO, Y., CHIHARA, G., TAGUCHI, T. Activity of lentinan against cancer and AIDS. **Journal of Immunotherapy,** v. 4, p. 203–213, 1989.

KAPTEYN, J. C.; RAM, A. F. J.; GROOS, E. M.; KOLLAR, R; MONTIJN, R. C.; VAN DEN ENDE, H.; LLOBELL, A.; CABIB, E.; KLIS, F. M. Altered extent of cross-linking of β1,6-glucosylated mannoproteins to chitin in *Saccharomyces cerevisiae* mutants with reduced cell wall β(1,3)-glucan content. **Journal of Bacteriology**, v. 179, n. 20, p. 6279-6284, 1997.

KIM, K.S.; YUN, H.S. Production of soluble β-glucan from the cell wall of *Saccharomyces cerevisiae*. **Enzyme and Microbial Technology**, v. 39, p. 496-500, 2006.

KLIS, F. M.; MOL, P.; HELLINGWERF, K.; BRUL, S. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. **FEMS Microbiology Reviews**, v. 26, n. 3, p. 239-256, 2002.

KLIS, F. M., BOORSMA, A., DE GROOT, P. W. J. Cell wall construction in *Saccharomyces cerevisiae*. **Yeast**, v. 23, p. 185-202, 2006.

KOGAN, G., STASKO, A., BAUEROVA, K., POLOVKA, M., SOLTES, L., BREZOVA, V., ET AL. Antioxidant properties of yeast (1-3)-b-D-glucan studied by electron paramagnetic resonance spectrometry and its activity in the adjuvant arthritis. **Carbohydrate Polymers**, v. 61, p. 18-28, 2005.

KOLLAR, R.; REINOLDS, B. B.; PETRAKOVA, E.; YEH, H. J. C.; ASHWELL, G.; DRGONOVA, J.; KAPTEYN, J. C.; KLIS, F. M.; CABIB, E. Architecture of the yeast cell wall. β-1,6-glucan interconnects mannoprotein, β-1,3-glucan, and chitin. **Journal of Biological Chemistry**, v.272, p.17762-17775, 1997.

LIU, X. Y.; WANG, Q.; CUI, S. W.; LIU, H. Z. A new isolation method of β-D-glucans from spent yeast *Saccharomyces cerevisiae*. **Food Hydrocolloids**, v. 22, p. 239-247, 2008.

LIU, D., ZENG, X., SUN, D., HAN, Z. Disruption and protein release by ultrasonication of yeast cells. **Innovative Food Science and Emerging Technologies,** v.18, p. 132-137, 2013.

MAGNANI, M.; CASTRO-GOMEZ, R. J. H. β-glucana from *Saccharomyces cerevisiae*: constitution, bioactivity and obtaining. **Semina**, v.29, n.3, p. 631-650, 2008.

MAGNANI, M., CALLIARI, C. M., MACEDO Jr.., F. C., MORI, M. P., SYLLOS- CÓLUS, I. M., CASTRO-GOMEZ, R. J. H. Optimized methodology for extraction of (1-3)(1-6)-β-D-glucan from *Saccharomyces cerevisiae* and in vitro evaluation of the cytotoxicity and genotoxicity of the corresponding carboxymethyl derivative. **Carbohydrate Polymers**, v. 78, p. 658-665, 2009.

MAGNANI, M., CASTRO-GOMEZ, R. J. H, AOKI, M. N., GREGÓRIO, E. P., LIBOS Jr., F., WATANABE, M. A. E. Effects of carboxymethyl-glucan from *Saccharomyces cerevisiae* on the peripheral blood cells of patients with advanced prostate cancer. **Experimental and Therapeutic Medicine**, v.1, n.5, p. 859-862, 2010.

MAGNANI, M., CASTRO-GOMEZ, R. J. H, AOKI, M. N., GREGÓRIO, E. P., LIBOS Jr., F., MORIMOTO, H. K., REICHE, E. M. V.,WATANABE, M. A. E. Analysis of peripheral T cells and the CC Chemokine Receptor (CCR5) Delta32 polymorphism in prostate cancer patients treated with Carboxymethyl**-Glucan**(CM-G). **Natural Product Research**, v. 26, n. 10, p. 945-951, 2012.

MAGNANI, M., CASTRO-GOMEZ, R. J. H, MORI, M. P., KUASNE, H., GREGÓRIO, E. P., LIBOS Jr., F., SYLLOS- CÓLUS, I. M. Protective effect of carboxymethyl-glucan (CM-G) against DNA damage in patients with advanced prostate cancer. Genetics and Molecular Biology, v.34, n.1, p.131-134, 2011b.

MANNERS, D. J.; MASSON, A. J.; PATTERSON, J. A.; BJORNDAL, H.; LINDBERG, B. The structure of a β(1-6)- D- glucan from yeast cell walls. **Biochemistry Journal, Washington**, v.135, n. 1, p.31-36, 1973.

MARTÍNEZ, J. P.; GIL, M. L.; RIBOT, J. L. L.; CHAFFIN, W, L. Serologic response to cell wall mannoproteins and proteins of *Candida albicans*. **Clinical Microbiology Reviews**, v. 11, n. 1, p. 121-141, 1998.

MELO, A. N. F., ARAÚJO, V. B. S., MARQUES, G. N., CASTRO-GOMEZ, R. J. H., SOUZA, E. L., MAGNANI, M. Characterization of french-type salad dressing prepared With mannoprotein from spent brewer's yeast. **Latin American Symposium of Food Science**, Unicamp, Campinas-SP, Brasil. November 3-6. 2013.

MORRIS, G. J., WINTERS L, COULSON G. E., CLARKE K. J. Effect of osmotic stress on the ultrastructure and viability of the yeast *Saccharomyces cerevisiae*. **Journal General Microbiology,** v. 132, p. 2023-2034, 1986.

MIADOKOVA, E.; SVIDOVA, S.; VLCKOVA, V.; DUHOVA, V.; PRAZMARIOVA, E.; TOTHOVA, K.; NAĎOVA, S.; KOGAN, G.; RAUKO, P. The role of natural biopolymers in genotoxicity of mutagens/carcinogens elimination. **Biomedical Papers of the Medical Faculty of the University Palacky, Olomouc**, v.149, n, p. 493-496, 2005.

NITSCHKE, M., COSTA, S. G.V.A.O. Biosurfactants in food industry. **Trends in Food Science & Technology**, v. 18, p. 252-259, 2007.

NIKZADE, V.; TEHRANI, M. M.; SAADATMAND-TARZJAN, M. Optimization of lowcholesterol-low-fat mayonnaise formulation: Effect of using soy milk and some stabilizer by a mixture design approach. **Food Hydrocolloids**, v. 28, p. 344-352, 2012.

ORLEAN, P. Biogenesis of yeast wall and surface components. In: The Molecular and Cellular Biology of the Yeast *Saccharomyces*. Cell Cycle and Biology (Pringle, J.R., Broach, J.R. and Jones, E.W., Eds.), p. 229-362. **Cold Spring Harbor Laboratory Press**, Cold Spring Harbor, NY. 1997.

PETRAVIĆ-TOMINAC, V., ZECHNER-KRPAN, V., GRBA, S., SREČEC, S., PANJKOTA-KRBAVČIĆ, I., VIDOVIĆ, L. Biological Effects of Yeast β-Glucans. **Agriculturae Conspectus Scientificus,** v. 75, n. 4, p. 149-158, 2010.

PELIZON, A. C., KANENO, R., SOARES, A. M. V. C., MEIRA, D. A., SARTORI, A. Immunomodulatory Activities Associated with β-Glucan Derived from *Saccharomyces cerevisiae*. **Physiological Research,** v. 54, p. 557-564, 2005.

PISKUR, J., LANGKJÆR, R. B. Yeast genome sequencing: the power of comparative genomics. **Molecular Microbiology**, v. 53, n. 2, p. 381–389, 2004.

QUIRÓS, M., GONZÁLEZ-RAMOS, D., TABERA, L., & GONZALEZ, R. A new methodology to obtain wine yeast strains overproducing mannoproteins. **International Journal of Food Microbiology**, v. 139, n. 2, p. 9-14, 2010.

RICE P.J., ADAMS E.L., OZMENT-SKELTON T., GONZALEZ A.J., GOLDMAN M.P., LOCKHART B.E., BARKER L.A., BREUEL K.F., DEPONTI W.K., KALBFLEISCH J.H. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. **J Pharmacol Exp Ther,** v. 314, p. 1079-1086, 2005.

ROSI, I., GIOVANI, G. Effect of some winemaking variables on the production of exopolysaccharides by Saccharomyces cerevisiae. In: Lonvaud-Funel, A., et al. (Ed.), **7th International Symposium of Oenology**, p. 324-328, 2003.

ROEMER, T. G., PARAVICINMI, M. A., PAWON, BUSSM, H. Characterization of the yeast 1,6-beta-glucan biosynthetic components, Kre6p and Sknlp, and genetic interactions between the PKCl pathway and extracellular matrix assembly. **Journal of Cell Biology**, v. 127, p. 567-579, 1994.

SALUK, J., BIJAK, M., PONCZEK, M. B., NOWAK, P., WACHOWICZ, B. (1-3)-D-Glucan reduces the damages caused by reactive oxygen species induced in human platelets by lipopolysaccharides. **Carbohydrate Polymers,** v. 97, p. 716-724, 2013.

SANDULA, J.; KOGAN, G.; KACURAKOVÁ, M.; MACHOVÁ, E. Microbial (1-3)-β-D-glucans, their preparation, physico-chemical characterization and immunomodulatory activity. **Carbohydrate Polymers,** v. 38, n. 3, p. 247–253, 1999.

SCENI, P., PALAZOLO, G. G., VASALLO, M. C., PUPPO , M. C., OTERO , M. A., WAGNER, J. R. Thermal and surface behavior of yeast protein fractions from *Saccharomyces cerevisiae*. **LWT - Food Science and Technology,** v. 42, p. 1098-1106, 2009.

SHEN, R., LUO, S., DONG, J. Application of oat dextrine for fat substitute in mayonnaise. **Food Chemistry,** v. 126, p. 65-71, 2011.

SHIM, S., SEO, S. H., YOUNGJA LEE, Y., MOON, G., KIM, M., PARK, J. Consumers' knowledge and safety perceptions of food additives: Evaluation on the effectiveness of transmitting information on preservatives. **Food Control**, v. 22, p. 1054-1060, 2011.

SGARBIERI, V. C.; ALVIM, I. D.; VILELA, E. S. D.; BALDINI, V. L. S.; BRAGAGNOLO, N. Produção piloto de derivados de levedura (*Saccharomyces* ssp.) para uso como ingrediente na formulação de alimentos. **Brazilian Journal of Food Technology**, v. 2, n. (1, 2), p. 119-125, 1999.

SIMONS, J. F.; EBERSOLD, M.; HELENIUS, A. Cell wall 1,6-β-glucan synthesis in *Saccharomyces cerevisiae* depends on ER glucosidases I and II, and the molecular chaperone BiP/Kar2p. **The EMBO Journal, Oxford**, v. 17, n. 2, p. 396-405, 1998.

TORABIZADEH, H.; SHOJAOSADATI, S.A.; TEHRANI, H.A. Preparation and Characterisation of Bioemulsifier from Sacccharomyces cerevisiae and its application in food products. **Lebensm.- Wiss.u.- Technology**, v.29. p.734-737, 1996.

TORRECILLAS, S., MAKOL, A., CABALLERO, M.J., MONTERO, D., ROBAINA, L., REAL, F., SWEETMAN, J., TORT, L., IZQUIERDO, M.S. Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. **Fish and Shelf Immunology,** v. 23, n. 5, p. 969-981, 2007.

TOWBIN, H., STAEHELIN, T., GORDON, J. Immunoblotting in the Clinical Laboratory. **Journal of Clinical Chemistry & Clinical Biochemistry,** Vol. 27, p. 495-501, 1989.

VETVICKA, V., VETVICKOVA, J. A comparison of injected and orally administered beta glucans. **Journal of the American Nutraceutical Association,** v. 11, p. 42-8, 2008.

VILELA, E. S. D.; SGARBIERI, V. C.; ALVIN, I. D. Determinação do valor protéico de células íntegras, autolisado total e extrato de levedura (*Saccharomyces* ssp.). **Revista Nutrição**, v. 13, n. 3, p. 185-192, 2000a.

WILLIAMS, D. L., MUELLER, A., BROWDER, W. Glucan-based macrophage stimulators. **Clinical Immunotherapy**, Local, v. 5, n. 5, p. 392-399, 1996.

WEITBERG, A. A phase I/II trial of beta-(1,3)/(1,6) D-glucan in the treatment of patients with advanced malignancies receiving chemotherapy. **Journal of Experimental & Clinical Cancer Research**, v. 27, p. 40-42, 2008.

WHITE, J. A., HART, R. J., & FRY, J. C. An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. **Journal of Automatic Chemistry**, 8, 170–177, 1986.

ZECHNER-KRPAN, V., PETRAVIC-TOMINAC, V., GOSPODARI, I., SAJLI, L., DAKOVIC, S., FILIPOVIC-GRCIC, J. Characterization of β-Glucans Isolated from Brewer's Yeast and Dried by Different Methods. **Biotechnology,** v. 48, n. 2, p. 189-197, 2010.

ZIOTNIK, H.; FERNANDEZ, M. P.; BOWERS, B.; CABIB, E. *Saccharomyces cerevisiae* mannoproteins form an external cell wall layer that determines wall porosity. **Journal of Bacteriology**, Washington, v. 159, n. 3, p. 1018-1026, 1984.

4 RESULTADOS E DISCUSSÕES

ARTIGO PUBLICADO NA REVISTA INNOVATIVE FOOD SCIENCE AND EMERGING TECHNOLOGIES

DOI 10.1016/j.ifset.2013.12.013

Innovative Food Science and Emerging Technologies xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the obtained mannoprotein as a stabilizer in mayonnaise

Vilma Barbosa da Silva Araújo [a], Adma Nadja Ferreira de Melo [a], Ariane Gaspar Costa [b], Raul H. Castro-Gomez [b], Marta Suely Madruga [a], Evandro Leite de Souza [c], Marciane Magnani [a,*]

[a] *Laboratory of Biochemistry of Foods, Department of Food Engineering, Federal University of Paraíba, João Pessoa, Brazil*
[b] *Department of Food Science and Technology, University of Paraná State, Londrina, Brazil*
[c] *Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba, João Pessoa, Brazil*

ARTICLE INFO

Article history:
Received 4 July 2013
Accepted 17 December 2013
Available online xxxx

Editor Proof Receive Date 22 January 2014

Keywords:
Brewery slurry
Cell wall
Emulsion formulation
Food additives

ABSTRACT

The aim of this study was to evaluate the viability of a novel method to extract both β-glucan and mannoprotein (MP) from the cell wall of spent brewer's yeast (*Saccharomyces uvarum*), particularly with the obtainment of MP by an additional step in a non-degrading procedure to extract β-glucan. The structure and composition of both obtained polymers were characterized, and the potential application of the MP as an emulsifier and stabilizing agent to replace xanthan gum (XG) in mayonnaise formulations was assessed. The yield obtained for β-glucan and MP was 10% and 4%, respectively. Infrared and nuclear magnetic resonance spectroscopy of β-glucan indicated a typical pattern of a glucose polymer with β-linkages. Analyses of the carbohydrate portion of MP revealed a retention factor of only 0.45, which indicates the presence of mannose; analyses of the protein portion of MP revealed 58-kDa and 64-kDa proteins that are largely composed (mg/g) of the amino acids valine (8.9%), aspartic acid (8.2%) and leucine (6.9%). The pH of the mayonnaise formulations containing different concentrations of MP (MP1: 0.6 g of MP/100 g; MP2: 0.8 g of MP/100 g; MP3: 1.0 g of MP/100 g) did not change ($P \geq 0.05$) during 28 days of refrigerated storage. The stability of the MP1, MP2 and MP3 formulations increased during the assessed time ($P \leq 0.05$) and after 28 days showed values higher than the formulation prepared with XG. All mayonnaise formulations prepared with MP exhibited high lightness (L^* value) and a tendency toward decreased yellow color (b^* value) during storage. Moreover, the formulations prepared with MP received similarly high scores ($P > 0.05$) for aroma, color, flavor and the overall evaluation without differences from that prepared with XG. Regarding the intent to purchase, the tasters generally reported the desire to purchase all of the tested mayonnaise formulations. The results presented herein demonstrated the feasibility of the proposed procedure to obtain both β-glucan and MP from spent brewer's yeast with a high yield and satisfactory purity. The obtained MP demonstrated good emulsifying and stabilizing properties, and its application to replace XG in the formulation of mayonnaise presented no negative effect on the sensory attributes of the product during refrigerated storage.
Industrial relevance: S. uvarum has been an interesting biotechnological tool for the production of beer using low fermentation, resulting in production of high amounts of brewer's yeast, which is commonly discarded. β-Glucan and mannoprotein (MP) obtained from spent brewer's yeasts have presented interesting biological properties, which could be applied for food production and conservation. Present study provides novel and valuable information concerning the viability of a followed method to extract β-glucan and MP from the cell wall of spent brewer's yeast (*S. uvarum*) discarded after fermentation processes, chiefly about the specific isolation of MP using an additional step in a non-degrading procedure that included sonication and proteolysis to extract β-glucan. The obtained MP revealed interesting emulsifying and stabilizing properties, and its use for the formulation of mayonnaise had no negative effect on the sensory properties of the product during refrigerated storage.
© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Yeast species from the *Saccharomyces* genus are largely employed in industrial fermentation processes. *Saccharomyces uvarum*, in particular, is the major biotechnological tool for the production of beer using low fermentation, and every year, tons of brewer's yeast are discarded worldwide (Darpossolo, Silas, Venancio, & Gomez, 2012; Ding, Wang, Xiong, Zhao, & Huang, 2013). The cell wall of spent brewer's yeast is primarily composed of β-glucans and mannoproteins, which represent up to 20% of the yeast cell dry weight. The immunostimulatory effects of yeast β-glucans have been previously studied and the beneficial effects

* Corresponding author. Tel.: +55 83 3216 7576; fax: +55 83 3216 7269.
E-mail address: magnani2@gmail.com (M. Magnani).

1466-8564/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ifset.2013.12.013

on the immune system after daily consumption have been reported (Magnani et al., 2010; Weitberg, 2008). It is known that the biological properties of β-glucans that are obtained from different sources correspond to the structure of the polymer, its molecular weight and the type of side chains (Bohn & BeMiller, 1985).

Early studies have also found interesting emulsifying and stabilizing properties of mannoproteins (MPs) obtained from the cell wall of brewer's yeast, and these properties are chiefly related to the original amphipathic structure of the MP molecule, in which the hydrophilic polymers of mannose are joined to proteins (Barriga, Cooper, Idziak, & Cameron, 1999; Cameron, Cooper, & Neufeld, 1988). MP demonstrated good emulsifying and stabilizing effects in vitro when assayed at different pH values and salt concentrations that are commonly applied by the food industry to formulate or preserve foods (Barriga et al., 1999; Cameron et al., 1988; Costa, Magnani, & Castro-Gomez, 2012); however, these properties remain to be assessed for the incorporation of MP in food formulations.

The methods typically applied to extract β-glucan and MP from the cell wall of brewer's yeast involve various acidic and alkaline washes and the application of high temperatures, which could cause degradation and alter the biological and/or technological properties of the obtained polymers (Freimund, Sauter, Kappeli, & Dutler, 2003; Liu, Wang, Cui, & Liu, 2008). The exposure to these extreme conditions, even for short time periods, could also result in unsatisfactory yield and limited purity of the obtained β-glucan and MP, in addition to waste production that is potentially harmful to the environment (Magnani et al., 2009). In an attempt to replace these harsh conditions that are typically applied to extract β-glucans and MP from the *Saccharomyces* cell wall, less aggressive methods involving hot water and enzyme treatments (Freimund et al., 2003) or hot water and high-pressure homogenization (Liu et al., 2008) have been described. An alternative method (Magnani et al., 2009) to the acidic and alkaline washes used to isolate β-glucan from the cell wall of brewer's yeast (*Saccharomyces cerevisiae*) using sonication and enzymatic treatment found high yields, and the obtained carboxymethyl glucan derivative (CM-G) revealed promising immunostimulatory effects in humans (Magnani et al., 2010).

Considering these aspects, the present study evaluated the viability of a followed method to extract β-glucan and MP from the cell wall of spent brewer's yeast (*S. uvarum*) discarded after five fermentation processes, particularly with the specific isolation of MP using an additional step in a non-degrading procedure that included sonication and proteolysis to extract β-glucan. The structure and composition of the obtained β-glucan and MP were characterized. Additionally, the potential application of the isolated MP as a stabilizing agent at different concentrations in mayonnaise was assessed. To the best of our knowledge, this is the first study reporting the isolation of both β-glucan and MP from the cell wall of *S. uvarum* discarded after several industrial fermentation processes using a followed extraction method.

2. Material and methods

2.1. Materials

Brewer's yeast slurry (*S. uvarum* cells) was kindly provided by an industrial brewery located in the city of João Pessoa (Paraíba, Brazil). The Protemax enzyme (EC 3.4.21.62) was provided by Prozyn® Bio Solutions for Life (São Paulo, Brazil). Glucose and mannose standards were supplied by Sigma-Aldrich (St. Louis, USA).

2.2. Slurry preparation

The brewer's yeast slurry was sieved through a 0.297 mm mesh, and distilled water was added (30%, w/v). To remove residual ethanol, the suspension was washed three times with distilled water (5000 ×g for 5 min at 10 °C) (Costa et al., 2012), NaCl was added (3%, w/v), and the suspension was autolyzed in a bath under stirring (120 rpm) at 55 °C for 24 h. The temperature was then increased to 85 °C and maintained for 5 min to inactivate the cellular enzymes. After the suspension reached room temperature, the yeast cell wall was obtained by centrifugation at 4500 ×g and 4 °C for 10 min.

2.3. Extraction of β-glucan and MP

β-glucan was extracted according to a previously described procedure (Magnani et al., 2009) with modifications in the enzyme used for proteolysis [substitution of the Protamex enzyme (Novozymes®, Latin America Ltda, Paraná, Brazil) with Protemax (Prozyn®, Bio Solutions for Life, São Paulo, Brazil)]. Briefly, the insoluble material from the autolyzed brewer's yeast slurry was diluted in sodium phosphate buffer (30% w/v; 0.02 M sodium phosphate buffer, pH 7.5), heated to 121 °C (1.5 atm) in an autoclave for 4 h and washed three times with distilled water (4500 ×g for 7 min at room temperature). To extract β-glucan, sonication (20 kHz; 150 W; 6 min), lipid extraction using petroleum ether (2 h under reflux) and proteolysis using the enzyme Protemax® N200 (5 h at 55 °C and pH 7.5; 0.4 U per gram of cell wall in a 20% aqueous suspension) were performed. After proteolysis, the insoluble residue was washed five times with distilled water, and the soluble protein was removed by centrifugation (4500 ×g for 5 min at room temperature) to obtain only β-glucan. The insoluble residue was dialyzed (48 h against distilled water under mild agitation with frequent water exchange), frozen at −20 °C and lyophilized.

To extract MP, the supernatant after the first centrifugation of the autolyzed sample was collected, absolute ethanol was added (3:1), and the mixture was maintained at 4 °C for 18 h to precipitate MP. The precipitate was obtained by centrifugation, washed with absolute ethanol (4500 ×g, 5 min, 10 °C), dialyzed (48 h against distilled water under mild agitation with frequent water exchange) and lyophilized.

The obtained β-glucan and MP were packed in hermetically sealed metalized BOPP bags, and after 28 days at 10 °C, 25 °C or 40 °C, the residual moisture of β-glucan and MP was determined by drying at 105 °C until a constant weight was achieved. The autolysis rate and the yield of β-glucan and MP were calculated based on the dry weight with respect to the initial weight of the spent cell.

2.4. Characterization of β-glucan and MP

2.4.1. Carbohydrate and protein determination

The total carbohydrate content of β-glucan and MP was determined by the phenol-sulfuric acid method according to Dubois, Gilles, Hamilton, Rebers, and Smith (1956) using glucose as a standard. The qualitative analysis of carbohydrates present in the MP structure was performed by thin layer chromatography (TLC) according to Costa et al. (2012). Crude protein of β-glucan and MP was measured using the micro-Kjeldahl method and a conversion factor of 6.25 (AOAC, 2005).

2.4.2. Infrared spectroscopy

The infrared spectra of β-glucan were obtained using a Shimadzu FT-IR spectrophotometer (model 3300). KBr pellets were used for preparation of the samples, and the deviation of the measurements was $\pm 2\ cm^{-1}$.

2.4.3. Nuclear magnetic resonance spectroscopy (NMR)

The ^{13}C and ^{1}H NMR spectra of β-glucan were obtained from 50 mg of the polymer dissolved in d_6-DMSO using a Varian Gemini spectrometer equipped with an NMR 300-OXFORD magnet operating at 75.449 MHz for ^{13}C and 300.059 MHz for ^{1}H. The chemical shifts were expressed in ppm with reference to the peaks of the solvent (δ_H 2.49 and δ_C 39.50 ppm for d_6-DMSO).

Please cite this article as: Silva Araújo, V.B., et al., Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the..., *Innovative Food Science and Emerging Technologies* (2014), http://dx.doi.org/10.1016/j.ifset.2013.12.013

ARTICLE IN PRESS

2.4.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The molecular weight of the protein fraction of MP at a concentration of 50 μg mL^{-1} was determined using SDS-PAGE and visualized with silver nitrate stain according to a previously described protocol (Costa et al., 2012). The marker 220–10 kDa (Invitrogen®) was used as a standard molecular weight.

2.4.5. High-performance liquid chromatography (HPLC)

The amino acid composition of MP was determined according to White, Hart, and Fry (1986). The samples were previously hydrolyzed in redistilled 6N hydrochloric acid followed by precolumn derivation of free amino acids using phenyl isothiocyanate (PITC). The separation of the derivatized phenylthiocarbamyl amino acids (PTC-AA) was performed using liquid chromatography (VARIAN, Waters 2690, California, USA).

2.5. Emulsifying activity and stability assays of MP

The emulsifying activity of MP was assessed as described by Martinez-checa, Toledo, Mabrouki, Quesada, and Calvo (2007). A range of MP concentrations was examined using the previously reported concentration for yeast MP (0.5 g/100 g), with up and down variations. Mixtures containing 5 mL of water, 5 mL of commercial soybean oil and MP at concentrations of 0.8 g/100 g (A); 0.6 g/100 g (B); 0.4 g/100 g (C); or 0.2 g/100 g (D) were stirred at 9500 ×g for 1 min (at room temperature); then, the obtained emulsion was centrifuged (3500 ×g, 5 min, 25 °C) in graduated tubes. The emulsifying activity (EA) was determined (in percent) considering the total volume of the solution (achieved in the tube by the mixture of the oil, water and MP) and the volume of the formed emulsion (all volumes were converted in emulsion means 100% of EA). The stability of the obtained emulsion (SA) was determined by heating the formed emulsion at 80 °C for 30 min and cooling it in running water for 10 min, followed by an additional centrifugation (3500 ×g; 25 °C; 30 min). The SA was determined (in percent) considering the final volume of the emulsion in graduated tube (after heating, cooling and centrifugation) in relation to the initially observed emulsion volume.

2.6. Chemical composition and stability evaluation of mayonnaise produced with MP

The mayonnaise formulations were prepared using the MP concentration (0.8 g of MP/100 g of emulsion) that demonstrated the best emulsifying and stabilizing properties as a reference. Three mayonnaise formulations were prepared according to the procedure described by Dikit, Musikasang, and H-kittikun (2010) using MP at concentrations of 0.6 g of MP/100 g of emulsion (MP1), 0.8 g of MP/100 g of emulsion (MP2) and 1.0 g of MP/100 g of emulsion (MP3). The ingredients used to prepare the mayonnaise consisted of soybean oil (65 g/100 g of emulsion), pasteurized egg powder (5 g/100 g of emulsion), vinegar (4 g/100 g of emulsion), sugar (2 g/100 g of emulsion), salt (1.5 g/100 g of emulsion) and water (22.5 g/100 g of emulsion). Assays to verify the stability, color, pH and chemical composition of the prepared mayonnaise formulations were performed after 1 (the product 24 h after preparation), 14 and 28 days of refrigerated storage (7 °C) and compared with the mayonnaise formulation prepared using xanthan gum (0.1 g/100 g of emulsion, the control) instead of MP. For each storage period, each mayonnaise formulation was heated at 80 °C for 30 min, cooled in running water (approximately 10 min) and centrifuged (3500 ×g; 30 min; 25 °C). The emulsion stability was determined by comparing the emulsion before and after heating, cooling and centrifugation according to the procedure described by Martinez-checa et al. (2007) (see details in 2.5 item). The color analyses were performed using a Minolta (CR-300, Minolta, Mahwah, New Jersey, USA) digital colorimeter to measure the CIELAB system parameters (Mun et al., 2009). The chemical composition (moisture, ash, fat, protein and carbohydrate) and pH were determined according to the methods described by the AOAC (2005). For all assessed parameters, the mayonnaise formulations were evaluated in three different replicate experiments, all analyses were performed in triplicate, and the results are presented as the mean of the replicates.

2.7. Sensory analyses of mayonnaise produced with MP

Sensory analyses of the mayonnaise formulations were performed using affective acceptance and preference tests with 60 untrained tasters after 1 and 28 days of refrigerated storage. All tests were performed after obtaining approval from the Ethics Committee for Research Involving Human Beings (Process Number 10734712.8.0000.5188, Federal University of Paraíba, João Pessoa, Brazil) and after microbiological analyses (total coliforms, thermotolerant coliforms, *Salmonella* spp. and coagulase-positive *Staphylococcus*) to ensure the safety of the different assayed mayonnaise formulations for human consumption according to the current legislation for microbiological criteria for food (APHA, 2001). The analyses were performed under controlled temperature and lighting conditions in individual booths. Each panelist received the samples of mayonnaise (50 mg) corresponding to the different treatments, which were served on a disposable white spoon coded with a random three-digit number. The samples were served simultaneously using a blind method of random sequence immediately after removal from cold storage. Tasters were asked to eat a salty biscuit and drink water between samples to avoid an aftertaste. For the acceptability of appearance, color, flavor, texture and the overall assessment, a nine-point structured hedonic scale was used that ranged from one (strongly disliked) to nine (strongly liked). The intent to purchase was assessed using a five-point structured hedonic scale ranging from one (definitely would not purchase) to five (definitely would purchase) (Nikzade, Mazaheri Tehrani, & Saadatmand-Tarzjan, 2012).

2.8. Statistical analysis

Statistical analyses were performed with descriptive statistics (mean and standard deviation) and inferential tests (ANOVA followed by Tukey's test) to determine significant differences ($P \leq 0.05$) between the treatments using the computer software Statistica 7.2.

3. Results and discussion

3.1. Extraction of β-D-glucan and MP

The autolysis rate of the brewer's yeast slurry (*S. uvarum* cells) was 40 ± 1%, which was lower than that reported by Liu et al. (2008) (48%) and Magnani et al. (2009) (53%), who used experimental conditions that were identical to those applied in this study. However, these authors used *S. cerevisiae* brewer's slurry and *S. cerevisiae* pressed mass, respectively, and it is known that the cell wall of different yeast species demonstrates different characteristics in composition and in response (changes) to environmental stress factors, such as the conditions found in fermentation processes (Kapteyn, Van Oen Enoe, & Klis, 1999). The total crude protein determined in the autolyzed suspension before treatment with hot water was 16 ± 0.5 g/100 g, whereas for the insoluble residue obtained by centrifugation after hot water treatment, this value was 1.1 ± 0.4 g/100 g. Regarding the obtained crude protein values, approximately 93% of the total protein was extracted by the applied hot water treatment. The β-glucan obtained after proteolysis (corresponding to the insoluble residue collected after washes by centrifugation) contained 97.21 ± 0.42% of the total carbohydrates, which was higher than the amounts reported by Xiaozhong, Jie, Baogui, and Wangxiang (2000) for the total carbohydrates in β-glucan obtained from *S. cerevisiae* cells using alkaline and acidic extraction (92%). However, the total carbohydrate content of the β-glucan

Please cite this article as: Silva Araújo, V.B., et al., Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the..., *Innovative Food Science and Emerging Technologies* (2014), http://dx.doi.org/10.1016/j.ifset.2013.12.013

observed in the present study was similar to the amounts obtained by Magnani et al. (2009) for β-glucan extracted from pressed mass *S. cerevisiae* cells (97%) using a similar procedure, but different enzyme to that applied in the present study.

3.2. Extraction yield and residual moisture of β-D-glucan and MP

The extraction yield of β-D-glucan was 10 ± 0.2% from *S. uvarum* cells, which is similar to the 11% yield obtained by Liu et al. (2008) and Magnani et al. (2009), who used similar methods to obtain β-glucan from *S. cerevisiae*. Additionally, a 4.16% yield of MP from *S. uvarum* cells was obtained, which is similar to the yield (4.0%) reported by Costa et al. (2012) for MP extraction from spent brewer's yeast (*S. uvarum*) using different experimental temperatures (75 °C, 85 °C, 95 °C). These results suggest the feasibility of the procedure proposed in the present study to obtain the two most important constituents of the brewer yeast cell wall, β-glucan and MP, with similar yields to those previously reported for each individually extracted polymer.

The residual moisture of β-glucan was 4.9 ± 0.1%, and this percentage did not change after the material was packed in metalized BOPP bags that were hermetically sealed and stored for 28 days at 10 °C, 25 °C or 40 °C, with no change in color (data not shown). Hromádková et al. (2003) found 12.7% residual moisture for lyophilized β-D-glucan that was extracted from *S. cerevisiae* using an alkaline treatment. Compared with previous reports (Magnani et al., 2009), we hypothesize that the β-glucan obtained using the methodology proposed herein possesses a lower water-holding capacity. The residual moisture of MP was 3.9 ± 0.9%, and no changes were observed after the material was packed in metalized BOPP bags that were hermetically sealed and stored for 28 days at 10 °C, 25 °C or 40 °C, suggesting the long-term stability of this polymer and its suitability in long-term applications.

3.3. Characterization of β-D-glucan and MP

3.3.1. Structural characterization of β-glucan

The infrared spectrum (Fig. 1) obtained for β-D-glucan showed an absorption at 891 cm^{-1}, which is characteristic of β-glycosidic linkages (Hromádková et al., 2003), and bands at 1040 cm^{-1} and 1080 cm^{-1}, which correspond to the stretching of CO and CC, respectively. Intense bands at the region from 3100 to 3600 cm^{-1} and at the region of 2925 cm^{-1} were also observed. The observed bands are related to functional groups of β-glucan (Magnani et al., 2009; Sandula, Kogan, Kacuraková, & Machová, 1999); no bands in the region corresponding to the amide group were observed.

The NMR spectrum revealed only the presence of anomeric carbons in the β configuration, with signals at approximately 103 ppm. The ^{13}C NMR spectra of β-glucan were similar to those published by Magnani et al. (2009) and Kogan, Alföldi, and Masler (1988) for an *S. cerevisiae* β-glucan. The ^{1}H NMR spectrum agreed with the reports of Freimund et al. (2003) for β-glucan extracted from *S. cerevisiae* using experimental conditions similar to those used in this study (data not shown).

3.3.2. Structural characterization of MP

The composition of the dialyzed and dried MP was determined to be 39 g of carbohydrate/100 g of MP and 58 g of protein/100 g of MP. Analyses of the MP carbohydrate portion using TLC, with respect to the monosaccharide, revealed a retention factor (Rf) of only 0.45, which indicates mannose. The SDS-PAGE profile of MP indicated proteins possessing molecular weights of 58 kDa and 64 kDa (Fig. 2). Similar results for MP from spent brewer's yeast were described in a previous study (Costa et al., 2012). The amino acid composition determined using high-performance liquid chromatography analysis revealed predominantly hydrophobic amino acids (Table 1), followed by neutral amino acids (which tend to form hydrogen bonds) and lower amounts of hydrophilic amino acids. The amino acid composition of MP, which is organized into an amphipathic structure, suggests strong stabilizing properties of this polymer because of its ability to bind apolar and polar regions of different molecules. In addition, it is known that low molecular weight proteins are able to provide systems possessing active surfaces (surface tension), whereas proteins of higher molecular weight are able to stabilize emulsions and produce viscous and stable emulsions (Rosenberg & Ron, 1999). The intermediate molecular weight proteins identified in the isolated MP suggest that this polymer is capable of forming emulsions with good stability over time.

The highest amino acid content (mg/g of protein) was observed for valine (8.9%), aspartic acid (8.2%) and leucine (6.9%), although other

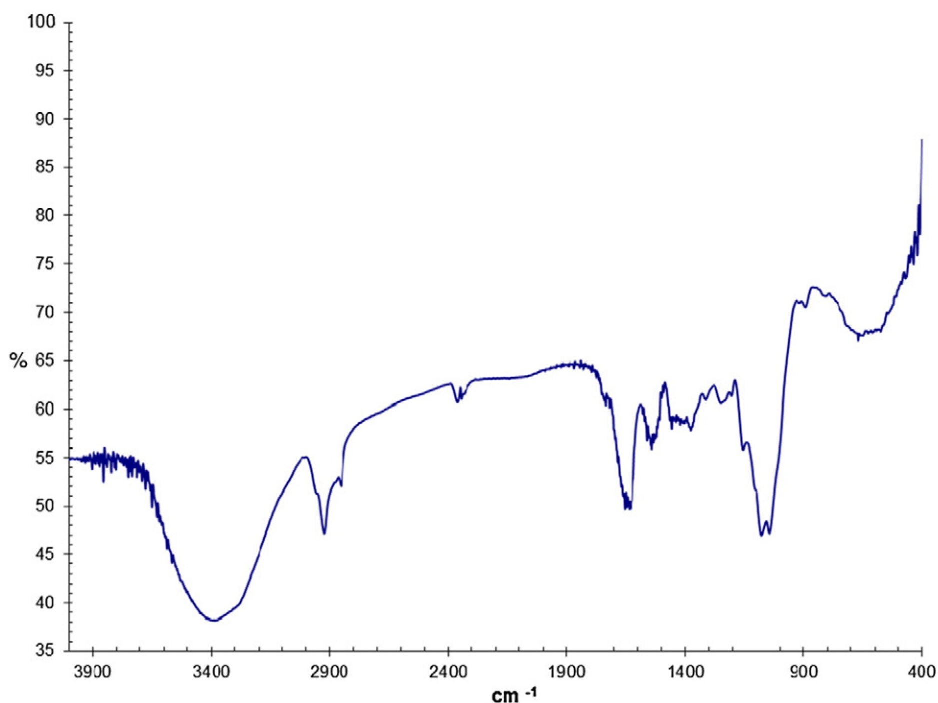

Fig. 1. Infrared spectrum of β-glucan extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes.

ARTICLE IN PRESS

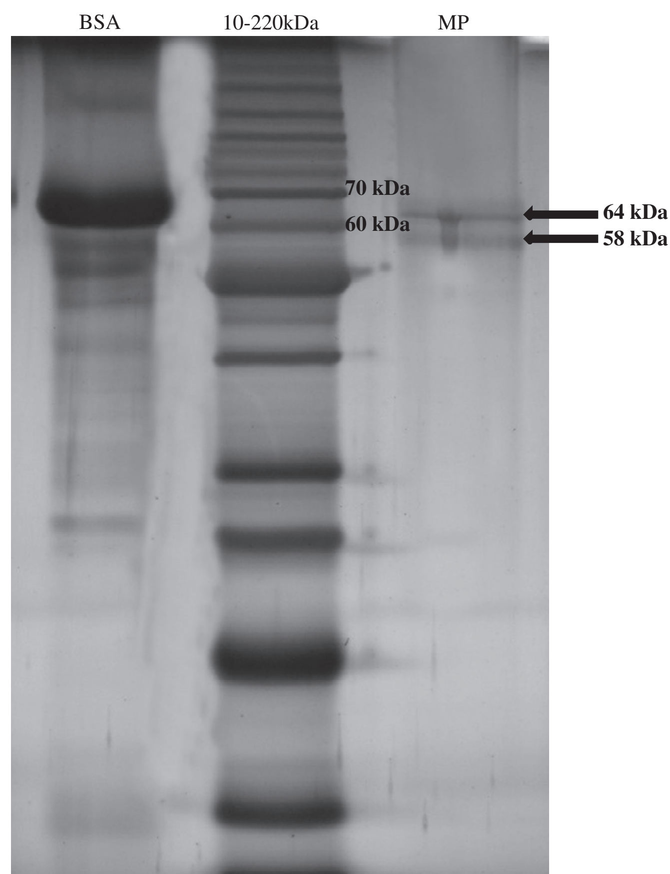

Fig. 2. SDS-PAGE profile of mannoprotein extracted from cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes.

essential amino acids, such as isoleucine, lysine, aspartic acid and histidine, were also found in minor amounts (Table 1). Regarding the potential of MP as an emulsifier and stabilizing compound for application in food matrices, the presence of glutamic acid in the evaluated MP is interesting because its salt, glutamate, is widely used as an additive to enhance the flavor of foods (Jinap & Hajeb, 2010); this finding may be a positive factor for maintaining the original taste of food products. The amino acid composition of MP determined in the present study differs in the amount and type of amino acids from that previously reported for MP from *S. uvarum* (Costa et al., 2012). However, the method used by researchers to obtain MP was not identical to that used in the present study. Additionally, the number of fermentation processes to which the yeast was subjected before the brewery slurry was discarded can also influence the composition of the cell wall constituents because the yeast alters the cell wall composition according to the conditions of the surrounding environment (Kapteyn et al., 1999).

Table 1
Amino acid composition of the mannoprotein extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes.

Amino acid (AA)	mg AA/g of protein
Aspartic acid	8.20 ± 0.16
Glutamic acid	5.56 ± 0.02
Serine	1.92 ± 0.17
Glycine	3.49 ± 0.04
Alanine + arginine	16.15 ± 0.23
Proline	5.20 ± 0.04
Tyrosine	2.31 ± 0.12
Histidine	4.72 ± 0.03
Threonine	3.33 ± 0.10
Valine	8.86 ± 0.09
Methionine	0.97 ± 0.07
Isoleucine	5.71 ± 0.03
Leucine	6.94 ± 0.08
Phenylalanine	1.48 ± 0.05
Lysine	5.26 ± 0.07

Table 2
Emulsifying activity (EA) and emulsion stabilizing activity (SA) of mannoprotein extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) that was discarded as brewery slurry after successive fermentation processes.

Sample[d]	EA (%)	SA (%)
A	47.7 ± 0.1[a]	98.2 ± 1.0[a]
B	45.2 ± 0.2[b]	97.0 ± 2.0[a]
C	43.9 ± 1.0[b]	96.7 ± 1.9[a]
D	38.2 ± 0.6[c]	97.0 ± 1.0[a]

EA: emulsifying activity; and SA: stabilizing activity. [a–c]For each trial, different superscript lowercase letters in an identical column denote differences ($P \leq 0.05$) between the mean values according to Tukey's test.

[d] The concentrations of mannoprotein (MP) in the samples (grams of MP for each 100 g of emulsion) are as follows: A, 0.8 g of MP/100 g of emulsion; B, 0.6 g of MP/100 g of emulsion; C, 0.4 g of MP/100 g of emulsion; and D, 0.2 g of MP/100 g of emulsion.

3.4. Emulsifying properties of MP

The emulsifying activity (EA) of MP increased with increasing MP concentration, with the highest EA obtained for 0.8 g of MP/100 g of emulsion; however, this behavior was not observed for the corresponding stabilizing activity (Table 2). These results could be related to the amino acid composition and, particularly, to the molecular weight (greater than 50 kDa) of the isolated MP, which suggests better stabilizing than emulsifying properties (Rosenberg & Ron, 1999). A similar EA and SA were reported in a previous study on MP from spent brewer's yeast (Costa et al., 2012). According to Barriga et al. (1999), the protein portion of MP from yeast is responsible for the emulsifying properties, whereas the carbohydrate portion is responsible for the increase in the solubility of the polymer and for the stability of the formed emulsion. Considering that in previous studies, the utilized method was applied to exclusively extract MP, the results presented herein reinforce the feasibility of MP isolation using the method proposed in this study because the MP structure is similar to the previously described MP (Costa et al., 2012; Freimund et al., 2003) that was extracted using different procedures.

3.5. Chemical composition, pH, stability and color during the storage of mayonnaise prepared with MP

The chemical composition of the formulations that were prepared using different amounts of MP (MP1: 0.6 g of MP/100 g of emulsion; MP2: 0.8 g of MP/100 g of emulsion; MP3: 1.0 g of MP/100 g of emulsion) was not altered during the examined storage period. Each 100 g of mayonnaise prepared with MP contained, on average,

Table 3
The observed stability of mayonnaise formulations during 28 days of refrigerated storage that were prepared with mannoprotein extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) after successive fermentation processes.

Formulation	Days of storage		
	Zero	14	28
MP1	94.23 ± 0.12[a]	96.46 ± 0.13[b]	97.93 ± 0.11[c]
MP2	94.22 ± 0.10[a]	96.81 ± 0.07[b]	98.00 ± 0.09[c]
MP3	97.72 ± 0.09[a]	98.58 ± 0.08[b]	99.75 ± 0.07[c]
XG (control)	94.56 ± 0.08[a]	98.84 ± 0.02[b]	97.45 ± 0.41[b]

MP1: 0.6 g of mannoprotein/100 g of mayonnaise; MP2: 0.8 g of mannoprotein/100 g of mayonnaise; MP3: 1.0 g of mannoprotein/100 g of mayonnaise; and XG: 0.1 g of xanthan gum/100 g of mayonnaise. [a–c]For each trial, different superscript lowercase letters in an identical row denote differences ($P \leq 0.05$) between the mean values according to Tukey's test.

Please cite this article as: Silva Araújo, V.B., et al., Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the..., *Innovative Food Science and Emerging Technologies* (2014), http://dx.doi.org/10.1016/j.ifset.2013.12.013

Table 4
Color analyses of different mayonnaise formulations during 28 days of refrigerated storage that were prepared with mannoprotein extracted from the cell wall of brewer's yeast (*Saccharomyces uvarum*) after successive fermentation processes.

	Color parameters								
	L*			a*			b*		
Days of storage	Zero	14	28	zero	14	28	Zero	14	28
MP1	90.98 ± 0.66^a	90.49 ± 0.75^a	90.06 ± 0.27^a	-0.96 ± 0.04^a	-0.64 ± 0.01^b	-0.62 ± 0.04^b	15.35 ± 0.37^a	13.66 ± 0.16^b	13.40 ± 0.06^b
MP2	91.44 ± 0.37^a	91.81 ± 0.48^a	90.14 ± 0.08^b	-1.02 ± 0.17^a	-0.77 ± 0.01^b	-0.64 ± 0.04^b	15.89 ± 0.50^a	$14.14 \pm 0{,}08^b$	14.02 ± 0.06^b
MP3	92.14 ± 0.26^a	91.57 ± 0.17^b	90.16 ± 0.16^b	-1.42 ± 0.15^a	-0.83 ± 0.02^b	-0.74 ± 0.05^c	16.29 ± 0.15^a	$14.51 \pm 0{,}04^b$	14.49 ± 0.10^b
GX (control)	91.54 ± 0.11^b	92.28 ± 0.21^a	92.74 ± 0.63^a	-0.67 ± 0.04^a	-0.46 ± 0.46^b	-0.47 ± 0.01^b	12.51 ± 0.38^a	13.33 ± 0.32^b	14.26 ± 0.14^c

MP1: formulation prepared with 0.6 g of MP/100 g of mayonnaise; MP2: formulation prepared with 0.8 g of MP/100 g of mayonnaise; MP3: formulation prepared with 1.0 g of MP/100 g of mayonnaise; and XG: formulation prepared with 0.1 g of xanthan gum/100 g of mayonnaise. [a–c]For each trial, different superscript lowercase letters in an identical column denote differences ($P \leq 0.05$) between the mean values according to Tukey's test.

25 g of moisture, 1.6 g of ash, 2.4 g of protein, 56 g of fat and 14 g of carbohydrates. The pH values of the mayonnaise formulations prepared with MP (MP1: 4.8 ± 0.05; MP2: 4.8 ± 0.03; MP3: 4.8 ± 0.02) did not change ($P \geq 0.05$) during the examined storage period; however, tended to decrease after 28 days of storage (MP1: 4.63 ± 0.01; MP2: 4.69 ± 0.03; MP3: 4.7 ± 0.02). Conversely, the pH values of the formulation prepared with XG tended to increase over the same storage assessed period (XG: 4.58 ± 0.02; after 28 days, XG: 4.70 ± 0.02). The stability of the MP1, MP2 and MP3 formulations increased ($P \leq 0.05$) during the assessed refrigerated storage time, which was not observed for the XG formulation, where was observed an increase in stability up to 14 days (Table 3). This behavior most likely result from the high stabilizing activity observed for MP (Table 2); however, other factors such as pH and the refrigeration temperature could also influence the observed stability because no differences were found in the stability of the mayonnaise formulations containing different concentrations of MP. Previous studies indicated that MP from brewer's yeast formed stable emulsions in a range of pH values and salt concentrations (Barriga et al., 1999; Cameron et al., 1988), and the pH has been cited as one of the most important limiting factors of the stabilization of emulsions that are prepared using proteins (Guo & Mu, 2011). The refrigeration temperature has also been cited as an important factor to maintain the desirable characteristics of emulsions (Magnusson, Rosén, & Nilsson, 2011). Good stabilizing agents must prevent the crystallization of the water and oil phases, which, depending on the size of the crystals, could result in destabilization of the system (Ghosh & Coupland, 2008). Presumably, the properties of MP improve the emulsion consistency and delay the coalescence of fat droplets.

The mayonnaise prepared with the three concentrations of MP exhibited high lightness (L* value ≥ 90), which did not differ ($P \geq 0.05$) among the assessed mayonnaise formulations or from the mayonnaise containing XG (Table 4). The lightness of the mayonnaise, which most likely resulted from the formation of crystals with similar size, is cited to positively influence the acceptance of the consumer (El-Bostany, Gaafar, & Salem, 2011; Mun et al., 2009). Over 28 days of refrigerated storage, an increase in green color (a* value) was observed for MP1, MP2, MP3 and XG. However, the yellow color (b* value) tended to decrease in the mayonnaise formulations prepared with MP during the assessed storage period, in contrast to the control formulation, which demonstrated increasing yellow color values. Early study assessing the use of constituents from yeast cell walls, such as β-glucan, in mayonnaise emulsions found a decrease in yellow color in these products and a parallel increase in their lightness (Santipanichwong & Suphantharika, 2007).

3.6. Sensory analyses of mayonnaise produced with MP

The three mayonnaise formulations prepared with MP received similar scores ($P \geq 0.05$) after 1 and 28 days of refrigerated storage for all assessed sensory attributes. The scores obtained for aroma (MP1: 6.8; MP2: 6.7; MP3: 7.0), color (MP1: 7.7; MP2: 7.5; MP3: 7.6), flavor (MP1: 7.4; MP2: 7.3; MP3: 7.8) and texture (MP1: 7.8; MP2: 7.6; MP3: 8.0) for all mayonnaise formulations prepared with MP corresponded to "liked much" and "liked" and did not differ ($P \geq 0.05$) in comparison to the scores given for the control mayonnaise (prepared with XG: 7.8). The average scores for the overall evaluation of the different mayonnaise formulations that were prepared with MP and the control (MP1: 7.9; MP2: 8.0; MP3: 8.0; XG 7.6) corresponded to "liked much" after the first day of storage and did not differ ($P > 0.05$) after the assessed refrigerated storage time (after 28 days; MP1: 8.0; MP2: 7.8; MP3: 8.1; XG 7.9). The high scores (approximately 8.0) for the color parameters of all formulations prepared with MP, which were similar to those of the control sample, are important because the analytical evaluation of the color revealed a decrease in yellow color during the assessed storage period; however, these changes were not negatively noted by the tasters.

When asked to report on the intent to purchase, the tasters reported the intent to purchase for all of the assessed formulations. The formulation prepared with 1.0 g of MP/100 g of emulsion (MP3) received the highest scores for the intent to purchase; however, no differences ($P > 0.05$) were observed among the three formulations prepared with MP or with the control sample after the first day (MP1: 3.4; MP2: 3.6; MP3: 4.1; XG 3.6) and after 28 days of refrigerated storage (MP1: 3.5; MP2: 3.4; MP3: 4.0; XG: 3.6). The higher average scores for the intent to purchase noted for MP3 in comparison to the other formulations could be related to the firmer consistency noted by the tasters. According to El-Bostany et al. (2011), the consistency is an attribute strongly perceived by the consumers of sauces and creams. These findings reveal that MP, in the concentrations assayed in this study, did not demonstrate negative effects on the sensory characteristics of the tested mayonnaise formulations.

4. Conclusions

The results presented herein demonstrated the feasibility of the proposed followed extraction method that obtain both β-glucan and MP, with a high yield and satisfactory purity, from the cell wall of *S. uvarum* discarded after several industrial fermentations. The obtained MP revealed interesting emulsifying and stabilizing properties, and its use to replace XG in mayonnaise formulation had no negative effect on the sensory properties of the product during refrigerated storage. These findings suggest the application of this protein as an alternative for the food industry to replace synthetic emulsifying/stabilizing agents used in foods, particularly in sauces such as mayonnaise.

Acknowledgments

The authors acknowledge the National Council for Scientific and Technological Development (CNPq, Brazil) for the financial support.

Please cite this article as: Silva Araújo, V.B., et al., Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the..., *Innovative Food Science and Emerging Technologies* (2014), http://dx.doi.org/10.1016/j.ifset.2013.12.013

References

AOAC (2005). *Official methods of analysis* (18th ed.). Gaithersburg, MD: AOAC Intl.

APHA (2001). *Compendium of methods for the microbiological examination of foods* (pp. 676) (4th ed.). Washington, DC: American public health association.

Barriga, J. A. T., Cooper, D.G., Idziak, E. S., & Cameron, D. R. (1999). Components of the emulsifier from *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology*, *25*, 96–102.

Bohn, J. A., & BeMiller, J. M. (1985). (1-3)-β-D-glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers*, *28*, 3–14.

Cameron, D. R., Cooper, D.G., & Neufeld, R. J. (1988). The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier. *Applied and Environmental Microbiology*, *54*, 1420–1425.

Costa, A. G., Magnani, M., & Castro-Gomez, R. J. H. (2012). Obtention and characterization of extract content mannoproteins from yeast discarded from brewery. *Acta Scientiarum — Biological Science*, *34*, 77–84.

Darpossolo, F. P. B., Silas, F. E., Venancio, E. J., & Gomez, R. J. H. C. (2012). *Saccharomyces uvarum* mannoproteins stimulate a humoral immune response in mice. *Brazilian Archives of Biology and Technology*, *55*, 597–602.

Dikit, P.S. M., Musikasang, H., & H-kittikun, A. (2010). Emulsifier properties of the mannoprotein extract from yeast isolated from sugar palm wine. *ScienceAsia*, *36*, 312–318.

Ding, J., Wang, Y., Xiong, S., Zhao, S., & Huang, Q. (2013). Optimised methodology for carboxymethylation of (1-3)-b-D-glucan from yeast (*Saccharomyces cerevisiae*) and promotion of mechanical activation. *International Journal of Food Science and Technology*, *48*, 253–259.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method form determination of sugars and related substances. *Nature*, *28*, 350–356.

El-Bostany, N. A., Gaafar, A.M., & Salem, A. A. (2011). Development of light mayonnaise formula using carbohydrate-based fat replacement. *Australian Journal of Basic and Applied Sciences*, *5*, 673–682.

Freimund, S., Sauter, M., Kappeli, O., & Dutler, H. (2003). A new non-degrading isolation process for 1,3-β-D-glucan of high purity from baker's yeast *Saccharomyces cerevisiae*. *Carbohydrate Polymers*, *54*, 159–171.

Ghosh, S., & Coupland, J. N. (2008). Factors affecting the freeze thaw stability of emulsions. *Food Hydrocolloids*, *22*, 105–111.

Guo, Q., & Mu, T. H. (2011). Emulsifying properties of sweet potato protein: effect of protein concentration and oil volume fraction. *Food Hydrocolloids*, *25*, 98e–106e.

Hromádková, K., Ebringerová, A., Sasinková, V., Sandula, J., Hribalová, V., & Omelková, J. (2003). Influence of the drying method on the physical properties and immunomodulatory activity of the particulate (1-3)-β-D-glucan from *Saccharomyces cerevisiae*. *Carbohydrate Polymers*, *51*, 9–15.

Jinap, S., & Hajeb, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite*, *55*, 1–10.

Kapteyn, J. C., Van Oen Enoe, H., & Klis, F. M. (1999). The contribution of cell wall proteins to the organization of the yeast cell wall. *Biochimica et Biophysica Acta*, *1462*, 373–383.

Kogan, G., Alföldi, J., & Masler, L. (1988). 13C-NMR spectroscopic investigation of two yeast cell wall β-D-glucans. *Bioplolymers*, *27*(7), 1055–1063.

Liu, X. -Y., Wang, Q., Cui, S. W., & Liu, H. -Z. (2008). A new isolation method of β-D-glucans from spent yeast *Saccharomyces cerevisiae*. *Food Hydrocolloids*, *22*, 239–247.

Magnani, M., Calliari, C. M., Macedo, F., Jr., Mori, M. P., Syllos-Cólus, I. M., & Castro-Gomez, R. J. H. (2009). Optimized methodology for extraction of (1-3)(1-6)-β-D-glucan from *Saccharomyces cerevisiae* and in vitro evaluation of the cytotoxicity and genotoxicity of the corresponding carboxymethyl derivative. *Carbohydrate Polymers*, *78*, 658–665.

Magnani, M., Castro-Gomez, R. J. H., Aoki, M. N., Gregório, E. P., Libos, F., Jr., & Watanabe, M.A. E. (2010). Effects of carboxymethyl-glucan from *Saccharomyces cerevisiae* on the peripheral blood cells of patients with advanced prostate cancer. *Experimental and Therapeutic Medicine*, *1*, 859–862.

Magnusson, E., Rosén, C., & Nilsson, L. (2011). Freeze–thaw stability of mayonnaise type oil-in-water emulsions. *Food Hydrocolloids*, *25*, 707–715.

Martinez-checa, F., Toledo, F. L., Mabrouki, K., Quesada, E., & Calvo, C. (2007). Characteristics of bioemulsier V2-7 synthesized in culture media added of hydrocarbons: chemical composition, emulsifying activity and rheological properties. *Bioresource Technology*, *98*, 3130–3135.

Mun, S., Kim, Y. L., Kang, C. G., Park, K. W., Shim, J. S., & Kim, Y. R. (2009). Development of reduced-fat mayonnaise using 4_GTase-modified rice starch and xanthan gum. *International Journal of Biological Macromolecules*, *44*, 400–407.

Nikzade, V., Mazaheri Tehrani, M., & Saadatmand-Tarzjan, M. (2012). Optimization of low-cholesterol-low-fat mayonnaise formulation: effect of using soy milk and some stabilizer by a mixture design approach. *Food Hydrocolloids*, *28*, 344–352.

Rosenberg, E., & Ron, E. Z. (1999). High and low molecular mass microbial surfactants. *Applied Microbiology and Biotechnology*, *52*, 154–162.

Sandula, J., Kogan, G., Kacuraková, M., & Machová, E. (1999). Microbial (1-3)-β-D-glucans, their preparation, physico-chemical characterization and immunomodulatory activity. *Carbohydrate Polymers*, *38*, 247–253.

Santipanichwong, R., & Suphantharika, M. (2007). Carotenoids as colorants in reduced-fat mayonnaise containing spent brewer's yeast β-glucan as a fat replacer. *Food Hydrocolloids*, *21*, 565–574.

Weitberg, A. (2008). A phase I/II trial of β-(1,3)/(1,6) D-glucan in the treatment of patients with advanced malignancies receiving chemotherapy. *Journal of Experimental and Clinical Cancer Research*, *27*, 40–41.

White, J. A., Hart, R. J., & Fry, J. C. (1986). An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. *Journal of Automatic Chemistry*, *8*, 170–177.

Xiaozhong, H., Jie, M., Baogui, Z., & Wangxiang, F. (2000). Isolation of β-(1,3)-D-glucan from baker's yeast by acid–alkali method. *Industrial Microbiology Chinese*, *30*, 28–31.

Please cite this article as: Silva Araújo, V.B., et al., Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the..., *Innovative Food Science and Emerging Technologies* (2014), http://dx.doi.org/10.1016/j.ifset.2013.12.013

ANEXOS

ANEXO 1- Parecer Comitê de Ética

UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
COMITÊ DE ÉTICA EM PESQUISA

CERTIDÃO

Certifico que o Comitê de Ética em Pesquisa, do Centro de Ciências da Saúde da Universidade Federal da Paraíba – CEP/CCS aprovou por unanimidade na 3ª Reunião realizada no dia 18/03/2013, o projeto de pesquisa intitulado: "OBTENÇÃO DE BIOEMULSIFICANTE E DE BIOMODULADOR A PARTIR DA PAREDE CELULAR DE LEVEDURA DESCARTADA EM CERVEJARIA PARA APLICAÇÃO EM ALIMENTOS" da Pesquisadora Vilma Barbosa da Silva Araújo. Prot. nº 001/13. CAAE: 10734712.8.0000.5188.

Outrossim, informo que a autorização para posterior publicação fica condicionada à apresentação do resumo do estudo proposto à apreciação do Comitê.

Drª Eliane Marques D. Sousa
Coordenadora CEP/CCS/UFPB
Mat. SIAPE: 0332618

Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba
Campus I – Cidade Universitária – Bloco Arnaldo Tavares – Sala 812
CEP 58051-900 – João Pessoa – PB - ☎ (83) 3216 7791

ANEXO 2- Ficha para análise sensorial da maionese

UNIVERSIDADE FEDERAL DA PARAÍBA – UFPB
CENTRO DE TECNOLOGIA – CT
PROGRAMA DE PÓS-GRADUAÇÃO EM CIENCIA E TECNOLOGIA DE ALIMENTOS - PPGCTA

Nome:_______________________________________Data:___/____/___

Faixa etária: até 20 anos (   ) até 30 anos (   ) acima de 30 anos (   )

1) Você está recebendo quatro amostras de maionese de forma codificada. Por favor, **prove** e **avalie** as amostras utilizando a escala abaixo para indicar o quanto você gostou ou desgostou para cada atributo quanto ao aroma, cor, sabor, textura, e aceitação global do produto.

(9) Gostei extremamente
(8) Gostei moderadamente
(7) Gostei regularmente
(6) Gostei ligeiramente
(5) Não gostei, nem desgostei
(4) Desgostei ligeiramente
(3) Desgostei regularmente
(2) Desgostei moderadamente
(1) Desgostei extremamente

CÓDIGO DA AMOSTRA	**ATRIBUTOS**				
	Aroma	Cor	Sabor	Textura	Aceitação Global

2) Por favor, a**valie as quatro amostras** segundo a sua **intenção de compra**, utilizando a escala abaixo:
(5) Compraria
(4) Possivelmente Compraria
(3) Talvez comprasse/ talvez não comprasse
(2) Possivelmente não compraria
(1) Não compraria

CÓDIGO DA AMOSTRA			

ANEXO 3- Aceite do artigo

Article title: Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (Saccharomyces uvarum) and application of the obtained mannoprotein as a stabilizer in mayonnaise

Reference: INNFOO1116
Journal title: Innovative Food Science and Emerging Technologies
Corresponding author: Dr. Marciane Magnani
First author: Dr. Vilma Barbosa da Silva Araújo
Online publication complete: 30-JAN-2014
DOI information: 10.1016/j.ifset.2013.12.013

Dear Dr. Magnani,

We are pleased to inform you that the final corrections to your proofs have been made. Further corrections are no longer possible. Your article is now published online at:

http://dx.doi.org/10.1016/j.ifset.2013.12.013
Please note that access to the full text of this article will depend on your personal or institutional entitlements.

This article can already be cited using the year of online availability and the DOI as follows: Author(s), Article Title, Journal (Year), DOI. You will be automatically notified by e-mail once the full bibliographic details are available.

Yours sincerely,
Elsevier Author Support

ANEXO 4- Artigo submetido à Revista Carbohydrate Polymers

Oral intake of carboxymethyl-glucan (CMG) derived from yeast (*Saccharomyces uvarum*) does not show immunostimulant properties but reduces malondialdehyde levels in healthy individuals

Running Title: Antioxidant effects of Carboxymethyl-glucan

Vilma Barbosa da Silva Araújo[a], Adma Nadja Ferreira de Melo[a], Vânia Maria Barboza da Silva[a], Raul H. Castro-Gomez[b], Alexandre Sérgio Silva[c], Evandro Leite de Souza[d], Marciane Magnani[a],*

[a]*Laboratory of Food Biochemistry, Department of Food Engineering, Federal University of Paraíba, João Pessoa, Brazil*
[b]*Department of Food Science and Technology, University of Paraná State, Londrina, Brazil*
[c]*Department of Physical Education, Federal University of Paraíba, João Pessoa, Paraíba, Brazil*
[d]*Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba, João Pessoa, Brazil*

*Corresponding author: Tel.: +55 83 3216 7576 Fax: +55 83 3216 7269.
E-mail: magnani2@gmail.com (M Magnani)

Abstract

Carboxymethyl-glucan (CM-G) is a water-soluble derivative of β(1-3)(1-6) glucan, a well-known immunostimulant and antioxidant compound. In this experimental, randomized and placebo-controlled study, the effects of oral CM-G intake over a 60 day period on the peripheral blood, cholesterol, glycemic index and malondialdehyde (MDA) levels of healthy men was assessed. Following CMG administration, no changes were observed in red and white blood cell, hematocrit, hemoglobin and platelet counts, or to cholesterol and glycemic indices. After 30 days of CM-G administration, the MDA levels decreased significantly ($p \leq 0.05$) in men

receiving CG-G orally. These results suggest that CM-G may act as an adjuvant in preventing oxidative damage in humans.

Key words: Carboxymethyl-glucan, glucan-derivatives, malondialdehyde, blood cells.

1. Introduction

β-glucans from yeast and some water-soluble derivatives have recognized immunomodulatory effects, in addition to reductions in cholesterol and blood sugar levels (Nicolosi et al., 1999; Demir, Klein, Mandel-Molinas & Tuzuner, 2007; Magnani et al., 2011b). Carboxymethyl-glucan (CM-G) is a water-soluble derivative of β(1-3)(1-6) glucan, derived from the *Saccharomyces cerevisiae* cell wall . CM-G has been of particular interest to researchers due to its bioactive properties when ingested as a food supplement and because of its long safety record (Weitberg, 2008; Magnani et al., 2010, 2011b).

CM-G has significant bioprotective properties, such as antimutagenic, antigenotoxic, antioxidant and anticancer effects (Slamenová *et al*., 2003; Kubala, Ruzickova & Nickova, 2003; Miadaková *et al*., 2005; Magnani et al., 2011b; Firat et al., 2013). The main proposed mechanism behind these protective effects is the capability to scavenge reactive oxygen species (ROS) at low concentrations (Kogan & Rauko, 2005), with activity compared to α-tocopherol (Babincová, Bacova, Machova & Kogan, 2002). Due to its immunomodulatory and antioxidant effects, CM-G has been studied in clinical trials as a component of therapy for a variety of diseases, including prostate cancer (Demir et al., 2007; Liu, Gunn, Hanses & Yan, 2009; Magnani et al., 2010; 2011b). However, because the majority of these studies involved patients with cancer or hyperlipidemia, especially men, information regarding the effects or benefits of CM-G intake for healthy individuals is still scarce.

Lipid peroxidation (LPO) mediated by ROS has been implicated in many diseases. Malondialdehyde (MDA), a product of LPO, has been adopted as a measure of free radical production and therefore an "index of LPO" (Toklu et al., 2006; Ozkan et al., 2010). Studies involving experimental models of induced oxidative damage demonstrated that local or systemic administration of β-glucan from yeast, through its antioxidant activity, could decrease MDA levels (Kogan et al., 2005; Ozkan et al., 2010).

As some researchers have reported that lifestyle changes lead to increased consumption of food and compounds thought to be associated with health, especially compounds that reduce oxidative damage in the body (Wootton-beard & Ryan, 2011; Abete et al., 2013), this study

assessed the effects of oral CM-G intake on the peripheral cells, cholesterol levels, MDA levels and blood glucose levels of healthy individuals.

2. Materials and methods

2.1 Individuals

The study was described as experimental, randomized and placebo-controlled and was conducted after the approval of the Committee on Ethical Research Involving Humans Beings of the Federal University of Paraíba (João Pesoa, Brazil), under the Process Number 10734712.8.0000.5188. A total of 26 healthy men ranging between 26 and 37 years of age (median age 29 ± 268 years) were included after giving their written informed consent. Exclusion criteria included the regular consumption of alcohol, tobacco smoke and/or the use of medications for chronic or congenital diseases. The inclusion criteria included an age greater than 22 years, normal blood cell levels, normal blood glucose and cholesterol levels, participation in physical activity at least two times a week and consumption of a diet including salad and fruit an average of three times a week. During the 60 days of CM-G intake, individuals were contacted weekly to monitor their condition and to report any adverse effects associated with the trial.

2.2 CM-G and Placebo

CM-G was obtained from the cell wall of *Saccharomyces uvarum* discarded from a brewery as slurry, with a substitution degree of 0.8, according to the procedure described by Magnani et al. (2009). The CM-G or placebo (starch) was divided into 50 mg portions, which were packed in metalized BOPP bags and hermetically sealed.

2.3 Blood samples

Peripheral venous blood samples were drawn for initial values from the subjects on day 1 of the study before the ingestion of CM-G with breakfast. Early each morning, individuals ingested a 50 mg CM-G or placebo capsule with breakfast. After 30 and 60 days, blood samples were recollected and analyzed. Both samples were taken while patients were fasting. Four-and-a-half milliliter Vaccutainer^{TM} tubes containing EDTA were used, and all the samples were processed immediately after collection by an automatic method, using Abbott Cell Dyn 3200 for blood cells counts. Blood glucose levels were determined by an automated spectrophotometric system (Baker Instruments, Allentown, PA), and total LDL- and HDL-cholesterol levels (mg/dL) were calculated according to the method described by Nicolosi et al.

(1999). The results obtained before and after CM-G or placebo intake were analyzed using the Wilcoxon signed-rank test and the *t* test for dependent paired samples. A P value ≤ 0.05 indicated significant differences.

3. Results and discussion

No changes were observed in the kidney and liver function (Table 1) of the men receiving CM-G, and no side effects associated with CM-G were recorded, reinforcing early findings regarding the safety of CM-G (Wietberg, 2008; Magnani et al., 2010).

Table 1. Results of liver and kidney function tests performed before and after carboxymethyl-glucan (CM-G) derived from *S. uvarum* intake with food for 60 days by healthy men.

Liver Function	**Before CMG**	**60 days after CMG**	**Reference Value (method)**
Transaminase aspartate amino transferase (TGO)	25.1 ± 1	24.9 ± 0.9	11 to 41 U/L (automated kinetics)
Transaminase alanine amino transferase (TGP)	10.2 ± 0.5	10.4 ± 0.8	7 to 52 U/L (automated kinetics)
Albumin	4.50 ± 0.75	4320 ± 0.50	3.35 to 5.62 g/dL (***Capillary electrophoresis***)
Direct bilirubin	0.07 ± 0.01	0.08 ± 0.02	Up to 0.3 mg/dL (colorimetric)
Indirect bilirubin	0.33 ± 0.12	0.32 ± 0.13	Up to 0.7 mg/dL (colorimetric)
Alkaline phosphatase	41.2 ± 10	40 ± 9	27 to 100 U/L (automated kinetics)
Kidney Function			
Urea	38 ± 9	37 ± 10	10 to 52 mg/dL (automated enzymatic)
Creatinine	0,85 ± 0.23	0,89 ± 0.23	1.30 mg/dL (automated kinetics)

After CM-G intake, the total leukocyte counts did not increase significantly ($p > 0.05$). Additionally, increasing trends for typical lymphocytes, monocytes and neutrophils were observed (Figure 1). These results are in accordance with the findings reported by Demir *et al.* (2009), who observed no changes in leukocyte counts after 14 days of oral β-glucan administration for women undergoing cancer treatment. In contrast, Magnani et al. (2011) observed a significant increase in total leukocyte counts, with an increase in typical lymphocytes, monocytes and neutrophils in men with advanced prostate cancer. Some models have demonstrated the ability of β-glucans and its derivatives administered by different routes

to raise blood cell counts after leukopenia secondary to cancer treatments (Kubala, Ruzickova & Nickova, 2003; Vetvicka, Dvorak & Vetvickova, 2007; Harada, Kawaminami & Miura, 2006; Magnani et al., 2011b; Weitberg, 2008). However, one hypothesis is that increases in blood cell counts in cancer patients receiving β-glucans are in part a physiological response to internal signals, such as low cells counts or inflammation. A previous study noted that the increase in blood cells was more pronounced in cancer patients with leukopenia when compared to those with normal counts before receiving CM-G (Magnani et al., 2011b).

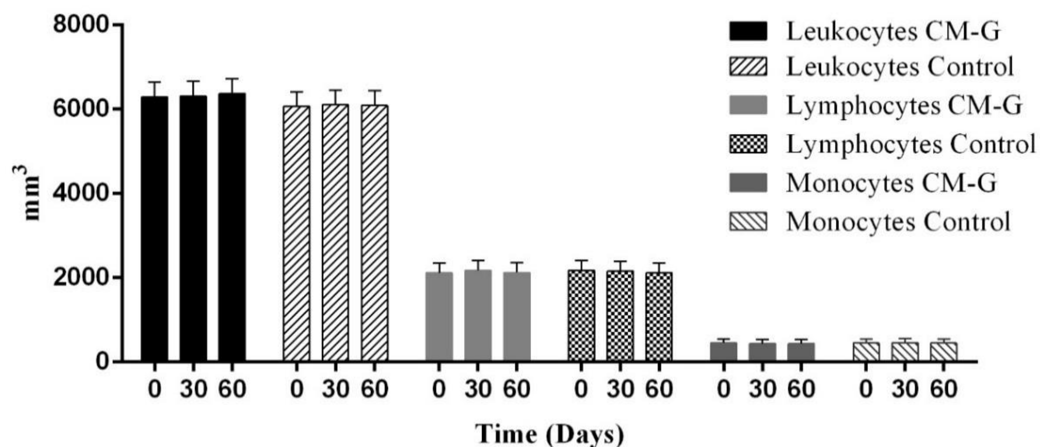

Figure 1 Total leukocyte, monocytes and typical lymphocytes counts before and after after carboxymethyl-glucan (CM-G) derived from *S. uvarum* intake with food for 30 and 60 days by healthy men.

Significant differences ($p \leq 0.05$) were not found in total (168.7±16.9 before; 167.8±16.9 after 60 days) HDL- (168.7±14.5 before; 167.8±14.9 after 60 days) or LDL- (37.4±14.9 before; 38.8±14.1 after 60 days) cholesterol levels after 60 days of CM-G intake. β-glucans from yeast possess anti-hyperlipidemia properties, and consumption of β-glucans is related to reductions in total cholesterol and LDL levels (Nicolosi et al., 1999). However, these effects are described for insoluble forms of glucans, which act as dietary fibers. In our study, two aspects could explain our finding of no changes in cholesterol levels: first, low amounts of CM-G were administered; second, the soluble form was used, which is able to pass from the gastrointestinal tract into systemic circulation. Orally administered soluble derivatives of β-glucan are absorbed through the gastrointestinal wall and pass into the circulatory system (Rice,

Adams & Ozment-Skelton, 2005), activating immune pathways such as Dectin-1, CR-3, SIGNR1, TLR-2/6 and 4 (Brown & Gordon, 2005).

After 30 or 60 days of CM-G intake, no significant increase ($p > 0.05$) in erythrocyte, hematocrit, hemoglobin or platelet counts was observed (Figure 2). Although some studies report that CM-G acts directly on myeloid progenitors, contributing to hematopoietic regeneration (Pospisil, Sandula & Pipalova 1991; Hofer & Pospisil, 1997), early studies have had interesting results regarding changes in platelets and other hematimetric indices following oral administration of β-glucan derivatives in cancer patients. Increases in hemoglobin and platelet levels in cancer patients following 28 days of oral β-glucan administration have been reported (Wietberg, 2008).

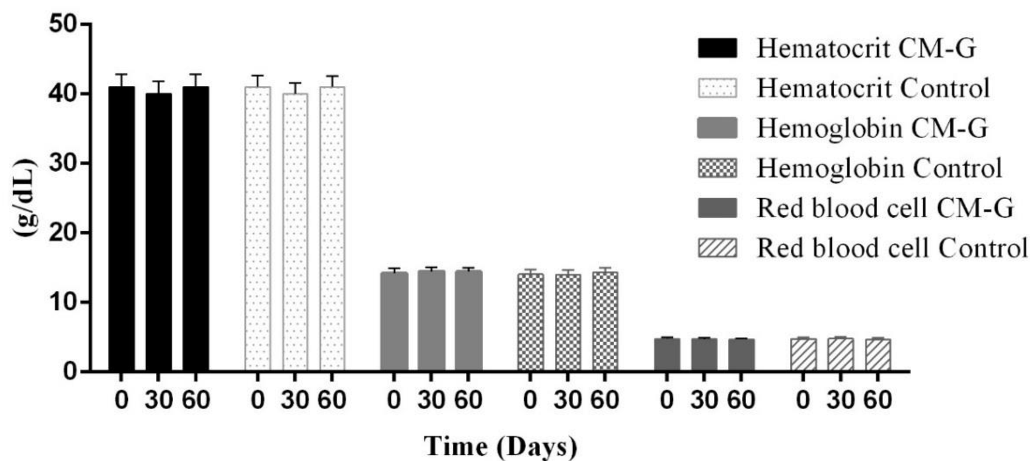

Figure 2 Hematological data (erythrocyte, hemoglobin, red blood cells) before and after after carboxymethyl-glucan (CM-G) derived from *S. uvarum* intake with food for 30 and 60 days by healthy men.

Similarly, Magnani et al. (2010) observed an increase in erythrocyte, platelet, hematocrit and hemoglobin levels after 28 days of CM-G administration in men with advanced prostate cancer, especially in patients initially presenting with values below the minimum reference value. This further supports the hypothesis that β-glucan's action is triggered by signals in the body, considering that in its soluble form β-glucan acts synergistically with *in vivo* myeloid growth factors and cell signaling, improving hematopoietic recovery and mobilizing progenitor cells in the peripheral blood (Patchen, Vaudrain & Correira, 1998; Turnbull, Patchen & Scadden, 1999; Magnani et al., 2011b).

The MDA levels of the group receiving CM-G were reduced ($p \leq 0.05$) after 30 and 60 days of intake when compared to the placebo group, which had no changes in MDA levels

(Figure 3). A decrease in MDA levels was also reported in a previous study of induced gastric damage (Ozkan, et. al., 2010), in a model of reactive oxygen species induced in platelets (Saluk, Bijak, Ponczek, Nowak & Wachowicz, 2013) and in burn-induced oxidative organ damage in rats (Toklu et al., 2006). Considering that MDA is an important measure of LPO, which is associated with oxidative damage (particularly in membranes), intake of CM-G could be a method to prevent oxidation, which naturally occurs in cells under the influence of many factors, including aging (Pawelec, Lustgarten, Ruby Gravekamp, 2009).

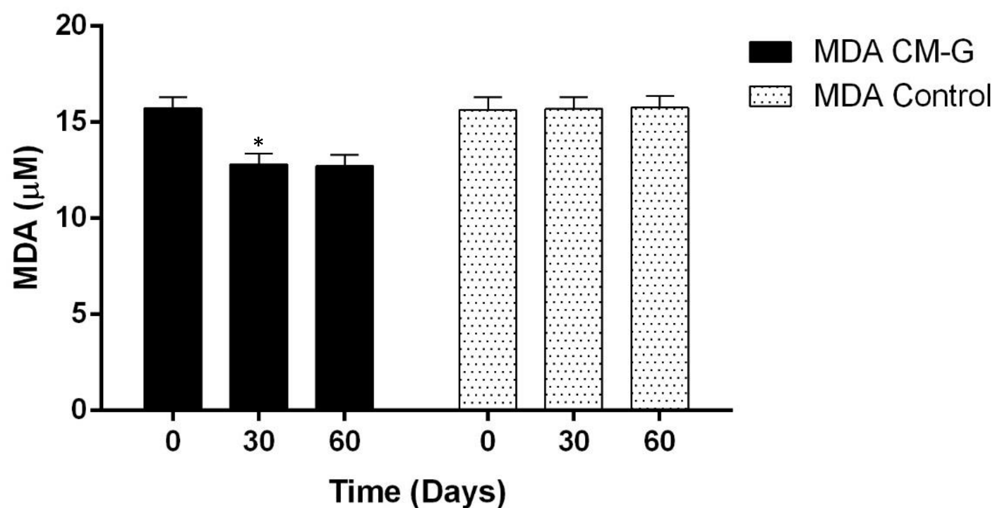

Figure 3 Malondialdehyde (MDA) levels before and after after carboxymethyl-glucan (CM-G) derived from *S. uvarum* intake with food for 30 and 60 days by healthy men.

Our findings are also important considering that CM-G was administered with food and in healthy individuals, suggesting that the antioxidant properties of CM-G derived from yeast are not related to disease-signaling in the body and are not compromised when ingested with foods. To the best of our knowledge, this is the first report of a decrease in blood MDA levels in healthy men following the intake of yeast-derived CM-G derived with food.

Acknowledgments

The authors thank the National Council for Scientific and Technological Development (CNPq-Brazil) for their financial support and CAPES for the master scholarship of the first author VBS Araújo.

References

Abete, I., Perez-Cornago, A., Navas-Carretero, S., Bondia-Pons, I., Zulet, M. A., Martinez, J. A. (2013). A regular lycopene enriched tomato sauce consumption influences antioxidant status of healthy young-subjects: A crossover study. *Journal of functional foods*, 5, 28-35.

Babincová M., Bacova Z., Machova E. & Kogan G. (2002). Antioxidant activity of carboxymethyl glucan: Comparative analysis. *Journal of Medicinal Food* 5:79-83.

Brown GD and Gordon S (2005). Immune recognition of fungal. *Cell Microbiology,* 7, 471-479.

Demir G., Klein H.O., Mandel-Molinas N. & Tuzuner N. (2007). β-glucan induces proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer. *International Immunopharmacology*, 7, 113-116.

Firat C., Samdancı E., Erbatur S., Aytekin A.H., Ak M., Turtay M.G., & Coban Y.K. (2013). β-Glucan treatment prevents progressive burn ischaemia in the zone of stasis and improves burn healing: An experimental study in rats. *Burns*, 39, 105-12.

Harada T., Kawaminami H., Miura N.N., Adachi, Y., Nakajima, T. et al (2006). Mechanism of enhanced hematopoietic esponse by soluble β-glucan SCG in cyclophosphamide-treated mice. *Microbiology Immunology,* 50, 687-700.

Hofer M. & Pospisil M. (1997). Glucan as stimulator of hematopoiesis in normal and gamma-irradiated mice. A survey of the authors'results. *International Journal of Immunopharmacology,* 19, 607-609.

Kogan G. & Rauko P. (2005). The role of natural biopolymers in genotoxicity of mutagens carcinogens elimination. *Biomedical Papers of the Medical Faculty of the University Palacky,* 149, 493-496.

Kubala L., Ruzickova J., Nickova K., Sandula, J., Ciz, M. & Lojek, A.et al (2003). The effect of (1→3)-β-D-glucans, carboxymethylglucan and schizophyllan on human leukocytes in vitro. *Carbohydrate Research,* 338, 2835-2840.

Liu J., Gunn L., Hanses R. & Yan J. (2009) Combined yeast-derived β-glucan with anti-tumor monoclonal antibody for cancer immunotherapy. *Experimental and Molecular Pathology*, 86, 208-214.

Magnani, M., Calliari, C.M., Macedo Jr. F.C., Mori, M. P., Colus, I. M. S. & Castro-Gomez, R. J. H. (2009). Optimized methodology for extraction of (1-3)(1-6)-β-D-glucan from Saccharomyces cerevisiae and in vitro evaluation of the cytotoxicity and genotoxicity of the corresponding carboxymethyl derivative. *Carbohydrate Polymers*, 78, 658-665.

Magnani, M., Castro-Gomez, R. J. H., Aoki, M. N., Gregório, E. P., Libos Jr., F & Watanabe, M. A. E. (2010). Effects of carboxymethyl-glucan from Saccharomyces cerevisiae on the peripheral blood cells of patients with advanced prostate cancer. *Experimental and Therapeutic Medicine*, 5, 859-862.

Magnani, M., Castro-Gomez, R. J. H, Mori, M. P., Kuasne, H., Gregório, E. P., Libos Jr., F. & Syllos- Cólus, I. M. (2011b). Protective effect of carboxymethyl-glucan (CM-G) against DNA damage in patients with advanced prostate cancer. *Genetics and Molecular Biology*, 1, 131-134.

Miadoková, E., Svidova, S., Vlckova, V., Duhova, V., Prazmariova, E. & Tothova, K., et al (2005). The role of natural biopolymers in genotoxicity of mutagens/carcinogens elimination. *Biomedical Papers of the Medical Faculty of the University Palacky, Olomouc,* 149, 493-496.

Nicolosi, R., Bell, S. J., Bistrian, B. R., Greenberg, I., Forse, R. A. & Blackburn, G. L (1999). Plasma lipid changes after supplementation with β-glucan fiber from yeast. *American Journal of Clinical Nutrition, New York*, 70, 208–212.

Ozkan O. V., Ozturk, O. H., Aydin, M., Yilmaz, N., Yetim, I. & Nacar, A. et al (2010). Effects of β-Glucan Pretreatment on Acetylsalicylic Acid–Induced Gastric Damage: An Experimental Study in Rats. *Current Therapeutic Research*, 71, 6.

Patchen M.L., Vaudrain T., Correira H., Martin, T. & Reese, D. (1998). In vitro and in vivo hematopoietic activities of Betafectin PGG-glucan. *Experimental Hematology,* 26, 1247-1254.

Pawelec G., Lustgarten J., Ruby C. & Gravekamp C. (2009). Impact of aging on cancer immunity and immunotherapy. *Cancer Immunology Immunotherapy,* 58, 1907-1908.

Pospisil M., Sandula J., Pipalova I., Hofer, M., & Viklická, S. (1991). Hemopoiesis stimulating and radioprotective effects of carboxymethylglucan. *Physiological Research,* 40, 377-380.

Rice P.J., Adams E.L., Ozment-Skelton T., Gonzalez, A.J., Goldman, M.P., Lockhart, B.E. & Barker, L.A. et al (2005). Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *Journal of Pharmacology and Experimental Therapeutics,* 314, 1079-1086.

Saluk, J., Bijak, M., Ponczek, M. B., Nowak, P. & Wachowicz, B (2013). (1-3)-D-Glucan reduces the damages caused by reactive oxygen species induced in human platelets by lipopolysaccharides. *Carbohydrate Polymers*, 97, 716– 724.

Slamenová, D., Laaj, J., Krizkova, L., Kogan, G., Sandula, J. & Bresgen, N. et al (2003). Protective effects of fungal (1 - 3)-β-D-glucan derivatives against oxidative DNA lesions in V79 hamster lung cells. *Cancer Letters, Amsterdam*, 198, 153-160.

Toklu, H. Z., Sener, G., Jahovic, N., Uslu, B., Arbak, S. & Yeðen, B. C. (2006). Beta-glucan protects against burn-induced oxidative organ damage in rats. *International Immunopharmacology*, 6, 156-169.

Turnbull, J.L., Patchen, M.L., & Scadden, D.T. (1999). The polysaccharide, PGG-glucan, enhances human myelopoiesis by direct action independent of and additive to early-acting cytokines. *Acta Haematologica,* 102, 66-71.

Vetvicka, V., Dvorak, B., Vetvickova, J., Richter, J., Krizan, J. & Sima, P. et al (2007). Orally administered marine (1→3)-beta-D-glucan Phycarine stimulates both humoral and cellular immunity. *International Journal Biological Macromolecules,* 40, 291-298.

Weitberg, A. (2008). A phase I/II trial of beta-(1,3)/(1,6) D-glucan in the treatment of patients with advanced malignancies receiving chemotherapy. *Journal of Experimental Clinical Cancer Research,* 27, 40.

Wootton-Beard, P. C., Ran, I. (2011). Improving public health: The role of Antioxidant-rich fruit and Vegetable Beverages. *Food research international*, 44, 3135-3148.