LUCIANA PEREIRA LOBATO

INTERAÇÃO DOS COMPONENTES DA AVEIA E DA SOJA SOBRE A COLESTEROLEMIA, IMUNOESTIMULAÇÃO E VIABILIDADE TECNOLÓGICA DE PRODUÇÃO DE ALIMENTOS PARA DIETAS ESPECIAIS

Londrina
2010

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Tese apresentada ao Programa de Pós – Graduação em Ciência de Alimentos, nível Doutorado, da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutor em Ciência de Alimentos.

Orientadora: Profa. Dra. Maria Victoria Eiras Grossmann

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Londrina, 17 de dezembro de 2010.

Dedico a

Maria Victoria Eiras Grossmann

Pelo exemplo de pessoa, professora, pesquisadora

Pela competência, serenidade e tranqüilidade

Por ser muito forte no enfrentamento de problemas, seus e dos outros

Por ter tornado esse trabalho possível, apesar das pedras no caminho

Por ter usado plurais nas dificuldades: "- **NÓS** vamos dar um jeito, Lu..."

Por acreditar nas minhas capacidades

Por sempre estar **LÁ** (... lá é o lugar onde eu precisava que estivesses)

Pelos sorrisos, abraços, conforto, segurança

Pela amizade e maternidade (quando necessária)

Pelo prazer e privilégio da convivência (ratificando)

Pelo significado que tens na minha vida

Pelo lugar que ocupa no meu coração...

Por ser tudo isso e nem imaginar que és tão importante pra mim...

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A minha família. Mãe, Pai, irmãs Alessandra e Carolina, sobrinhos Mariana, Carlos Henrique e Lara. Ratificando depoimentos anteriores, eu os escolheria novamente para essa jornada. "*Como é grande meu amor por vocês...*"

À Cleunice, grande presente dessa jornada. Pela ajuda, por me mostrar os caminhos, pelos florais, caronas, atendimentos especiais e terapias. Pela amizade...

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Ao CNPq, pela concessão da bolsa de estudos.

Paciência e Prodígio

O Homem perguntou ao Trabalho:
- Qual o elemento mais resistente que encontraste, observando a Natureza?
- A pedra, respondeu o Trabalho.
A água que corria brandamente em derredor, escutou o que se dizia e, em silêncio, descobriu um meio de pingar sobre a pedra e, com algum tempo, abriulhe grande brecha, através da qual a água passava de um lado para outro.
O Homem anotou o acontecido e indagou da água sobre o instrumento que ela usara para realizar aquele prodígio.
A água humilde respondeu simplesmente:
- Foi a ***Paciência****.*

Emmanuel
(Psicografia de Chico Xavier)

LOBATO, Luciana Pereira. **Interação dos componentes da aveia e da soja sobre a colesterolemia, imunoestimulação e viabilidade tecnológica de produção de alimentos para dietas especiais.** 2010 208 f. Tese (Doutorado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2010.

RESUMO

Doenças cardiovasculares estão entre as maiores causas de mortalidade e morbidade, tanto em países desenvolvidos quanto naqueles em desenvolvimento. Entre as recomendações para diminuição dos riscos está a redução do colesterol e subfrações séricas. Há inúmeras estratégias farmacológicas e não farmacológicas para normalização do perfil lipídico, entre elas está o consumo de compostos bioativos como os componentes da soja e da aveia, separadamente. A combinação da proteína vegetal com fibra solúvel tem sido estudada e demonstra potencialização do efeito sobre a diminuição do colesterol. A partir disso, o objetivo deste estudo foi de avaliar o efeito combinado de componentes do farelo de aveia e da farinha de soja sobre a colesterolemia de indivíduos dislipidêmicos e de ratos, sobre o sistema imunológico de ratos, assim como a viabilidade tecnológica de produção de um ingrediente expandido extrusado e de produtos (barras de cereais) com conteúdo de compostos hipocolesterolêmicos que atenda às recomendações de ingestão diária. Foi desenvolvido um ingrediente extrusado com 37,5% de farelo de aveia, 37,5% de farinha de soja e 25% de amido de milho, com adição de 4,5% de inulina, como coadjuvante do processo. A mistura, com 25% de umidade, foi processada a 160ºC. O produto obteve boa aceitação sensorial (7,1 em escala de 9 pontos) e é indicado para ser usado em granolas e barras de cereais. Barras de soja, com possível aplicação em dietas hipocolesterolêmicas, contendo 34,25 g de proteína/100g e 100,39 mg isoflavonas/100g foram desenvolvidas. As barras foram utilizadas em ensaio clínico, com 79 pacientes, durante 45 dias. Esses pacientes foram distribuídos em 4 grupos experimentais: 1) aveia, 2) soja, 3) aveia+soja e 4) grupo controle. Apenas o grupo controle seguiu dieta restrita em gordura saturada e colesterol. Em todos os grupos houve diminuições de subfrações de colesterol, no entanto, não foi verificado efeito sinérgico ou aditivo na regularização do perfil lipídico quando aveia e soja foram ingeridas juntas. No experimento com ratos Wistar, foi verificado que a ingestão de colesterol ou uma moderada hipercolesterolemia pode reduzir a resposta imune. Foi demonstrado que o farelo de aveia, mas não a farinha de soja, parece reverter parcialmente essa diminuição da resposta imune quando ingerido por 45 dias.

Palavras-chave: β-glucana. Isoflavona. Proteína vegetal. Colesterol. Sistema imunológico. Extrusão. Barras de cereais.

LOBATO, Luciana Pereira. **Interaction of components of oat and soy on cholesterol, immunostimulation and technological feasibility of producing of food for special diets.** 2010 208 f. Tese (Doctor Degree in Food Sciene) – Universidade Estadual de Londrina, Londrina, 2010.

ABSTRACT

Cardiovascular diseases are among the major causes of morbidity and mortality both in developed and in developing countries. Among the recommendations for diminish of risk is the reduction of serum cholesterol and subfractions. There are numerous pharmacological and nonpharmacological strategies for normal lipid profile, among them are consumption of bioactive components such as soy and oatmeal, whatever. The combination of vegetable protein with soluble fiber has been studied and demonstrated potentiation of the effect on lowering cholesterol. From this, the objective of this study was to evaluate the combined effect of components of oat bran and soy flour on cholesterol in dyslipidemic subjects and mice on the immune system of mice, as well as the technological feasibility of producing an ingredient expanded and extruded products (cereal bars) content of hypocholesterolemic compound that meets the recommendations for daily intake. We developed an ingredient with 37.5% of extruded oat bran, 37.5% soybean meal and 25% corn starch, with the addition of 4.5% inulin as an adjuvant procedure. The mixture with 25% moisture was processed at 160°C. The product has good acceptability (7.1 on 9-point scale) and is intended to be used in granola and cereal bars. Soy bars, with possible cholesterol-lowering diets containing 34.25 g and 100.39 mg protein/100g isoflavones/100g were developed. The bars were used in a clinical trial with 79 patients during 45 days. These patients were divided into four groups: 1) oats, 2) soybean, 3) soybean and oat + 4) control group. Only the control group followed a diet restricted in saturated fat and cholesterol. In all groups there were decreases of subfractions of cholesterol, however, there were no synergistic or additive effects in regulating lipid profile when oats and soybeans were eaten together. In experiments with rats it was found that cholesterol intake or a moderate hypercholesterolemia may reduce the immune response. It was shown that oat bran, but not soy flour, seems to partially reverse this decline in immune response when ingested for 45 days.

Keywords: β-glucan. Isoflavones. Vegetable protein. Cholesterol. Immune system. Extrusion. Cereal bars.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1 INTRODUÇÃO

O interesse no uso da alimentação como determinante da saúde cada vez mais se fortalece e há consenso da estreita relação entre alimentação-saúde-doença. A partir disso, novos conceitos sobre as necessidades de nutrientes fisiológicos especiais, efeitos benéficos de compostos não nutrientes, aumento da expectativa de vida, são fatores que vêm estimulando a produção de novos alimentos. Há muitos aspectos positivos demonstrados por pesquisas científicas motivando o uso correto da alimentação e a produção de alimentos específicos para manutenção da saúde.

Os alimentos funcionais vêm atender a demanda deste mercado. Estes são alimentos que possuem substâncias (compostos bioativos) capazes de auxiliar na redução de riscos de doenças. Entre as categorias de ingredientes utilizados para o desenvolvimento de alimentos funcionais estão os ácidos graxos ômega-3, os fitosteróis, as fibras solúveis e as proteínas de soja.

Nos últimos anos, têm sido enfatizada a necessidade da perda de peso e redução da ingestão de gorduras saturadas e colesterol. Um aconselhamento adicional tem sido a prática de exercícios, que tem papel fundamental na redução de lipídeos séricos (STEFANICK et al., 1998) e, consequentemente, na saúde. A regularização do perfil lipídico a níveis normais se faz de extrema importância devido a sua estreita relação com o desenvolvimento de inúmeras doenças, principalmente as doenças cardiovasculares.

Há inúmeras estratégias farmacológicas, não farmacológicas e suas combinações para diminuição do colesterol sérico dos indivíduos. A terapia farmacológica é extensamente utilizada e o padrão é o uso de 10 a 20 mg de inibidor da hidroximetil glutaril coenzima A (HMG-CoA) redutase hepática, representado pelo grupo farmacológico das estatinas, que podem reduzir as lipoproteínas de baixa densidade, conhecido como LDL, em 21 a 44% ou mais (JENKINS et al., 1999). Há outras drogas terapêuticas disponíveis para esse fim e muitas vezes são necessárias combinações de 2 ou mais desses fármacos hipolipemiantes para atingir o objetivo de regularização do perfil lipídico. No entanto, há possibilidade de aparecimento de efeitos colaterais indesejáveis,

como miopatias, frequentemente relatadas por usuários de estatinas, além dos valores gastos com a terapia farmacológica crônica.

Outra possibilidade de terapia é a utilização de compostos bioativos que possuam ação hipolipemiante, em substituição total ou em associação com medicamentos. A partir disso, são desenvolvidas inúmeras pesquisas com alimentos, ou seus componentes, que possam ser incluídos na dieta normal que venham colaborar com eficácia na diminuição do colesterol, para redução ou eliminação do uso de medicamentos. Fibras solúveis e também proteínas da soja têm sido demonstradas ter esse efeito, independentemente (ANDERSON, et al., 1992; JENKINS et al., 1993; KRITCHEVSKY, 1995; ANDERSON; JOHNSTOONE; COOK-NEWELL, 1995). Além disso, foi verificado que a combinação de compostos bioativos pode promover uma ação sinérgica. Jenkins et al. (1999) demonstraram os benefícios da combinação de proteína vegetal da soja e fibra solúvel na diminuição de lipídeos séricos (colesterol), em um estudo metabólico com 31 homens e mulheres com hiperlipidemia que seguiam dieta restrita em gorduras saturadas e colesterol. Além disso, Van Horn et al. (2001) relatou um efeito sinérgico do uso de aveia (flocos, farelo) e soja (proteína isolada) na diminuição do colesterol de mulheres hipercolesterolêmicas.

Contudo, nas dietas destes estudos foram incluídos os ingredientes, em sua maioria, sem a interferência de uma matriz alimentar e seu processamento. A inclusão destes ingredientes em alimentos viria a facilitar o acesso aos benefícios destes componentes. Além disso, há estudos que demonstram os efeitos benéficos de β-glucanas, proteínas de soja ou isoflavonas sobre as respostas imunológicas em animais experimentais e humanos e na diminuição do risco de desenvolvimento de alguns cânceres, individualmente. Todavia, não há estudos do efeito combinado destas substâncias sobre o perfil lipídico quando os indivíduos não seguiram dietas restritas em consumo de gorduras saturadas e colesterol. Além disso, a avaliação dessa combinação sobre o sistema imunológico também não foi estudada.

2 OBJETIVOS

2.1 Objetivo Geral

- Investigar o efeito combinado da aveia ou do farelo de aveia (contendo β-glucanas) e da soja ou da farinha de soja (contendo proteínas e isoflavonas) sobre colesterolemia, sistema imunológico e a viabilidade tecnológica de produção de ingrediente e alimento para dietas especiais.

2.2 Objetivos Específicos

- Caracterizar o farelo de aveia e a farinha de soja quanto à composição química;

- Desenvolver um ingrediente expandido, por extrusão, contendo farinha de soja e farelo de aveia aplicando o planejamento experimental de misturas;

- Avaliar as características físicas e físico-químicas dos ingredientes extrusados;

- Produzir barras (similares às de cereais) com altos teores de proteínas e β-glucanas, destinadas a dietas especiais e para utilização em estudo clínico;

- Caracterizar as barras quanto à composição química, características físicas e análise sensorial;

- Avaliar o efeito individual e combinado de componentes do farelo de aveia e da farinha de soja na colesterolemia de indivíduos dislipidêmicos.

- Avaliar o efeito combinado de componentes da aveia e/ou da soja sobre a colesterolemia e imunoestimulação em ratos Wistar.

3 ESTRUTURAÇÃO DA TESE

Os resultados obtidos neste estudo estão apresentados na forma de artigos científicos já estruturados nas normas das revistas para as quais foram enviados ou aquelas selecionadas para posterior publicação. Segundo normas ditadas pelo Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, na tese de doutorado, estruturada de tal forma, devem ser ainda apresentadas uma revisão bibliográfica geral dos assuntos relatados nos artigos científicos e uma conclusão geral.

3.1 Artigos Científicos

Serão 4 artigos:

1. Artigo 1- relata os resultados obtidos no desenvolvimento de ingrediente expandido produzido por extrusão
2. Artigo 2 - relata os resultados obtidos no desenvolvimento de barras de soja e estudo clínico realizado com esse produto
3. Artigo 3 - relata resultados obtidos em estudo clínico realizado com pacientes que consumiram soja, aveia, soja+aveia ou dieta hipocolesterolêmica durante 45 dias, para avaliação do perfil lipídico
4. Artigo 4 - relata resultados obtidos em estudo biológico com ratos Wistar que consumiram soja, aveia, soja+aveia adicionadas de colesterol durante 45 dias, para avaliação do perfil lipídico e imunoestimulação.

4 REVISÃO BIBLIOGRÁFICA

4.1 Alimentos Funcionais

Há décadas, desde que surgiu o conceito no Japão, por volta de 1980, há discussões internacionais em torno da definição dos alimentos funcionais e da diferenciação destes com nutracêuticos, alimentos para fins especiais e medicamentos. A Portaria n^{O}398 de 30/04/99, da Secretaria de Vigilância Sanitária do Ministério da Saúde, no Brasil, fornece a definição legal de alimento funcional, como sendo: "todo aquele alimento ou ingrediente que, além das funções nutricionais básicas, quando consumido como parte da dieta usual, produz efeitos metabólicos e/ou fisiológicos e/ou benéficos à saúde, devendo ser seguro para consumo sem supervisão médica".

A Agência Nacional de Vigilância Sanitária (ANVISA), também em 1999, publicou duas resoluções: (1) Resolução ANVISA/MS n^{0}18, de 30/04/1999 (republicada em 03/12/1999): aprova o regulamento técnico que estabelece as diretrizes básicas para análise e comprovação de propriedades funcionais e/ou de saúde alegadas em rotulagem de alimentos; (2) Resolução ANVISA/MS n^{0}19, de 30/04/1999 (republicada em 10/12/1999): aprova o regulamento técnico de procedimentos para registro de alimento com alegação de propriedades funcionais e ou de saúde em sua rotulagem. Nessas resoluções, faz-se distinção entre alegação de propriedade funcional e alegação de propriedade de saúde, como segue: (1) Alegação de propriedade funcional: é aquela relativa ao papel metabólico ou fisiológico que uma substância (seja nutriente ou não) tem no crescimento, desenvolvimento, manutenção e outras funções normais do organismo humano; (2) Alegação de propriedade de saúde: é aquela que afirma, sugere ou implica a existência de relação entre o alimento ou ingrediente com doença ou condição relacionada á saúde. Não são permitidas alegações de saúde que façam referência à cura ou prevenção de doenças.

Desta forma, os alimentos que surgirem no mercado deverão trazer em seu rótulo qual é o benefício para a fisiologia do organismo ou porque reduz o risco de certa doença, informação que deverá ser comprovada através de pesquisas

científicas. Neste contexto, é importante ratificar que aos alimentos funcionais se atribui somente a possível redução do risco de doenças e, assim, promoção da saúde, e não prevenção de doenças.

O consumo desses alimentos e suplementos alimentares tem expandindo rapidamente. Entre os compostos bioativos com alegação de saúde já aprovados pelos órgãos de fiscalização e controle, como o *Food and Drugs Administration* - FDA e a Agência Nacional de Vigilância Sanitária – ANVISA, para redução do risco de doenças cardiovasculares (DCV), destacam-se os ácidos graxos ômega 3, fitosteróis, fibras solúveis e proteína de soja. Segundo a *World Health Organization* (WHO, 2003) a estimativa era de que até 2010 as DCV seriam a principal causa de mortalidade em países desenvolvidos. Entre as recomendações para redução do risco de desenvolver as DCVs está a diminuição do colesterol total e da Lipoproteína de Baixa Densidade.

4.2 Dislipidemias

4.2.1 Lipoproteínas

Sposito et al. (2007) formularam a IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose onde consta esclarecimentos sobre composição e funções das lipoproteínas.

As lipoproteínas permitem solubilização e transporte dos lípides, que são substâncias geralmente hidrofóbicas, no meio aquoso plasmático. São compostas por lípides e proteínas denominadas apolipoproteínas, que possuem diversas funções no organismo como formação intracelular das partículas lipoprotéicas, ligantes a receptores de membrana ou co-fatores enzimáticos. Existem quatro grandes classes de lipoproteínas separadas em dois grupos: (i) as ricas em TG, maiores e menos densas, representadas pelos quilomicrons, de origem intestinal, e pelas lipoproteínas de densidade muito baixa ou “very low density lipoprotein” (VLDL), de origem hepática; e (ii) as ricas em colesterol de densidade baixa ou “low density lipoprotein” (LDL) e de densidade alta ou “high density lipoprotein”

(HDL). Existe, ainda, uma classe de lipoproteínas de densidade intermediária ou “intermediary density lipoprotein” (IDL).

Os quilimicrons são responsáveis pelo transporte dos lípides absorvidos pelo intestino originários da dieta e da circulação entero-hepática. No fígado, o conteúdo de colesterol é regulado por três mecanismos principais: a) síntese intracelular do colesterol; b) armazenamento após esterificação; c) excreção pela bile. Na luz intestinal, o colesterol é excretado na forma de metabólitos ou como ácidos biliares. Metade do colesterol biliar e aproximadamente 95% dos ácidos biliares são reabsorvidos e retornam ao fígado pelo sistema porta (ciclo êntero-hepático). O transporte de lípides de origem hepática ocorre por meio das VLDL, IDL e LDL. Os triglicérides das VLDL, assim como os dos quilomícrons, são hidrolisados pela lipase lipoprotéica, seus ácidos graxos liberados para os tecidos e metabolizados. Uma parte das VLDL dá origem às IDL, que são removidas rapidamente do plasma. O processo de catabolismo continua, envolvendo a ação da lipase hepática e resultando nas LDL, que permanecem por longo tempo no plasma. Esta lipoproteína tem um conteúdo apenas residual de triglicérides e é composta principalmente por colesterol e uma única apolipoproteína. As LDL são removidas pelo fígado através dos receptores B/E. A expressão desses receptores é a principal responsável pelo nível de colesterol no sangue e depende da atividade da enzima hidroxi-metil-glutaril (HMG) CoA redutase que é a enzima-chave intracelular para síntese do colesterol hepático.

O processo de esterificação do colesterol, que ocorre principalmente nas HDL, é fundamental para sua estabilização e transporte do plasma, no centro desta partícula. A HDL transporta o colesterol até o fígado onde este é captado por receptores. O circuito de transporte do colesterol dos tecidos periféricos para o fígado é denominado transporte reverso do colesterol. Neste transporte, é importante a ação do complexo “ATP Binding Cassete” A1 (ABC-A1) que facilita a extração do colesterol da célula pelas HDL. A HDL também tem outras ações que contribuem para a proteção do leito vascular contra a aterogênese, tais como a remoção de lípides oxidados da LDL, inibição da fixação de moléculas de adesão e monócitos ao endotélio e estimulação da liberação de óxido nítrico. O acúmulo de quilomícrons e/ou de VLDL no compartimento plasmático resulta em hipertrigliceridemia e decorre da diminuição da hidrólise dos triglicérides destas

lipoproteínas pela lipase lipoprotéica ou do aumento da síntese de VLDL. O acúmulo de lipoproteínas ricas em colesterol como a LDL no compartimento plasmático resulta em hipercolesterolemia (SPOSITO et al., 2007).

Na Figura 1 está representada a composição das lipoproteínas em quantidades de triglicérides, fosfolipídeos, ésteres de colesterol, colesterol e proteínas, assim como seus tamanhos e densidades. Pode-se observar que quanto maior a proporção de triglicérides ligada à lipoproteína, menos densa ela se apresenta e maior será o caráter aterosclerótico desta molécula.

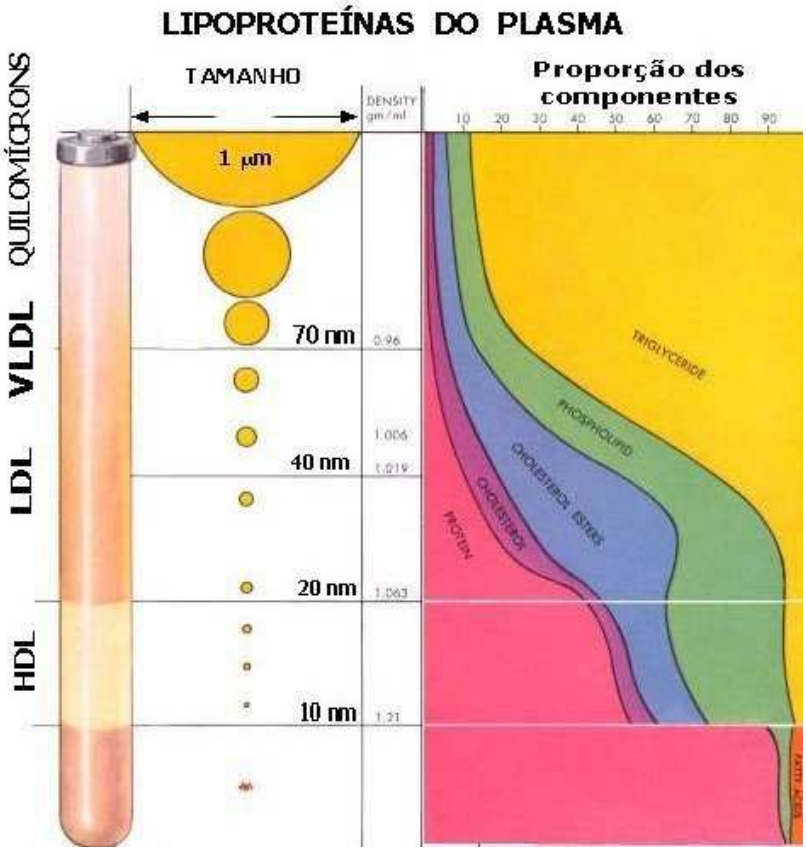

Figura 1 – Representação da composição das lipoproteínas do plasma. HDL – Lipoproteína de alta densidade, LDL – Lipoproteína de baixa densidade, VLDL – Lipoproteína de muito baixa densidade.
Fonte: http://anatpat.unicamp.br/talipoproteina.html.

4.2.2 Doenças Cardiovasculares e Dislipidemias

Doenças cardiovasculares (DCV) continuam sendo uma das maiores causas da mortalidade nos países desenvolvidos, tanto na população em geral como naqueles portadores de doenças crônicas (TANDRA; VUPPALANCHI, 2009). No entanto, segundo a IV Diretriz Brasileira Sobre Dislipidemias e Prevenção da Aterosclerose (2007) durante os últimos trinta anos presenciamos declínio razoável da mortalidade por causas cardiovasculares nesses países, enquanto elevações relativamente rápidas e substanciais têm ocorrido em países em desenvolvimento, dentre os quais o Brasil. De acordo com as projeções da *WHO* esta tendência de elevação na ocorrência de doenças cardiovasculares tende a persistir, agravando ainda mais o quadro de morbidade e mortalidade elevadas nestes países.

Níveis aumentados de colesterol plasmático, particularmente LDL, são associados com elevado risco de doenças ateroscleróticas, como doenças coronárias, infarto do miocárdio e doenças vasculares periféricas (CASTELLI et al., 1992). Correlação entre diminuição do colesterol e mortalidade e a positiva influência da diminuição do colesterol sobre a redução da progressão de DCV estão bem estabelecidas. A *WHO* relatou que os altos níveis de colesterol contribuem em 56% dos casos de doenças coronárias em todo o mundo e causam aproximadamente 4,4 milhões de mortes por ano (KAVEY et al., 2003). Segundo Anderson e Konz (2001) mudanças no perfil lipídico estão associadas a mudanças no risco de doenças cardiovasculares nas seguintes proporções: +1% colesterol total, +2–3% risco; +1% LDL-C, +1.2–2.0% risco; −1% HDL-C, +3% risco; sendo que para triglicerídeos o risco não é bem estimado.

A aterosclerose é uma das maiores causas de morbidade e mortalidade nos países desenvolvidos. É uma doença inflamatória crônica de origem multifatorial que ocorre em resposta à agressão endotelial, acometendo principalmente a camada íntima de artérias de médio e grande calibre. A formação da placa aterosclerótica inicia-se com a agressão ao endotélio vascular em resposta a diversos fatores como envelhecimento, toxinas, infecções virais, reações imunológicas, hipertensão arterial, tabagismo, elevação de lipoproteínas aterogênicas (LDL, IDL, VLDL, remanescentes de quilomícrons), além de

produtos da lipoperoxidação presentes na dieta e nas partículas de LDL oxidadas (FRANKEL, 2005; MATSUURA et al., 2006; FÖRSTERMANN, 2008).

A partir desses dados, verifica-se a extrema importância na regularização do perfil lipídico, pois, consequentemente, será diminuído o risco do desenvolvimento de DCVs e outras doenças. Mudanças de hábitos de vida e alimentares e a inserção de compostos bioativos na dieta têm sido demonstrados por pesquisas científicas e na prática clínica serem fatores importantes para esses objetivos serem atingidos.

4.2.3 Dieta, Compostos Bioativos e Dislipidemias

A composição da dieta humana tem papel importante nas concentrações de lipídeos e lipoproteínas sanguíneas. A redução da ingestão de gorduras saturadas e colesterol têm sido o primeiro passo na terapia alimentar na redução de desordens cardíacas (KERCKHOFFS et al., 2002). Na Tabela 1, encontram-se descritas as recomendações dietéticas para o tratamento de dislipidemias, segundo a IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose (2007)

Tabela 1 – Recomendações dietéticas para o tratamento das hipercolesterolemias.

Nutrientes	**Ingestão recomendada**
Gordura total	25 a 35% das calorias totais
Ácidos graxos saturados	≤ 7% das calorias totais
Ácidos graxos polinsaturados	≤ 10% das calorias totais
Ácidos graxos monoinsaturados	≤ 20% das calorias totais
Carboidratos	50 a 60% das calorias
Proteínas	Cerca de 15% das calorias totais
Colesterol	< 200 mg/dia
Fibras	20 a 30 g/d
Calorias	Ajustado ao peso desejável

Fonte: IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose (2007)

Os conteúdos alimentares de gorduras saturadas e de colesterol influenciam diferentemente os níveis lipídicos plasmáticos, em especial a colesterolemia. A maioria da população absorve aproximadamente metade do colesterol presente na luz intestinal, enquanto uma minoria é hiperresponsiva. A absorção de gordura saturada, no entanto, não é limitada e, por isso, sua ingestão promove efeito mais intenso sobre a colesterolemia (SPOSITO et al., 2007).

Na IV Diretriz sobre Dislipidemias e Prevenção de Aterosclerose há recomendações de utilização de alguns alimentos ou compostos bioativos como tratamento não medicamentoso das dislipidemias e medidas de prevenção da aterosclerose como, por exemplo, fitosteróis, ácidos graxos insaturados, proteína de soja, fibras e antioxidantes.

A utilização dessas terapias não farmacológicas são, na sua maioria, utilizadas de maneira informal, sendo pouco aplicada na prática clínica, ou seja, pouco recomendada por médicos. O estudo de interações entre alimentos funcionais ou compostos bioativos e estes com medicamentos se torna muito importante e urgente, pois o consumo já está acontecendo. Há inúmeros trabalhos demonstrando o efeito de alimentos consumidos independentemete para redução do colesterol, no entanto, interações ocorrem e podem tanto potencializar o efeito como diminuí-lo. Há estudos que mostram, por exemplo, que o consumo de fibras solúveis pode diminuir a ação de medicamentos hipocolesterolêmicos devido ao seu mecanismo de ação ser, provavelmente, por diminuição de absorção.

4.3 Compostos Bioativos e Imunoestimulação

O corpo humano protege a si mesmo contra a entrada de substâncias ou organismos reconhecidos como “estranhos” ou “não próprios”. Este processo de proteção e eliminação é chamado imunidade. A imunidade é mediada por mecanismo humoral (extracelular) ou celular (intracelular) e, em ambos os tipos de imunidade os linfócitos, um dos tipos de leucócitos importantes na defesa do

organismo, desempenham um papel importante. Quando as células do sistema imune encontram um antígeno ocorre uma resposta humoral, uma resposta celular, ou ambas (KEITH; JEEJEEBHOY, 1997).

Imunidade humoral é mediada por linfócitos B que, após estimulação por antígenos, proliferam e se diferenciam em células produtoras de anticorpos no plasma. Imunidade celular é mediada por células T, que são derivadas do timo e que, uma vez ativadas, podem secretar citocinas para recrutar outras células T, agindo como células citotóxicas para destruir células infectadas ou induzir células B a tornarem-se células do plasma e secretar anticorpos. Linfócitos reconhecem somente antígenos que podem se ligar aos receptores de células T ou imunoglobulinas na superfície, portanto este processo é muito preciso e específico para cada antígeno (Figura 2).

Figura 2 – Representação da especificidade de um anticorpo ligando-se ao antígeno. Adaptado de: http://pt.wikipedia.org/wiki/Imunoglobulina

Além disso, linfócitos T carreiam os antígenos CD4 e CD8. Os que carreiam antígenos CD4 são chamados de “T helper” e os que carreiam CD8 são os “T suppressor” (KEITH; JEEJEEBHOY, 1997). Na Figura 3, verifica-se uma representação da cadeia de eventos na resposta imune humoral e celular frente a

antígenos diferentes, para resposta humoral (apenas com antígenos CD4) e resposta celular (com antígenos CD4 E CD8).

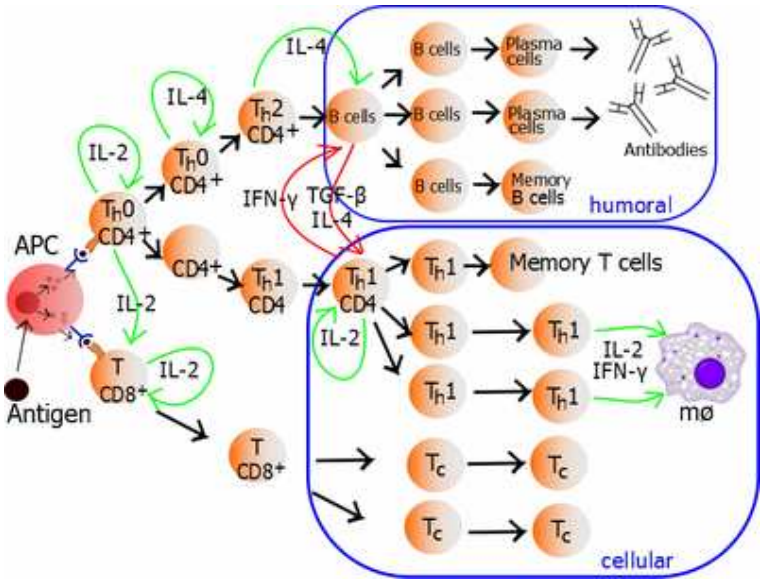

Figura 3 – Representação da cadeia de eventos na resposta imune humoral e celular frente a um antígeno. **Fonte:** http://en.wikipedia.org/wiki/T_helper_cell

Anticorpos (Ac) são moléculas capazes de neutralizar agentes infecciosos. Iniciam seus efeitos biológicos ligando-se de forma não covalente aos antígenos (Ag). Quase sempre surgem em resposta a um Ag. Ac também são conhecidos como Imunoglobulinas (Ig). Igs com atividades de Ac combinam especificamente com a substância que induziu sua formação. São produzidas pelos linfócitos B. Há cinco classes de Igs que têm funções específicas (SEADI, 1998):

- IgG – Presente em maior quantidade no soro humano (70 a 75% do total das Igs). Difunde-se muito mais do que qualquer outra Ig no espaço extravascular. É capaz de ultrapassar a placenta, neutraliza toxinas (produzidas por bactérias), bactérias e vírus, e facilita a fagocitose;
- IgM – Representa 10% do total de Igs no soro normal. Juntamente com a IgD, apresenta-se na superfície de linfócitos B. É o primeiro Ac a se manifestar em uma infecção primária. Não atravessa a placenta e fica confinada ao espaço intravascular devido ao

tamanho da molécula, o que a torna eficiente na captura de microorganismos;

- IgA – É o principal Ac nas secreções, como saliva, lágrimas, fluídos nasais, colostro, intestino, etc. Protege as mucosas bloqueando a entrada de microorganismo do meio externo para os tecidos;
- IgE – Em pessoas normais existem apenas traços de IgE. É uma importante mediadora da hipersensibilidade do tipo 1, ou seja, alergia e anafilaxia. Liga-se a receptores de mastócitos e basófilos e, ao reagir com o Ag, propicia o lançamento de histamina;
- IgD – A concentração é pequena no soro, com menos de 1% do total das Igs. É a principal Ig da superfície dos linfócitos B, juntamente com a IgM, onde parece ter algum papel regulatório.

A resposta imune é dependente da replicação celular e da síntese de compostos protéicos ativos. Desta forma, é fortemente afetada pelo status nutricional, que determina a habilidade metabólica celular e a eficiência com que a célula reage aos estímulos, iniciando e perpetuando o sistema de proteção e autoreparação orgânicas. Calorias, aminoácidos, algumas vitaminas e minerais são nutrientes para os quais já se estabeleceu a estreita relação existente entre seu status orgânico e o funcionamento do sistema imune (BRUNETTO et al., 2007). Muito tem se pesquisado a influência da alimentação, compostos nutrientes e não nutrientes na imunidade tanto em modelos animais quanto em humanos, devido a essa possibilidade de estreita relação.

O interesse nos efeitos fisiológicos e farmacológicos das fibras alimentares tem sido crescente (MACKEOWN-EYSSEN; BRIGHT-SEE, 1984; EDWARDS, 1995; SCHENEEMAN; TINKER, 1995) e, devido aos diversos efeitos sobre as várias vias metabólicas, é possível que também influenciem nas funções imunes, como uma conseqüência de mudanças na estrutura da mucosa e microflora do intestino (LIM et al., 1997). A fermentação de fibras por bactérias intestinais leva a um meio mais ácido no intestino, inibindo a conversão de ácidos biliares primários aos seus metabólitos secundários (JACOB, 1988). Ácidos biliares aumentam a produção de IgE por linfócitos de linfonodos mesentéricos (MLN) e impedem a

produção de IgA, IgG e IgM a concentrações séricas relativamente altas, 400-500 µmol/L, que podem ser encontradas nos estados de enfermidade (LIM et al., 1994). Similar regulação da produção de imunoglobilinas é induzida por ácidos graxos insaturados (YAMADA et al., 1996).

Embora a ocorrência de mudanças na microflora intestinal possa influenciar nas funções imunes, as informações disponíveis sobre a atividade imunorregulatória das fibras ainda é insuficiente. Uma vez que a regulação da produção de Ig é também realizada por citocinas, o efeito das fibras alimentares na produção de Ig e citocinas nos linfócitos mesentéricos (MLN) merece mais estudos (LIM et al, 1997). Tem sido demonstrado que vários componentes alimentares que não fibras alimentares podem modificar a produção de Ig (HERALD et al., 1994; LIM et al., 1994, 1995, 1996; PENE et al., 1988, YAMADA et al., 1993, 1996). A modificação da produção de Ig pode afetar a incidência de várias doenças, a partir da indução da hipersensibilidade (Vollenweider et al., 1991) e imunossupressão (NEWBLE et al.1975).

Recentes evidências sugerem que isoflavonas da soja modulam a função imune positiva ou negativamente. A isoflavona genisteína tem sido muito estudada em relação aos seus efeitos sobre a imunidade. Genisteína suprime a resposta imune específica para antígenos *in vivo* e a proliferação de linfócitos *in vitro*. No entanto, genisteína melhora a resposta citotóxica mediada por "*natural killer*" (NK) e células T citotóxicas e a produção de citocinas pelas células T. Assim, o efeito da genisteína na imunidade é dependente do tipo de célula imunológica. Devido ao seu efeito exclusivo sobre a função imune, genisteína tem sido usada para tratamento de doenças em modelos animais e tem sido verificado que inibe respostas inflamatórias alérgicas. No entanto, estudos epidemiológicos da associação do consumo da soja ou isoflavonas com desordens alérgicas são limitados (SAKAI & KOGISO, 2008). Miyake et al. (2005) conduziram um estudo sobre a relação entre a ingestão de produtos de soja e isoflavonas com a prevalência de rinite alérgica. Esses autores observaram que há possibilidade de que a alta ingestão de soja e isoflavonas seja associada com redução da prevalência de renite alérgica. No entanto, mais investigações são necessárias para estabelecer essa relação.

4.4 Aveia: Aspectos Funcionais

A Aveia (*Avena sativa* L.) é um cereal de excelente valor nutricional. Destaca-se dentre os outros cereais por seu teor e qualidade protéica, variando de 12,4 a 24,5% no grão descascado; e por sua maior porcentagem de lipídios, que variam de 3,1 a 10,9%, distribuídos por todo o grão e com predominância de ácidos graxos insaturados (WEBSTER, 1986; LÀSZTITY, 1998; DE SÁ et al., 2000). O conteúdo de carboidratos (incluindo celulose e polissacarídeos não amiláceos) na aveia pode chegar a 75-80% do peso seco, sendo o amido o componente principal. Contêm ainda altas proporções de polissacarídeos não amiláceos, principais constituintes das fibras almentares, dentre estas destacando-se as beta-glucanas (LÀSZTITY, 1998; DE SÁ et al., 2000).

Para a aveia poder ser utilizada na alimentação humana é necessário o seu processamento que envolve processos mecânicos, térmicos e alteração de umidade e são eles: descascamento, estabilização e tostagem, corte, flocagem (flocos médios ou finos) ou moagem (farelo ou farinha) (De SÁ; DE FRANCISCO; SOARES, 1998).

O farelo de aveia consiste de camadas externas do grão, que contém a maior parte da fibra alimentar. A fibra solúvel da aveia consiste, principalmente, de polissacarídeo linear (1-3)(1-4)-β-D-Glucana que são componentes estruturais das paredes celulares dos grãos, com maior concentração na camada sub-aleurona, no endosperma amiláceo adjacente ao embrio e na camada de aleurona (WOOD, 1993, DE SÁ, et al., 1998).

O conteúdo total da fibra nos grãos varia de 60 a 90g/kg, dependendo da variedade da cultivar, local de crescimento, condições climáticas e fertilização (MÄLKKI; VIRTANEN, 2001). Aproximadamente, metade do total destes valores é fibra solúvel, sendo a outra metade insolúvel. A fibra insolúvel é muito similar à das fibras alimentares de outros grãos de cereais, em composição, e consistem principalmente, de celulose, hemiceluloses e lignina (MARLETT, 1993). Os menores componentes que, em menor proporção, acompanham a fibra alimentar de aveia são ácido fítico, componentes minerais, componentes fenólicos, vários

com propriedades antioxidantes, e lignanas, que são precursores de fitoestrógenos (MÄLKKI; VIRTANEN, 2001).

β-glucanas da aveia têm, no seu estado nativo, uma cadeia de, aproximadamente, 20.000 unidades glicosídicas, com peso molecular de 3 milhões (WOOD et al., 1991). Na parede celular do endosperma da aveia e da cevada, β-glucanas com ligações β-1,3 e β-1,4 (Figura 4) estão presentes como polímero complexado de polissacarídeo e proteína (BHATTY, 1993), enquanto as glucanas das paredes celulares de leveduras e fungos consistem de moléculas de glicose com ligações β-1,3 (β-1,3-D-glicose) com resíduos de glucopiranosil com ligações β-1,6 (MANNERS; MASSON; PATTERSON, 1973).

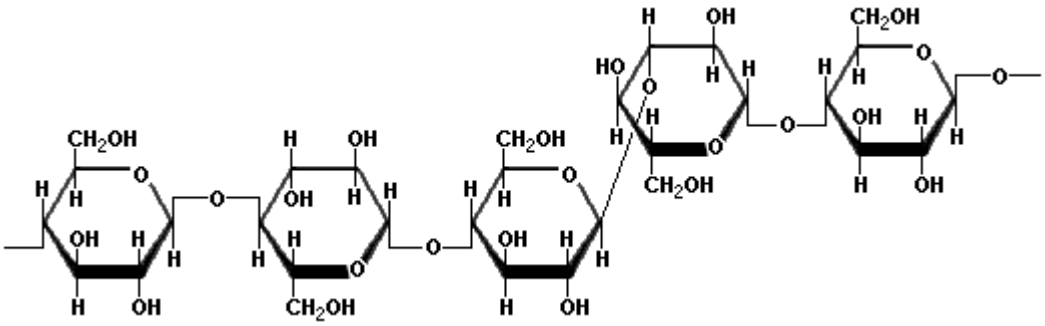

Figura 4 – Estrutura de beta-glucana com ligações β-1,3 e β-1,4.

Vários efeitos fisiológicos benéficos têm sido atribuídos à ingestão de fibras solúveis, como diminuição do índice glicêmico, diminuição de níveis de colesterol, maior produção de ácidos graxos de cadeia curta, absorção de nutrientes, diminuição do risco de desenvolvimento de alguns cânceres, entre outros.

β-Glucanas com tamanhos moleculares variáveis e estruturas secundárias, isoladas de várias fontes naturais, têm sido estudadas por sua habilidade de ativar mecanismos de defesa do hospedeiro contra infecções microbianas e parasitárias (YUN et al., 2003). A maioria dos estudos de β-glucanas como imunoestimulantes têm se concentrado em extrações de leveduras e fungos, havendo informação limitada na utilização de β-glucanas de cereais como profilático ou agente terapêutico (YUN et al., 2003). Czop & Austen (1985)

demonstraram que β-glucanas de cevada reconhecem os receptores específicos em macrófagos humanos. Embora não haja estudo para definir esta mesma atividade em β-glucanas de aveias se presume que reconheça os mesmos receptores de macrófagos humanos e de ratos, devido à estrutura química idêntica de β-glucanas de cevada e aveia (YUN et al., 2003).

De Groot et al. (1963) foram os primeiros a demonstrar que o consumo diário de 300 g de pão contendo 140g de aveia por 3 semanas, resultaria em uma diminuição de 11% no colesterol total sérico em humanos. Braaten et al. (1994) demonstraram que o feito hipocolesterolêmico dos produtos com aveia pode ser atribuído ao seu principal componente da fibra alimentar, as β-glucanas. Em 21 de janeiro de 1997, o U.S. Food and Drug Administration (FDA) aprovou a alegação de saúde “uma dieta rica em fibras solúveis de aveia (farelo e farinha de aveia) e pobre em gorduras saturadas e colesterol pode reduzir o risco de doenças cardíacas”. O FDA concluiu que, ao menos 3 g/d de β-glucanas de aveia deveriam ser consumidas para atingir uma redução clínica relevante nos níveis de colesterol sérico (FDA, 1996 e 1997).

Vários mecanismos de ação são propostos a ação das fibras alimentares na redução dos lipídeos séricos (THEUWISSEN; MENSINK, 2008, JONES, 2008):

- Fibras solúveis se ligam aos ácidos biliares durante a formação de micelas no lúmen intestinal. Consequentemente fibras solúveis podem aumentar a excreção de ácidos biliares;
- Fibras solúveis podem formar uma camada de gel não miscível no lúmen intestinal agindo como barreira física diminuendo a absorção e reabsorção de gorduras, incluindo colesterol e ácidos biliares. A partir disso, mais colesterol e ácidos biliares são eliminados através do íleo no intestino grosso. O resultado disso é a redução da absorção
- Ocorre diminuição da circulação enterohepática de colesterol e ácidos biliares. Como resultado, a conversão hepática de colesterol em ácidos biliares aumenta, então ocorre uma diminuição de colesterol livre e um aumento na síntese de colesterol endógeno;
- Aumento na atividade da 7-alfa-hidroxilase (enzima limitante na síntese de ácidos biliares do colesterol) e HMG-Coa redutase (enzima que controla o

metabolismo que sintetiza o colesterol) para compensar as perdas de ácidos biliares e colesterol armazenados no fígado;

- Aumento da expressão de transportadores de membrana ABCG5 e ABCG8. Transportadores hepáticos ABCG regulam secreção do colesterol no intestino;
- Receptores hepáticos de LDL aumentam sua atividade para restaurar o armazenamento de colesterol hepático, que leva à diminuição das concentrações de LDL sérico;
- Atenua a resposta glicêmica pós-prandial devido ao aumento da viscosidade intestinal, reduzindo níveis de insulina circulante e inibindo a síntese de colesterol, assim como de gorduras.
- Melhora a produção de ácidos graxos de cadeia curta (AGCC) por fermentação no intestino grosso. Os AGCC podem influenciar no metabolismo lipídico.

A partir disso, parece que fibras solúveis podem agir em ambos os níveis intestinais e hepáticos para redução da síntese e ou absorção do colesterol (JONES, 2008).

4.5 Soja: Aspectos Funcionais

A soja é uma leguminosa (*Glycine max* L.) proveniente do sudoeste asiático, composta de macronutrientes como lipídeos, carboidratos e proteínas. Os lipídeos da soja (18-20%), que são privados de colesterol, contêm aproximadamente 15% de gorduras saturadas, 61% de poliinsaturadas e 24% de monoinsaturadas. Carboidratos compõem, aproximadamente, 30% do grão, com 15% sendo de carboidratos solúveis (sacarose, rafinose e estaquiose) e 15% de carboidratos insolúveis (fibra alimentar). O conteúdo protéico da soja varia de 36 a 46% dependendo da variedade (CEDERROTH; NEF, 2009). Soja também contém micronutrientes, que incluem isoflavonas, fitatos, saponinas, fitosteróis, vitaminas e minerais. Estudos têm demonstrado que há uma grande variabilidade

no conteúdo e composição de isoflavonas, devido às diferentes variedades e condições ambientais (WANG; MURPHY, 1994, CALDWELL et al., 2005).

A soja é consumida como um alimento tradicional na Ásia há muito tempo e, em contraste, nos países do oeste, a soja ainda não foi incluída na dieta da maioria da população, mesmo representando uma boa fonte de proteínas, fibra alimentar e uma variedade de fitoquímicos. As proteínas de soja são únicas entre as proteínas vegetais, pois tem alto valor biológico e aminoácidos essenciais. São abundantemente ricas em lisina, que se encontra deficiente na maioria dos cereais (DHINGRA; JOOD, 2004). Proteínas de reserva são predominantes, tais como a globulina 7S (β-conglicinina) e globulina 11S (glicinina), que representam, aproximadamente, 80% do conteúdo total de proteína, assim como as proteínas menos abundantes, globulinas 2S, 9S e 15S (GARCIA et al., 1997). Segundo alguns estudos, β-conglicinina, mas não glicinina é capaz de melhorar o perfil lipídico sérico em animais e humanos, na ausência de fitoestrógenos (MORIYAMA et al., 2004; KOHNO et al., 2006).

A suplementação com soja em alimentos a base de cereais, na forma adequada, poderia aumentar o conteúdo protéico e melhorar a disponibilidade de lisina (RASTOGI; SINGH, 1989; RAO; RAO,1997; RIAZ, 1999; SHARMA et al., 1999). Mejia (2006), ainda, relata que a soja é uma ótima fonte de peptídeos bioativos, que são aminoácidos de cadeia curta produzidos pela digestão ou processamento da soja. Estes peptídeos possuem propriedades antioxidantes, antihipertensivas e anticarcinogênicas.

Há vários efeitos benéficos da inclusão da soja (proteínas e isofavonas) na dieta, como efeito hipocolesterolêmico, regulação da razão insulina/glucagon sanguínea, regulação de fatores de transcrição de genes envolvidos na síntese do colesterol, regulação do metabolismo lipídico hepático, redução da lipotoxicidade no fígado, efeito sobre a síntese de ácidos biliares e colesterol intestinal e efeito sobre as funções renais (TORRES; TORRE-VILLALVAZO; TOVAR, 2006) além de possuir efeitos preventivos para osteoporose e vários tipos de cânceres (SAKAI; KOGISO, 2008).

O consumo de proteína de soja por ratos têm sido associado com um aumento na atividade do receptor hepático apo-B/E; este receptor está envolvido na absorção de VLDL, lipoproteína de densidade intermediária e LDL (DAVIS et

al., 1993). Hipóteses do mecanismo de ação incluem isoflavonas (fitoestrógenos) e as proteínas (composição de aminoácidos). As isoflavonas da soja são referidas como fitoestrógenos devido ao fato de se ligarem ao receptor de estrógeno e afetarem os processos mediados por este hormônio (MOLTENI; BRIZIO-MOLTENI; PERSKY, 1995) e tem essa capacidade de se ligar a esses receptores devido à semelhança estrutural com o hormônio estradiol (estrogênio 17β-estradiol) (Figura 5). Dois tipos de receptores de estrógenos (RE) têm sido encontrados nos núcleos: REα e REβ (KUIPER et al., 1996). O primeiro é expresso em vários tecidos e órgãos e, similarmente, o segundo tem sido encontrado também nos ossos (ARTS et al., 1997), no sistema cardiovascular (MÄKELA et al., 1999) e no cérebro (SHUGHRUE; LANE; MERCHENTHALER, 1997).

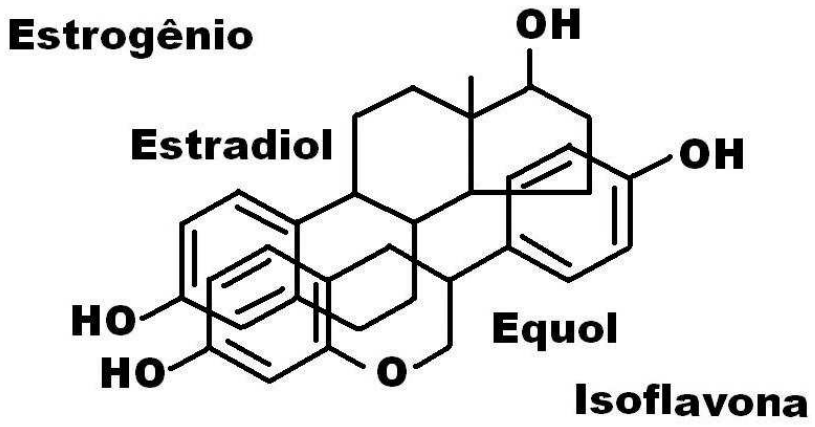

Figura 5 – Representação da semelhança entre as estruturas químicas de isoflavona (equol) e estrogênio (estradiol). Fonte: Setchell & Cassidy (1999)

As isoflavonas da soja contêm genisteína, daidzeína, gliciteína e seus respectivos conjugados glicosídicos (Figura 6), tendo diferentes afinidades de ligação com os REs baseado em suas estruturas e tipos de REs. Genisteína tem mais alta afinidade do que os outros derivados, e afinidade significativamente maior por REβ do que por REα (KUIPER et al., 1997).

As isoflavonas da soja podem exercer tanto efeitos estrógenos antagonistas quanto agonistas (MIKSICEK, 1995), e têm efeitos inibitórios sobre a tirosina-quinase, topoisomerase e angiogênese, que pode reduzir o risco de

câncer (ADLERCREUTZ; MAZUR, 1997). A proteína de soja contendo isoflavonas tem sido relatada ter vários efeitos benéficos sobre a saúde cardiovascular. Em um estudo, 80 mg de isoflavonas na dieta diária preveniram perda óssea da espinha lombar em mulheres em perimenopausa (ALEKEL et al., 2000), e tem sido observado que o consumo de isoflavonas extraídas da soja por ratas ovarioctomizados previne a diminuição da densidade óssea (LEE et al., 2004). Vários estudos também têm demonstrado que as isoflavonas da soja podem melhorar a função cognitiva (recepção, aprendizagem e memória, pensamento e expressão) em humanos e ratos (FILE et al., 2001; PAN et al., 2000), mas os mecanismos ainda não estão bem esclarecidos. Pan, Anthony & Clarkson (1999) sugerem que as isoflavonas da soja ajam mimetizando os efeitos estrógenos no cérebro. No entanto, as propriedades agonistas do estrógeno das isoflavonas da soja podem não ser explicadas pelos efeitos cerebrais (LEE; LEE; SOHN, 2005).

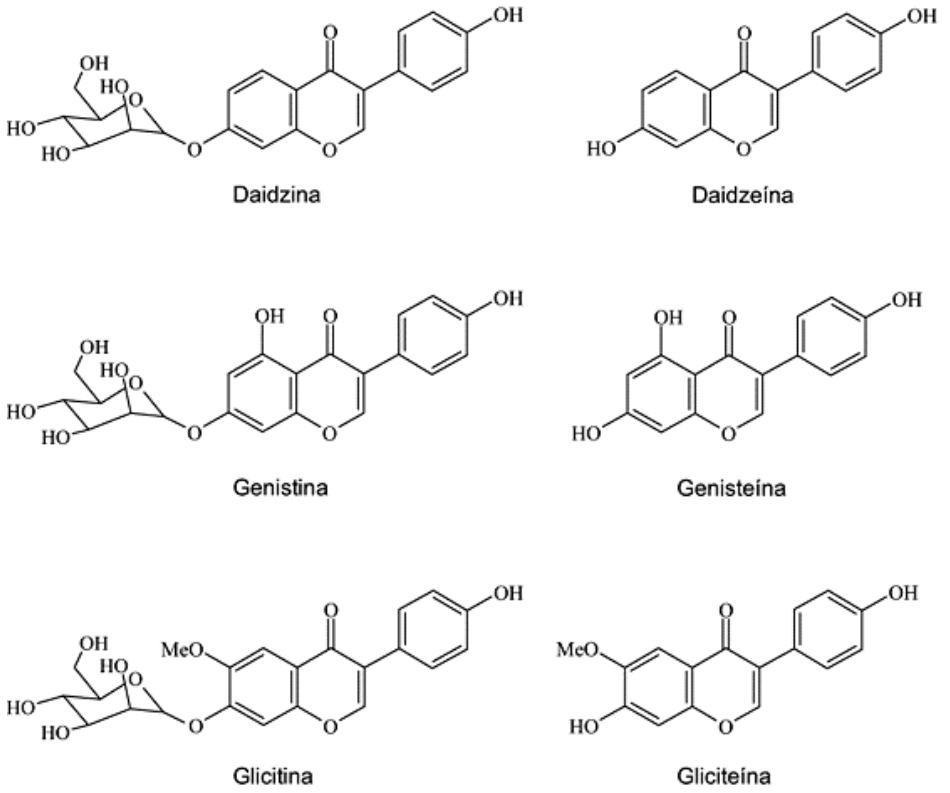

Figura 6 - Estruturas químicas dos glicosídeos e agliconas presentes na soja. Fonte: César et al. (2007).

Há vários componentes da soja que tem sido propostos como envolvidos na diminuição do colesterol como segue: proteína de soja (parece estar diretamente envolvida na inibição da absorção de colesterol micelar), conteúdo de aminoácidos, razão lisina/arginina (parece modificar a composição biliar), saponinas e isoflavonas (JONES, 2008) e globulina 7S. Há também vários mecanismos de ação propostos (POTTER, 1995; TORRES et al, 2006):

- Proteína de soja aumenta a excreção fecal de ácidos biliares;
- Aumento da conversão hepática do colesterol ácidos biliares;
- Aumento da síntese de ácidos biliares;
- Aumento da atividade da HMG-CoA redutase;
- Aumento da atividade dos receptores LDL;
- Aumento na remoção de LDL e VLDL por hepatócitos;
- Diminuição da circulação enterohepática de colesterol e ácidos biliares;
- Diminuição das concentrações de insulina sérica pos-prandial;
- Redução da razão insulina/glucagon pode ser responsável por parte do efeito hipocolesterolêmicos da proteína de soja, provavelmente devido a baixa razão lisina:arginina;
- Aumento nos níveis de tiroxina (hormônio da tireóide);
- Efeitos estrogênicos das isoflavonas;
- Influência no status oxidativo.

A partir disso, proteína de soja e outros compostos, como isoflavonas, podem agir em vários sistemas fisiológicos no corpo para reduzir colesterol e subfrações, mas devido a interações complexas entre proteína de soja e isoflavonas, os mecanismos sobre o metabolismo lipídico não são bem entendidos (TORRES et al., 2006).

De acordo com Barnes, Kirk & Coward (1994) a biodisponibilidade e o metabolismo de diferentes isoflavonas depende da forma química, sendo as agliconas a forma mais ativa para utilização para diminuição do risco de desenvolvimento de doenças. Este grupo de compostos tem sido relatado ter

ambas as atividades estrogênicas e antiestrogênicas (CASSIDY; BINGHAM; SETCHELL, 1995) dependendo dos níveis de estradiol sérico (CEDERROTH; NEF, 2009).

Por exercer efeito estrogênico, aumenta a possibilidade desta classe de fitoquímicos poder afetar os metabolismos lipídico e da glicose (CEDERROTH; NEF, 2009). Estudos em humanos e roedores demonstram que proteínas de soja ou fitoestrógenos derivados da soja podem ser benéficios para prevenção da obesidade e diabetes por agirem sobre o tecido adiposo (BHATENA; VELASQUEZ, 2002; VELASQUEZ; BHATENA, 2007).

4.6 Combinação de Compostos Bioativos: Presente na Aveia e na Soja

Tem sido amplamente estudado o efeito da interação entre compostos bioativos (JENKINS et al.,1999, BOURQUE et al., 2003; CASTRO et al., 2007) sobre o perfil lipídico e diminuição de riscos cardiológicos.

A literatura demonstra e confirma o papel da aveia na redução de colesterol (JENKINS et al., 1993; GLORE et al., 1994) e, cada vez mais, os benefícios antiaterogênicos têm sido relatados quando a soja é usada na substituição/reposição de outras fontes de proteína (ANDERSON; JOHNSTONE; COOK-NEWELL, 1995).

Jenkins et al. (1999) demonstraram os benefícios da combinação de proteína de soja e fibra solúvel na diminuição de lipídeos séricos (colesterol), em um estudo metabólico com 31 homens e mulheres com hiperlipidemia que seguiam dieta restrita em consumo de gorduras saturadas e colesterol. Segundo este estudo, após 2 e 4 semanas, houve diminuição da LDL (±6,7%), apolipoproteína B (± 8,2%) e razão de colesterol LDL:HDL (±6,3%). Ainda, foram verificadas diferenças de alguns efeitos entre os sexos, sendo reduções maiores entre os homens participantes do experimento. Também foi verificada a interferência do uso de alguns medicamentos nos efeitos. Houve aumento da produção de butirato, um ácido graxo de cadeia curta (± 24%).

Van Horn et al. (2001) estudaram o efeito sinérgico de aveia (flocos e farelo) e soja (proteína isolada) na redução no colesterol total e LDL em 127 mulheres em estágio de pós-menopausa com moderada hipercolesterolemia. Após 3 semanas de dieta, houve redução do colesterol total e LDL, triglicerídeos, assim como perda de peso, em relação à dieta com trigo e soja.

Torres et al. (2009) avaliaram a associação de 25 g de proteína de soja e 15 g de fibras solúveis de aveia sobre o perfil lipídico e polimorfismos de genes em indivíduos que seguiram dietas restritas em gordura saturada e obtiveram redução no colesterol total, LDL e TG independentemente do genótipo

Na Figura 7 encontram-se compilados alguns dos mecanismos propostos para a ação hipocolesterolêmica das fibras solúveis e da proteína de soja. É possível verificar que tanto as fibras solúveis quanto a proteína de soja agem em vários sistemas do organismo e que a diminuição do colesterol provavelmente ocorra por várias ações em cascata.

A aveia ou a fibra solúvel parece agir mais no lúmem intestinal na forma de impedimento físico na absorção e reabsorção de colesterol e outros nutrientes. A soja ou a proteína de soja e isoflavonas parecem agir mais em eventos hepáticos na estimulação de receptores de LDL e em reações enzimáticas. Porém, mesmo havendo muitos estudos pesquisando os efeitos hipocolesterolêmicos de ambos os compostos, o mecanismo ainda não foi estabelecido.

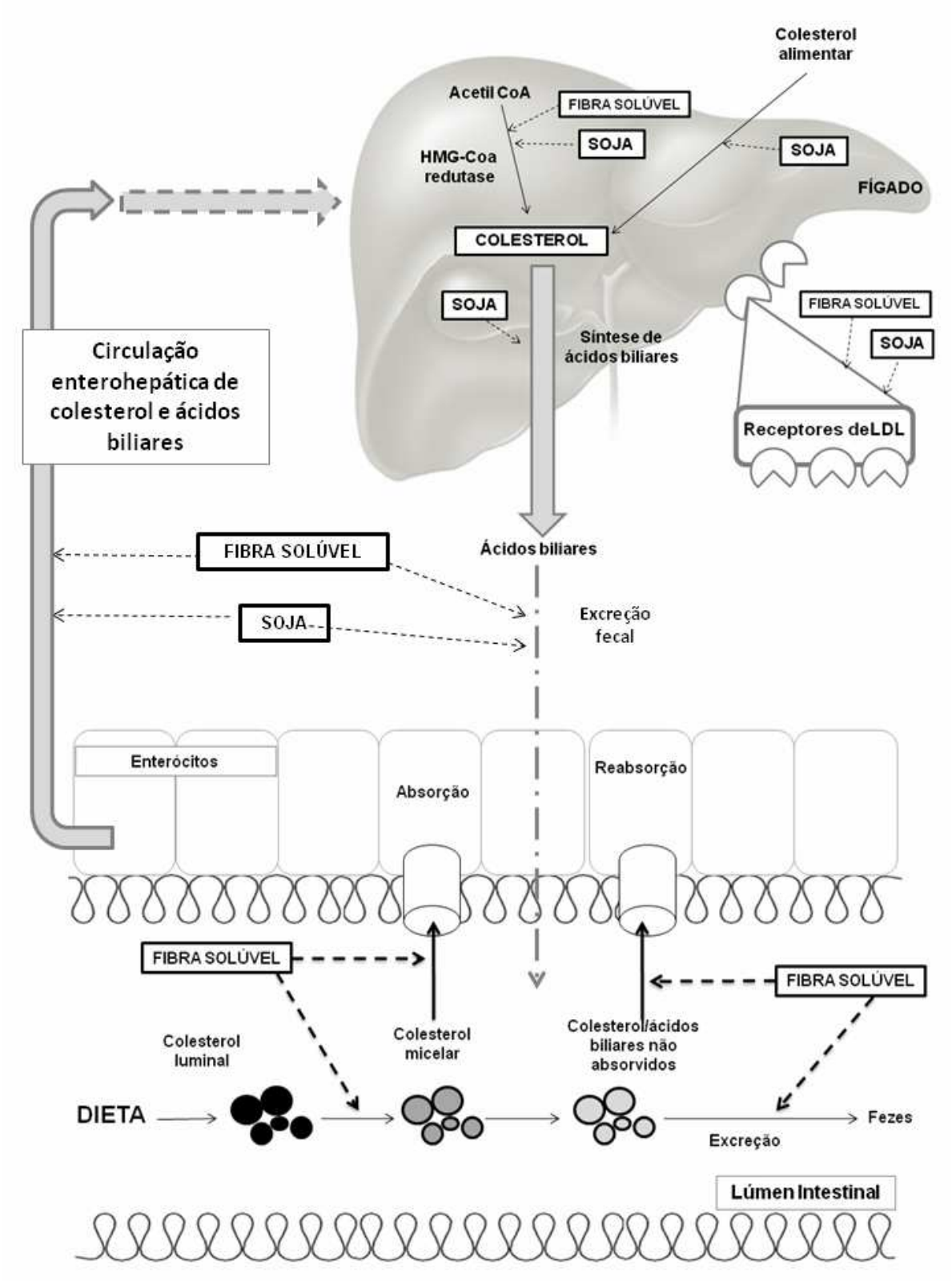

Figura 7 – Mecanismos de ação propostos para proteína de soja e fibras solúveis. Mecanismos da soja baseados em relatos de Potter (1995) e da aveia baseados em Theuwissen & Mensink (2008).

4.7 Desenvolvimento de Produtos Potencialmente Funcionais

A diminuição de riscos de desenvolvimento de doenças, entre elas as cardiovasculares, pode ser alcançada com mudanças de hábitos alimentares. O desenvolvimento de novos alimentos e ingredientes pode facilitar esse processo por tornar mais diversificadas as opções de produtos com essas potencialidades funcionais.

A utilização de ferramentas versáteis e de baixo custo como a extrusão é uma ótima alternativa para diversificação desses produtos, no entanto a adição de ingredientes não tradicionais deve ser bem estudada e formulada devido à grande influência dessa adição nas características finais do produto. Esses novos ingredientes extrusados com alto teor de fibras e proteínas, por exemplo, poderiam ser adicionados em granolas e barras de cereais, entre outros.

Além disso, outra forma de atender essa diversificação de produtos é a inserção de ingredientes funcionais em alimentos de conveniência, como as barras de cereais, muito bem aceitas pelos consumidores devido ao seu apelo de saúde. Barras com maiores teores de fibras e proteínas já estão disponíveis no mercado, no entanto, ainda há a preocupação com as características finais dos produtos pela modificação das características sensoriais pela inserção de ingredientes não convencionais.

4.7.1 Extrusão e Produtos Funcionais Extrusados

A extrusão é um processamento amplamente utilizado para produção e modificação ou melhoramento da qualidade de vários produtos (HARPER, 1981; COLONNA et al., 1989; FRAME, 1994). O processo de extrusão ganha destaque e expansão na indústria de alimentos por ser uma importante técnica que apresenta vantagens quando comparado a outros sistemas tradicionais de processamento de alimentos, tais como: versatilidade, baixo custo, alta produtividade, não geração de resíduos durante ou após o processamento, entre outras (SOUZA et al., 2007).

O controle do processo de extrusão é fundamental, pois não somente permite a obtenção de produtos com características tecnológicas variadas, mas também melhora a eficiência e economia da operação. Há inúmeras variáveis que podem influenciar nesse processo positiva ou negativamente. Entre as características dos materiais, influenciam no processo a composição química o conteúdo de umidade inicial. Entre as características do processo propriamente dito podem influenciar a temperatura das diferentes zonas de aquecimento, perfil e velocidade do parafuso, tempo de residência, forma da matriz e energia mecânica específica, entre outros. Na Figura 8 é mostrada, de forma esquemática, uma extrusora, suas principais funções e seus produtos.

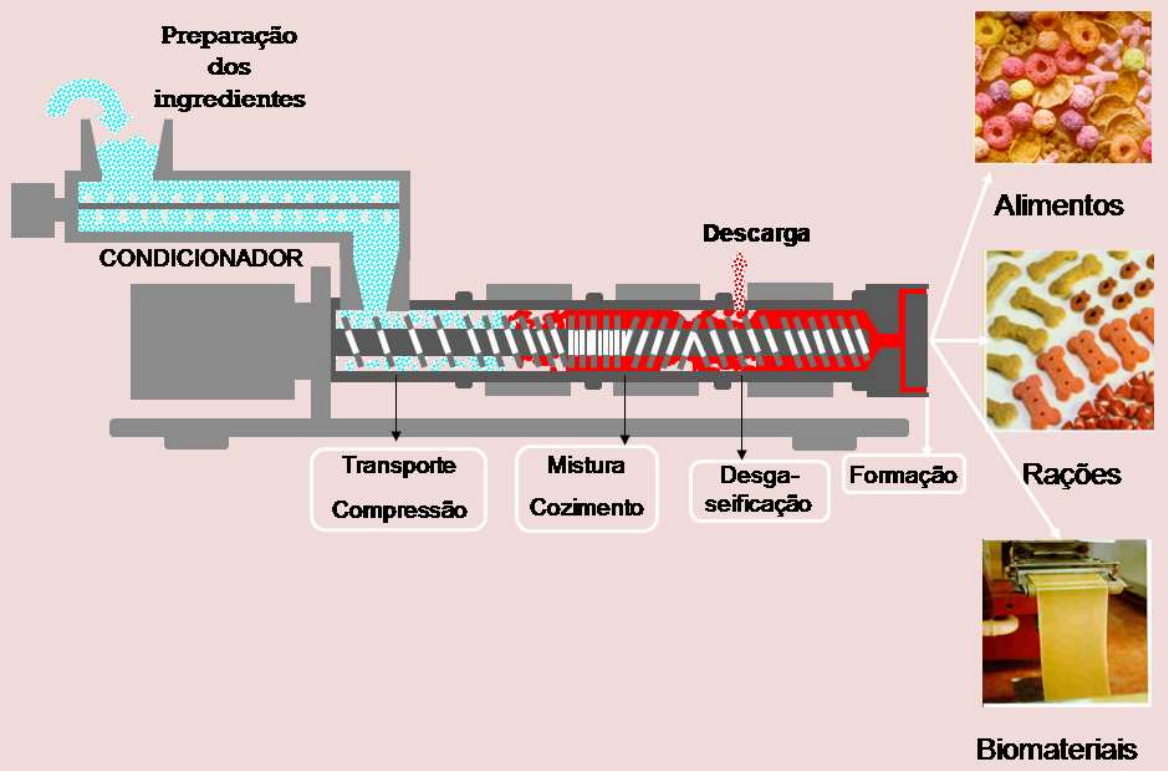

Figura 8 - Esquema da extrusora, principais funções e produtos. Adaptado de Embrapa (2009).

Inúmeras também são as transformações que ocorrem nos materiais extrusados. O amido, de acordo com as condições do processo, sofre transformações químicas que causam intumescimento e ruptura dos grânulos, modificações das estruturas cristalinas provocando solubilidade e viscosidade em

água fria (EL-DASH et al., 1984; CHEFTEL, 1986). A extrusão de materiais amiláceos convencionalmente resulta na gelatinização de amido, desnaturação de proteínas e formação de complexos entre amido, lipídios e proteínas (Ho & Izzo, 1992; Bhatnagar & Hanna, 1994a,b). Um esquema do que acontece com materiais amiláceos na saída da extrusora é mostrado na Figura 9, onde ocorre uma expansão do produto por diferença de pressão e temperatura com o ambiente e, logo após, uma contração.

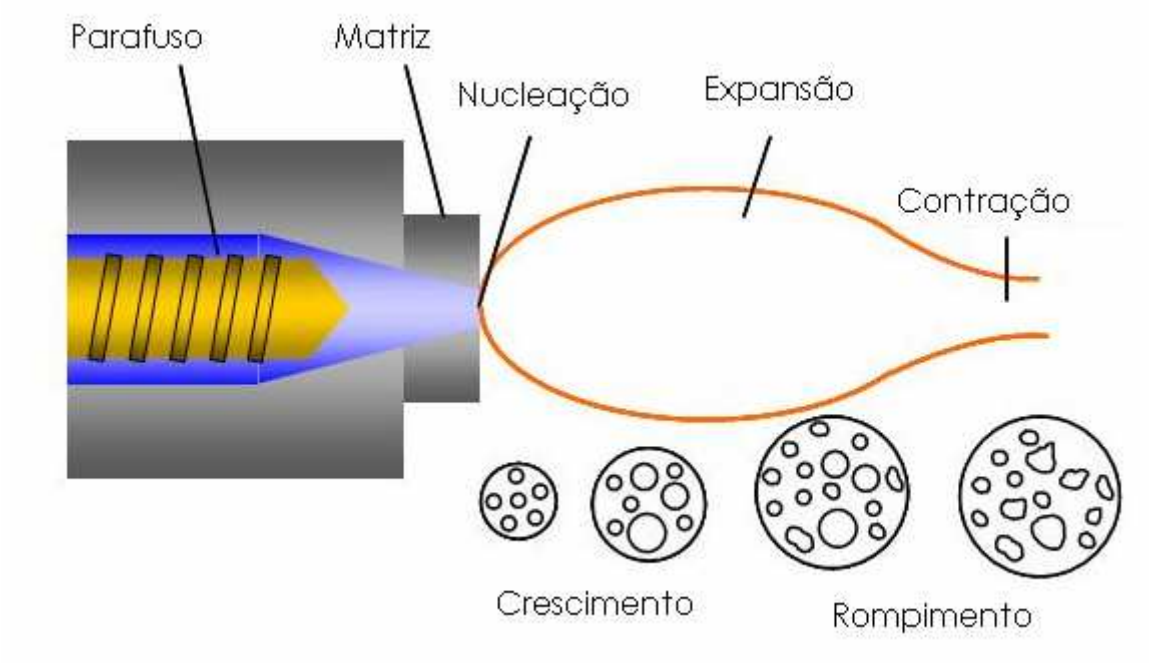

Figura 9 – Representação do processo que ocorre com materiais amiláceos após saída de extrusora com alta pressão e alta temperatura. Adaptado de: Kokini et al. (1992)

O tratamento térmico em materiais amiláceos induz modificações físicas e químicas dos grânulos de amido e seus constituintes, produzindo mudanças texturais e reológicas, aumentando a digestibilidade e a disponibilidade do amido como fonte energética (CHEFTEL, 1986).

Tem sido demonstrado que lipídeos podem formar complexos com amilose, com a porção de hidrocarbono do lipídeo localizada dentro da cavidade helicoidal da amilose (BANKS; GREENWOOD, 1972). A ligação de lipídeos com amido

acarreta mudanças nas propriedades físico-químicas de extrusados. No entanto, estas ligações são complexas e têm demonstrado variar com o tipo, quantidade e balanço hidrofílico-lipofílico de lipídeos e os materiais a serem extrusados (FAUBION et al., 1982). Battacharya & Hanna (1988) relataram que a diminuição do conteúdo lipídico resultou em maior expansão dos extrusados que têm maior capacidade de ligação à água na extrusão de glúten de milho com proteína concentrada de soja desengordurada.

A extrusão é uma ferramenta promissora no processamento de cereais, não só para o consumo humano, mas também para várias aplicações industriais. Entre as aplicações alimentares da extrusão, pode citar-se a produção de cereais matinais e expandidos, farinhas e amidos pré-gelatinizados, produtos texturizados, inativação de fatores antinutricionais e enzimas, produtos de confeitaria, bebidas e alimentos ricos em fibras. Para o uso do processo da extrusão com produtos com alto teor de fibras ou alto teor de proteínas, as condições do processo, como temperatura, umidade devem ser alteradas. Alterações nas propriedades funcionais dos alimentos devem ser monitoradas após o processo de extrusão, que devido às severas condições de temperatura, pressão e cisalhamento podem modificar as características de composição de nutrientes e não nutrientes.

Os efeitos benéficos à saúde a partir da ingestão da soja podem ser mais facilmente alcançados com o desenvolvimento de produtos aceitáveis. A realização dos benefícios à saúde pode depender da estabilidade das isoflavonas durante o processamento, assim como o perfil específico dos derivados das isoflavonas no produto final (MAHUNGU et al., 1999). Tem sido relatado que a quantidade e a forma das isoflavonas no produto final depende da temperatura (WU, 1994; KUDOU et al., 1991) e do tipo e intensidade das condições do processamento (MAHUNGU et a., 1999). Extrusados de misturas de cereais são um veículo nutricional promissor para incorporação de soja, como cereais matinais e “snacks”

Mahungu et al. (1999) avaliaram o efeito da temperatura e agitação, requeridas durante a extrusão, sobre o perfil de isoflavonas em misturas de farinha de milho/proteína concentrada de soja (80:20). Estes verificaram que houve uma grande influência da extrusão qualitativamente no perfil de

isoflavonas, enquanto que a quantidade foi pouco alterada. Os níveis totais de genisteína e seus derivados tenderam a diminuir, enquanto que para a daidzeína tenderam a aumentar, influenciados principalmente pela temperatura.

A incorporação de fibras alimentares em produtos extrusados limita a expansão e reduz a crocância, portanto diminuindo a vida útil. Quase que invariavelmente, tem sido demonstrado que o aumento da concentração de fibras em formulações de extrusados reduz a expansão de produtos. Mendonça et al. (2000) relataram que o aumento da proporção de farelo de milho resultou em menor expansão e em características texturais indesejáveis. Em geral tem sido observado que a redução na expansão resulta em produtos que são densos, duros e não crocantes (LUE; HSIEH; HUFF, 1991).

4.7.2 Barras de Cereais

A barra de cereal é um produto elaborado pela mistura de cereais, tais como flocos de arroz, milho, aveia e outros cereais com uma calda aglutinante preparada com açúcares, xarope de glicose e gordura vegetal, com a opção de agregar frutas desidratadas. É um alimento nutritivo de sabor adocicado e agradável, fonte de vitaminas, sais minerais, fibras, proteínas e carboidratos complexos (SKLIUTAS, 2002).

De acordo com Murphy (1995), os cereais em barras são multicomponentes e podem ser muito complexos em sua formulação. Como tal, muito cuidado deve ser tomado na combinação dos vários ingredientes para garantir que eles se complementem mutuamente nas características de sabor, textura e propriedades físicas particularmente no ponto de equilíbrio de umidade relativa. Cereais em barras estão inseridos em uma categoria particular de *countlines*, termo que descreve uma classe de produtos de confeitaria de forma retangular que são vendidos em embalagens individuais. Este é o setor de mais rápido crescimento no mercado de confeitaria.

Barras de cereais foram introduzidas há mais de duas décadas como uma alternativa “saudável” de confeito, quando consumidores se mostravam mais interessados em saúde e dietas (BOWER; WHITTEN, 2000). Alternativa saudável

às barras de chocolate, o produto foi direcionado no Brasil inicialmente aos adeptos de esportes radicais e com o tempo, conquistou outros setores (FREITAS; MORETTI, 2006).

Um crescimento no consumo de barras de cereais vem incentivando as indústrias a produzirem produtos diferenciados, agregando cada vez mais valor nutricional (alto teor de proteínas, fibras, vitaminas e sais minerais), e investir na melhoria da qualidade sensorial dos produtos (DUTCOSKI, 2004).

Enquanto no Brasil se consumiu US$ 4 milhões em barras de cereais por ano, os Estados Unidos deram conta de US$ 2,9 bilhões (FRANCAL FEIRAS, 2003). Segundo Palazzolo (2003), o catalisador para o crescimento no segmento de barra de cereais nos Estados Unidos foram produtos inovadores e um foco em conveniência e saúde. Pehanich (2003) reportou que barras nutricionais e energéticas vêm ganhando o mercado consumidor nos segmentos diet, “para mulheres”, “atletas de fim de semana”, “esportistas, e outros.

A importância de se disponibilizar, através de cereais em barras, energia e proteínas de bom valor biológico, resulta do fato de que crianças e adolescentes apresentam uma grande preferência por estes tipos de produtos e, devido a isto, poderiam cumprir uma função destacada em seu desenvolvimento físico e mental. Nas últimas décadas, tem-se dado muita atenção ao consumo de alimentos naturais com alto teor de fibras e baixos níveis de aditivos (ALBERTA AGRICULTURE, FOOD AND RURAL DEVELOPMENT, 2003).

Segundo Pszczola (2003), a solução para os problemas atuais de saúde está na utilização de ingredientes saborosos, saudáveis e funcionais para reformular a alimentação básica habitual do indivíduo. A popularidade das barras de cereais está relacionada a características como conveniência e nutrição. Além disso, está relacionada ao acréscimo do teor de fibras recomendado na ingestão diária devido ao baixo consumo de fibras implicarem em um fator de risco para diversas doenças (MURPHY, 2001).

Um trabalho de Boustani & Mitchell (1990) examinou a opinião de consumidores de barra de cereais e constatou que respostas associadas com alimento saudável, e apelos relacionando “saúde” e “sabor”, são as razões de compra do produto. “Snacks” e produtos açucarados continuam sendo uma fatia

dominante na dieta desses consumidores, ancorados, ambos processadores e consumidores, na imagem saudável destes produtos.

Alguns estudos sobre barra de cereais vêm reportando características e preferências de consumidores em análise sensorial e crescimento do mercado. O crescimento do segmento de barra de cereais nos Estados Unidos foi catalisado por produtos inovadores focados em saúde e conveniência, conforme o relato de Boustani e Mitchell (1990), que investigou os apelos envolvidos no marketing de barras de cereais.

As fibras insolúveis, tais como farelos, têm sido tradicionalmente utilizadas em produtos como barras de cereais, pães, massas e cereais matinais, mas a palatabilidade desses produtos fica muito limitada aos níveis em que estas fibras são incorporadas. As fibras solúveis são de maior interesse na formulação de alimentos “saudáveis” porque estas são mais palatáveis. Em alguns casos, ainda, podem ser utilizadas em sistemas alimentícios para espessar, adicionar viscosidade ou geleificar (DREHER, 1999).

Além das barras de cereais mais tradicionais há as barras com alto teor de proteínas, inicialmente direcionada a esportistas, agora consumida também pela população mais consciente em relação à alimentação adequada. Essas barras contêm 15 a 35% de proteína, que consiste de misturas de diferentes fontes de proteínas, como as de soja e do soro do leite. A vida útil dessas barras é frequentemente limitada pela tendência de tornarem-se inaceitavelmente duras durante o armazenamento (LOVEDAY et al., 2009). O exato mecanismo para explicar a ocorrência dessas modificações ainda não está elucidado, mas há algumas hipóteses. Loveday et al. (2010) sugerem como causa a ocorrência de reações de Maillard, reações físico-químicas, reorganização da microestrutura, podendo ocorrer agregação afetando a solubidade das barras de proteína durante o armazenamento, influenciando diretamente na dureza do produto. Ainda são sugeridas ocorrências de reações disulfídicas, reações cruzadas, agregação e formação de rede (ZHOU, LUI & LABUZA, 2008), ordenação da estrutura secundária das proteínas, migração de umidade e menor hidrofobicidade na superfície de proteínas (BAIER et al., 2007).

ARTIGOS CIENTÍFICOS

Artigo 1

“Extruded puffed functional ingredient with oat bran and soy flour”

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Extruded puffed functional ingredient with oat bran and soy flour

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Keywords: extrusion cooking, dietary fiber, vegetable protein, corn starch

Abstract

Extrusion cooking is a food processing technique that is used worldwide to transform various ingredients. The aim of this work was to apply extrusion to develop a functional puffed ingredient with defatted soy flour and oat bran and the minimum amount of corn starch required to attain good textural properties. The proportions of the feed ingredients and the processing conditions (extruder temperature, moisture, and inulin percentage as a technological coadjutant) were optimized. Applying mixture experimental design to study the effect of feed ingredients on expansion and textural properties of extruded product, the formula containing 250 g/kg corn starch, 375 g/kg soy flour, and 375 g/kg oat bran was selected as the best between tested. Using this blend and applying incomplete factorial design, the best process conditions (250 g/kg moisture; 45 g/kg inulin and 130 ºC) were chosen. Based on the results of the process, optimization step temperatures higher than 130 ºC were tested and showed that 160 ºC increased the radial expansion ratio and decreased hardness the most. The obtained puffed product had 212.6 g/kg fiber, 281.0

g/kg protein, and a caloric value of 319.1 kcal/100 g. It was well accepted by the panelists in the sensory evaluation, mainly in terms of texture.

Key words: vegetable protein, dietary fiber, extrusion cooking, mixture design

1. Introduction

Extrusion, classified as a high temperature/short time process, is an important food processing technique used worldwide for the production and modification or improvement of quality of various products. In the extruder, the food mix is thermomechanically cooked at high temperature under pressure and shear stress generated in the screw barrel assembly. The cooked melt is then texturized and shaped in the die. The thermomechanical action during extrusion brings about starch gelatinization, protein denaturation, and inactivation of enzymes, microbes and many anti-nutritional factors. All this occurs in a shear environment, resulting in a plasticized continuous mass (Battacharya & Prakash, 1994).

The product properties are influenced by extruder operating parameters, such as barrel and die temperature, screw speed, screw configuration, and die shape, as well as raw material formulation, namely the moisture, protein, starch, and lipid contents (Moraru & Kokini, 2003). Furthermore, ingredients such as sugar, salt, protein, and fiber can affect extrusion system variables, as well as product characteristics, such as texture, structure, expansion, and sensory attributes (Jin, Hsieh & Huff, 1994). There is abundant literature describing the effects of ingredient incorporation on extruded product microstructure and physicochemical properties (Kokini, Ho & Karwe, 1992; Jin, Hsieh & Huff, 1995; Mendonça, Grossmann & Verhé, 2000; Moraru & Kokini, 2003).

Dietary fibers have a number of health benefits. However, their incorporation into extruded puffed snack foods and breakfast cereals limits puffing and reduces crispness, therefore decreasing bowl life. Almost invariably, it has been found that increasing fiber

concentration in the extrudate formulations reduces the expansion volume of extruded foods. Mendonça et al. (2000) reported that co-extrusion of corn bran and cornmeal with increased bran content resulted in less radial expansion and undesirable product textural characteristics. In general, it has been observed that reduction in expansion during extrusion results in products that are dense, tough, and non-crispy (Lue, Hsieh & Huff, 1991).

Adding cereal fibers to extruded products has been limited to a maximum of 100-300g/kg fiber substitution for flour due to increase in product hardness and decrease in consumer acceptance (Hsieh, Mulvaney, Huff, Lue & Brent 1989; Lue, Hsieh, Peng & Huff, 1990; Jin et al., 1995). Liu, Hsieh, Heymann & Huff (2000) have studied the addition of up to 200g/kg fibers to extruded products because of the numerous health benefits of high food fiber content.

Several researchers have reported about the addition of soy protein to extruded starch products with conflicting results. Faubion and Hoseney (1982) reported that wheat starch with 10–80g/kg soy protein isolate expanded more than pure starch. However at 100g/kg soy protein isolate, expansion decreased. Zasypkin and Lee (1998) showed that increasing the proportion of soybean flour to 100g/kg in a wheat flour-soybean flour blend resulted in a decrease in expansion ratio at 160g/kg barrel moisture content, and an increase at 170–180g/kg moisture content. Further addition of soy flour up to 400g/kg led to continuous decrease in expansion ratio. Beyond 400g/kg, the trends for expansion depended again on moisture content and also on a possible phase inversion occurring beyond that level of soy flour. Li, Zhang, Jin and Hsieh (2005) reported that the addition of soybean flour to corn meal in the range from 0 to 400g/kg increased expansion ratio. The different results could be explained by the differences in blends, proportion of the ingredients, and process conditions.

Mixing different ingredients to make a puffed ready-to-eat product using the extrusion process is difficult. Oat bran, for example, has a high level of fat and soluble gum. However, the negative effects of fibers or proteins in the extruded dough or the

products can be minimized if some additives are used, such as monoglycerides, modified starches, modified gelatin, oligofructose, or inulin.

Inulin has already been used as an additive in extrusion (Ascheri, Couri & Madeira, 2006). It was selected from among other additives to be used as an extrusion coadjutant in this investigation based on preliminary experiments to determine optimum extruder operating conditions. Inulin can provide a number of functional properties to extruded cereal, including greater expansion (Niness, 1999).

Consumption of either soluble fiber or vegetable protein decreases serum cholesterol (Anderson, Garrity, Wood, Whitis, Smith, & Oeltgen, 1992; Jenkins et al., 1993; Anderson, Johnstone, & Cook-Newel, 1995). Jenkins et al. (1999) investigated the combined effect of protein (soy) and soluble fiber added to a standard cholesterol-lowering diet. They concluded that this combination reduced both LDL cholesterol and the LDL: HDL cholesterol ratio in diets with low saturated fat and cholesterol ingestion in the diet. Therefore, extrusion cooking of soy flour in combination with nutritionally complementary cereal grains, such as oat, is of even greater interest, because it can be used to produce nutritionally balanced ingredients in the well-accepted form of an extrudate.

The objective of this study was to develop a potential functional extruded ingredient combining defatted soy flour and oat bran by selection of the best proportions among ingredients and optimization of process parameters. Preliminary studies showed that it is impossible to obtain a product with good sensory characteristics extruding only defatted soy flour and oat bran with the lowest amount possible of corn starch in the formulation. Furthermore, the process was possible only with the addition of inulin, because of its contribution to better mass flow during the process.

2. Material and Methods

2.1. Ingredients

Oat bran (225 g/kg dietary fiber [95 g/kg soluble and 130 g/kg insoluble], 267 g/kg protein and 75 g/kg lipids, db) was provided by SL Alimentos (Mauá da Serra, Brazil). Defatted soy flour (160 g/kg dietary fiber [10 g/kg soluble and 150 g/kg insoluble], 555 g/kg protein and 10 g/kg lipids, db) was purchased from Vitao Alimentos (Curitiba, Brazil), Inulin Beneo ST® (920g/kg purity, DP ≥ 10) and corn starch were obtained from Clariant (São Paulo, Brazil) and Unilever (Mogi das Cruzes, Brazil), respectively.

2.2. Sample Preparation

Corn starch, oat bran, and defatted soy flour constituted a ternary mixture and were blended in predetermined ratios, as stated by the experimental design in the first step. To each blend, 50g/kg inulin (calculated on total formulation) was added to promote better extrusion flow. The blended samples were conditioned to 250g/kg moisture by spraying a calculated amount of water and mixing continuously in a mixer. The samples were sealed in plastic bag and stored at 4 ºC overnight. The feed material was then allowed 1 hour to equilibrate at room temperature prior to extrusion. This preconditioning procedure was employed to ensure uniform mixing and hydration and to minimize variability. In other steps of the study, the ingredient proportion in the ternary mixture was fixed at corn starch (250g/kg), oat bran (375g/kg), defatted soy flour (375g/kg), and the moisture and inulin percentage was varied according to the experimental design.

2.3. Extrusion

A single screw laboratory extruder (BGM, EL-25 model, Brazil) was used. The barrel diameter (D) and the length-to-diameter ratio (L/D) were 25 mm and 26:1. The extruder had four zones with electrical resistance band heaters and thermocouple sensors to monitor the temperature, a 3:1 compression ratio screw, and a die with six 2-mm diameter holes. In the first step (formula optimization), the processing temperatures were 80, 100**,** 120, and 120 ºC at zones 1, 2, 3, and die, respectively. In the second and third steps (process optimization), the temperatures of zone 3 and of the die were varied according to experimental design. The screw speed was fixed at 70 rpm and the extruder was operated at a steady state for each set of conditions. Attainment of these conditions was judged by constant amperage. Samples (approximately 1000 g) were collected and dried overnight at 80 ºC in a forced-air convection oven to moisture values of around 30g/kg. Part of the extrudates was coarsely or finely ground for further physical and chemical determinations. The other part, used in texture tests, was hand cut, placed in polyethylene bags, sealed, and stored until testing.

2.4. Product Characteristics

2.4.1. Radial Expansion Ratio (RER)

RER was calculated by dividing the average cross-section area of extrudate (mm) (obtained with calipers) by the extruder die cross-section area (mm). Each treatment was measured 15 times.

2.4.2. Specific Length (SL)

Specific Length was calculated by dividing the average length (mm) of extrudate by its weight (g) (Alvarez-Martinez, Kondury, & Harper, 1988). The extrudates were cut by hand in pieces of about 5 cm in length. Each treatment was measured 15 times.

2.4.3. Specific Volume

The specific volume of each extrudate, the ratio between its volume and weight, was expressed as cm^3/g. The volume was obtained using the rapeseed displacement method (method 10-05) (AACC, 2000).

2.4.4. Textural properties

The hardness (peak force during first compression) and fracturability (force at yield break) of extrudates (Bourne, 1978) were determined using a TA.XT2 Texture Analyzer (Stable Micro System, U.K.) with a 5-blade Kramer shear cell probe and the software XTRAD. The samples were cut by hand in pieces with the size of the cell (about 7.5 cm). Approximately 80 pieces of each sample were put horizontally and downright with the knives in multiple layers in the cell to fill up to a standard height of 2 cm. The knives had a crosshead speed of 1 mm/s, were 10 mm apart and had a 0.05-N force threshold.

2.4.5. Proximate Composition

Moisture (method 44-15), crude fat (method 30-25), ash (method 08-01), and crude protein (method 46-10) contents in g/100 g, dry weight basis, were analyzed by official AACC methods (AACC, 2000). Dietary fiber (total, soluble, and insoluble) was analyzed by method 985.29 of AOAC (2000). The nitrogen-protein conversion factor used to calculate total protein content was 6.25, whereas the carbohydrates were calculated by subtraction.

2.5. Acceptance Test

Sensory evaluation of the final formulation extrudate in terms of general appearance, color, flavor, taste, and texture was performed by 50 individuals who were faculty, staff members and students of the Universidade Estadual de Londrina. The sample was presented in pellets. A verbally anchored 9-point structured hedonic scale (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike, 5-neither like nor dislike, 6-like, 7-like moderately, 8-like very much, and 9-like extremely) was used to evaluate overall acceptability. The panelists tasted the extrudate sample and chose among the nine points of the scale one that best matched their opinion of the product.

2.6. Experimental Design for Extrusion

2.6.1. First Step – Formula Optimization

The effects of ingredient proportions on product characteristics were studied with a simplex-centroid design for three-component mixtures expanded with internal points with constraints (Statistica, 2006). The constraints (lower and upper) for each ingredient were established in previous tests (200-300g/kg for corn starch; 200-500g/kg for both soy flour

and oat bran). The experiment consisted of nine different formulations and two replications of the central point. The pseudo-components were calculated as: $P_{CS} = C_{CS} – 0.20/0.40$; $P_{OB} = C_{OB} – 0.20/0.40$; $P_{SF} = C_{SF} – 0.20/0.40$, where P = pseudo-component concentration and C = real component concentration. The formulation proportions expressed as real and pseudo-components are presented in Table 1. After data collection, Scheffé's canonic model for three components (eq. 1) was used to model the responses (Barros Neto, Scarminio & Bruns, 2007)

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \qquad \textbf{(eq. 1)}$$

where Y is the dependent variable, β_i is the regression parameter for each linear component, β_{ii} is the binary interaction coefficient, X_1 is the corn starch content, X_2 is soy flour, and X_3 is oat bran.

The dependent variables hardness, fracturability, radial expansion ratio, specific volume, and specific length were analyzed. The adjusted models were subjected to variance analysis (ANOVA) to assess the significance ($p < 0.05$), variation coefficient, determination coefficient (R^2), and lack of fit. Data analysis and contour plots were carried out using the Statistica program version 7.1 (Statistica, 2006).

For each of the response variables, model summaries were analyzed for linear, quadratic, and cubic models and the most accurate model was chosen. The proportion of ingredients for optimal extrusion performance was calculated using a multiple response method called desirability employing Statistica 7.1 (2006). This optimization method incorporates desires and priorities for each of the variables. RER was specified at the maximum level and hardness was specified at the minimum in desirability. Optimization was carried out for the formulation first and the optimized formulation was used in the process optimization.

Table 1 – Experimental design for formula optimization of the ternary mixture of corn starch, defatted soy flour, and oat bran in terms of actual proportions and in pseudo-components.

Formulation	Proportion of components in ternary mixture					
	Actual concentration*			Pseudo-components		
	Corn Starch (c_1)	Soy Flour (c_2)	Oat Bran (c_3)	Corn Starch (X_1)	Soy Flour (X_2)	Oat Bran (X_3)
1	0.30	0.50	0.20	0.25	0.75	0
2	0.25	0.25	0.50	0.125	0.125	0.75
3	0.20	0.30	0.50	0	0.25	0.75
4	0.20	0.40	0.40	0	0.50	0.50
5	0.20	0.50	0.30	0	0.75	0.25
6	0.30	0.35	0.35	0.25	0.375	0.375
7	0.25	0.375	0.375	0.125	0.4375	0.4375
8	0.25	0.50	0.25	0.125	0.75	0.125
9	0.30	0.20	0.50	0.25	0	0.75
10	0.25	0.375	0.375	0.125	0.4375	0.4375
11	0.25	0.375	0.375	0.125	0.4375	0.4375

Source: Statistica, 2006

$c_1 + c_2 + c_3 = 1$

*Real concentration - proportion of ingredients (starch, soy flour, and oat bran) in g/kg

2.6.2. Second Step – Improvement of Product by Additive Inclusion and Selection of the Best Operating Conditions

The aim of this step was to improve the characteristics of the formulation chosen in the first step by studying the effects of inulin and the process conditions. The independent variables were inulin content (30, 40, and 50g/kg), feed moisture (230, 250, and 270g/kg), and extruder zone 3 and die (110, 120. and 130 ºC) temperatures. The variables were combined according to an incomplete factorial design (3^{3-1}) with nine treatments and one repetition in a central point. The three levels of each variable, established according to preliminary tests, were coded as -1, 0, and 1 for statistical analysis. The dependent variables were expansion ratio, specific length, specific volume, hardness, and fracturability.

Analysis of variance was conducted to determine significant differences among treatments for each response variable using Statistica 7.1 (2006). The best model was determined following the stepwise regression procedure and the surface plots were drawn using the same software. Calculation of optimal formula and processing conditions for extrusion performance also used desirability and was carried out with Statistica 7.1 (2006). RER was specified at the maximum level in desirability and hardness was fixed to an intermediate level.

2.6.3. Third Step – Extruder Temperature Tests – Process Optimization

The results obtained in the second step demonstrated a tendency for lower hardness and higher RER with the increase in the extruder temperature. In the third step, higher temperatures (130, 140, 150, and 160 ºC) in zone 3 and die were tested while the other conditions defined in the second step (45g/kg of inulin and 250g/kg of moisture) were maintained. Analyses of variance were conducted to determine significant differences among treatments for each response variable (hardness, RER) using Statistica 7.1 (2006).

3. Results

3.1. First step – Proportion of the Ingredients

The quadratic models adjusted for extrudate hardness and radial expansion ratio (RER) are presented in Table 2. All the models were significant ($p < 0.05$), presenting coefficients of determination (R^2) of 0.851 and 0.907. Only for hardness was the lack of fit significant ($p < 0.05$); however, Box and Draper (1987) proposed that the significance test for lack of fit is not relevant when the mean square of pure error is very low, as observed in this case. Analysis of variance of predictive polynomial regressions showed that only soy flour, oat bran, and the interaction between these ingredients contributed significantly to the hardness model. RER was significantly affected by linear effects of corn starch, soy flour, oat bran, and the interaction of corn starch and oat bran (Table 2). It was not possible to fit significant models to the results of specific volume, SL, and fracturability.

Table 2 - Models and goodness of fit obtained for hardness and radial expansion ratio (RER) using statistical evaluation

	Parameter	Equation	R^2	p**	Lack of fit (p)
Mixture Design (First Step)	Hardness	Y_h = -96.66CS **+ 394.40SF + 219.35OB – 617.07SFxOB**	0.907	0.0015	0.0042*
	RER	Y_{RER} = **1.76CS + 1.05SF + 1.13OB – 0.94CSxOB**	0.851	0.0103	0.2917
Factorial Design (Second Step)	Hardness	Y_h = **158.60** + 11.58M – 11.74M^2 **+ 32.48T – 49.09In** + 22.24In^2 **– 53.12MxT – 76.66MxT^2**	0.990	---	---
	RER	Y_{RER} = 1.13 – 0.02M **+ 0.14T** – 0.028In	0.837	---	---

CS = Corn Starch, SF = Soy Flour, OB = Oat Bran; M = Moisture, T = Temperature, In = Inulin; RER = Radial Expansion Ratio;

Bolded coefficients are statistically significant; * significant value; **p = Probability level

The increase of corn starch decreased hardness, while increase of oat bran and soy flour increased hardness (Figure 1A). Hardness values ranged from 125.53 to 288.03 N, and the lowest value corresponded to the sample containing maximum starch (300g/kg) and equal concentrations of the other two components (350g/kg). The hardest sample was sensorily undesirable.

Stanley (1986) suggested that food texture is a result of its microstructure, which depends on the influence of physical forces on chemical components. The texture of extruded food products is greatly influenced by the extrudate composition. Generally, the addition of fiber to an extruded product results in increased product density and hardness. Great differences have been observed with increasing amounts of fiber (Hsieh, Huff, Lue & Stringer, 1991; Jin et al., 1995; Mendonça et al., 2000; Yanniotis, Petraki & Soumpasi, 2007) as a result of its effect on air cell wall thickness. In many cases, hardness higher than 200 N is not a desirable attribute for expanded snacks.

Above a critical concentration, the fiber disrupts the continuous structure of the melt, hindering its elastic deformation during expansion. Fibers can also bind some of the water present in the matrix, thus reducing its availability for expansion (Moraru & Kokini, 2003), and therefore modifying the hardness.

Lue et al. (1991) reported that inert component (fiber) in feed material resulted in poor formation and low retention of expanded air pockets. Moore, Samei, Vanhecke, and Bouvier (1990) found that the number of air cells per unit area increased greatly while average cell size decreased as the concentration of bran was raised.

Conway and Anderson (1973) researched different extrusion-cooked blends of cereal-protein; in the mixtures with yellow corn snacks meal with up to 222g/kg soy protein isolate, 280g/kg soy protein concentrate, and 400g/kg soy flakes (all products defatted). All blends had texture similar to that of commercial extruded products. Expansion was sufficient to provide a fairly uniform cell structure, the products were friable and crisp and could be readily ground or directly consumed.

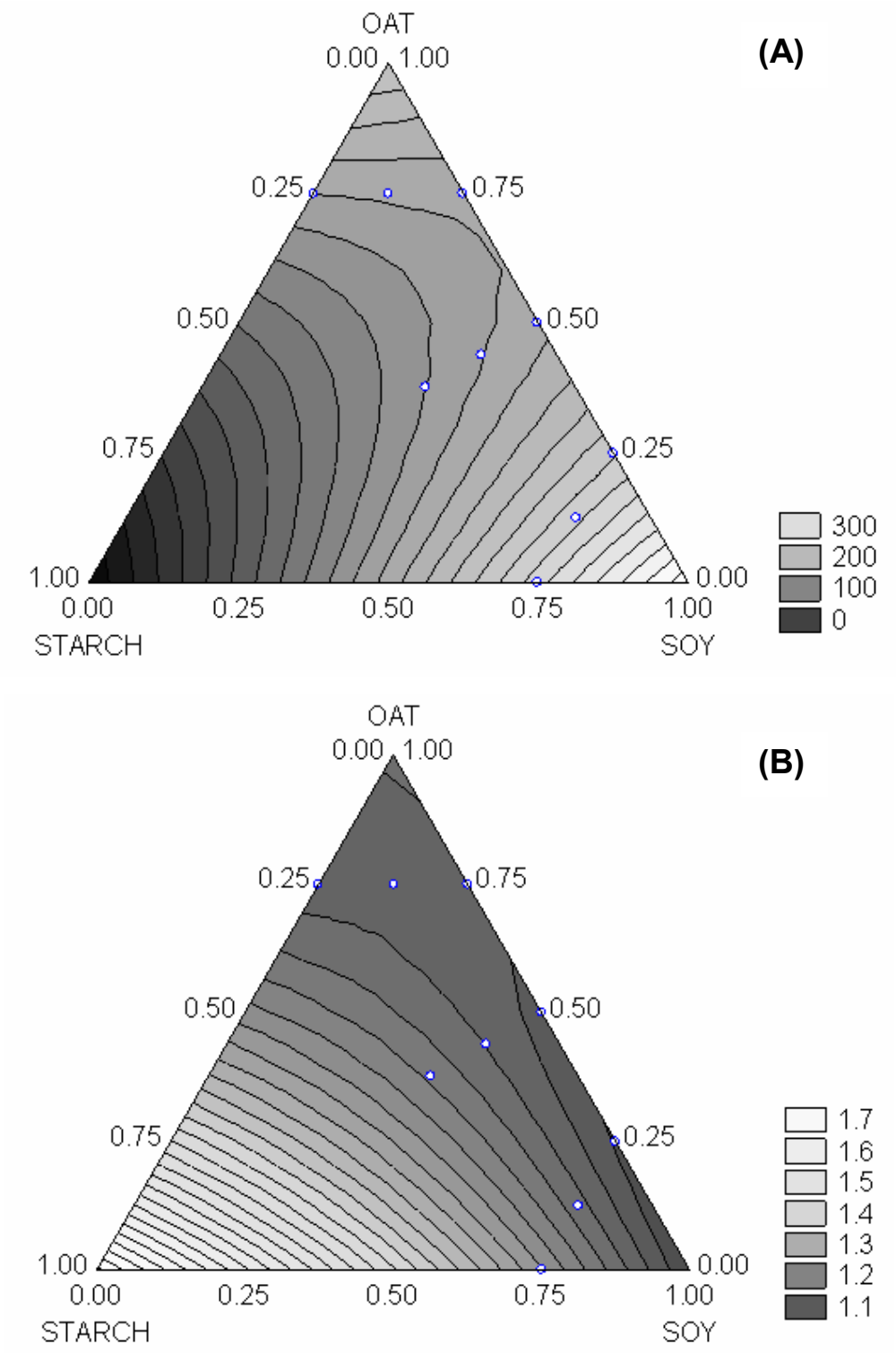

Figure 1 – Contour plot for hardness (A) and radial expansion ratio (B) of extruded product containing corn starch, soy flour, and oat bran. Experimental area is delimited by points and expressed as pseudo-components.

A higher fiber effect would be expected in extruded oat bran/soy flour/corn starch mixtures. Dilution of the protein in feed materials with cereals or starches reduces the possibility of formation of a continuous protein phase and, thereby, lessens texturization (Harper, 1979).

The increase of corn starch increased the RER, while the oat bran and soy flour had the opposite effect (Figure 1B). RER values varied from 1.07 to 1.24. Volumetric expansion, either radial or longitudinal, of extruded starch-based materials is a consequence of extensive flash-off of internal moisture and flow properties of molten mass (Chinnaswamy & Hanna, 1988). The latter depends on the degree of gelatinization, which is determined by processing conditions and raw material composition (Jin et al., 1994; Hsieh et al., 1991; Hsieh, Peng & Huff, 1990).

The deleterious effect of soy flour and oat bran on radial expansion is explained by the action of inert components, such as fibers, which rupture air cell walls and external surface in extrudates, thereby preventing the full expansion of air bubbles (Lue et al., 1990).

The effect of fibers on extrudate expansion seems to be concentration-dependent. Mendonça et al. (2000) reported that the RER of extruded corn starch/corn bran mixtures decreased with increasing corn bran content for similar moisture and temperature combinations.

Increasing fiber content results in a less expanded and more compact extrudate texture. The air bubbles were smaller, and the cell walls were thicker at 300g/kg fiber level than at 100g/kg (Jin et al., 1995). Guy (1985) suggested that since bran interferes with bubble expansion, which reduces cell wall extensibility and causes premature rupture of steam cells at a critical thickness, steam could exit easily during flashing.

Lue et al. (1990) studied the effects of oat fiber and wheat bran on the internal structure of corn meal extrudate. They found that increasing oat fiber content produced extrudates with a denser structure and smaller average cell size. However, air cells were

not visually distinguishable in extrudates with 100-300g/kg of red wheat bran. This variation in extrudate properties may involve mechanisms related to the physicochemical states and compositions of the different feeds.

Faubion and Hoseney (1982) reported that the effect of proteins on expansion depends on their type and concentration. Most studies reported that the addition of protein extrusion mixture can increase expansion up to a determined percentage. However, above the critical concentration it decreases expansion. As demonstrated by Fernandes, Wang, Ascheri, Oliveira, and Costa (2002), the increase of the soy flour content in the mixture from 200 to 300g/kg can decrease the RER approximately in half. El Dash (1982) reported that the addition of soy products, especially soy concentrate, decreased the expansion ratio. Conway and Anderson (1973) evaluated many corn meal and soy product blends and proposed that the addition of soy products tends to decrease the expansion of extruded blends. Expansion properties were the best for combinations of corn meal with more refined soy products, such as soy isolate and concentrate. This probably resulted from the lesser dilution of the cereal base by high protein content ingredients.

Proteins have an effect on expansion through their ability to affect water distribution in the matrix and through their macromolecular structure and conformation, which affect the extensional properties of the extruded melts. They also contribute to extensive networking through covalent and nonbonding interactions that take place in extrusion (Madeka & Kokini, 1992; Li & Lee, 1996)

Applying the desirability optimization technique to select the ingredient proportion that yields an extruded with higher RER and lower hardness showed that the highest corn starch concentration was the best. However, considering that one of the objectives of this study is to develop an extruded with the lowest starch amount possible, and verifying in Figure 1A that it is possible to reduce this level to an intermediate level (250g/kg), in the area of the central point, while still maintaining the same hardness range, this point was chosen as the best formula. Reducing the starch level even more to the lowest amount is

undesirable due to the resulting increase in hardness. There are no studies in the literature on extruded products combining oat bran, soy flour, and corn starch for data comparison.

Second Step – Effects of Process Parameters

The models were adjusted for extrudate hardness and RER and are presented in Table 2. All the models were significant ($p < 0.05$) and had no lack of fit ($p > 0.05$). The coefficients of determination (R^2) varied from 0.837 to 0.990. Analysis of variance of predictive polynomial regressions showed that temperature, inulin percentage, the linear interaction between moisture and temperature, and the interaction between moisture and quadratic effect of temperature contributed significantly to the hardness model, while temperature level was significant for the RER model (Table 2). It was not possible to fit significant models to the results of specific volume, SL, and fracturability.

Analyzing Figure 2A, it observed that an increase in temperature increased the hardness. However, at a higher amount of inulin and at a higher temperature, the hardness showed intermediate values, varying from 100.12 to 216.55 N. The samples with low hardness were very fragile. These observations are important in order to choose the condition that yields the best texture. We chose the intermediate value of hardness of about 125 N as the most desirable.

Although inulin is an ingredient, in this work it was considered a technological coadjutant, and thus evaluated as a process parameter. The number of fructose units linked to a terminal glucose, n, of inulin, can vary from 2 to 70 (De Leenheer & Hoebregs, 1994). This means that inulin is a mixture of oligomers and polymers. This low degree of polymerization can lend a lubricating effect to inulin, which explains its positive effect on extrusion observed in this work.

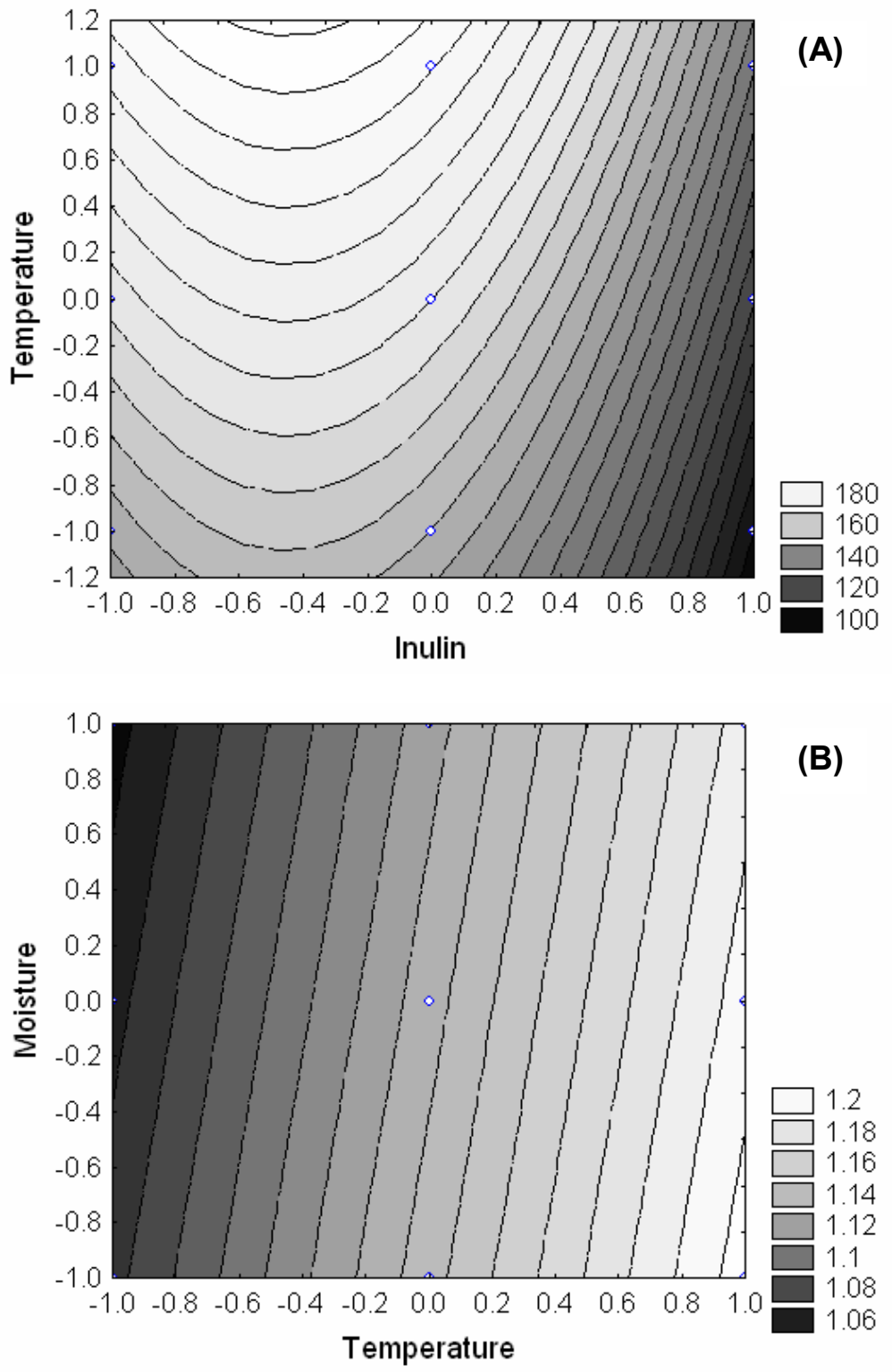

Figure 2 – Contour plot for extruded Hardness (A) (Temperature vs. Inulin) and RER (B) (Moisture vs. Temperature) for blends containing corn starch, soy flour, and oat bran.

Niness (1999) reported that addition of up to 300g/kg inulin to breakfast cereal extrusion feed increased the expansion and improved bowl life, taste, and texture. In this work, a small amount (30-50g/kg) of inulin decreased hardness and improved extrusion by leading to a better batter flow in the extruder. Ascheri et al. (2006) reported that addition of 100g/kg inulin to rice flour extrudates increased the RER, but decreased expansion above this concentration.

Only temperature had a significant effect on the RER (Table 2 and Figure 2B). When the temperature was increased, the RER also increased, reaching values ranging from 1.03 to 1.22. Battacharya and Prakash (1994) reported a significant quadratic effect of barrel temperature in the range of 75 to 185 ºC on the expansion of rice flour extrudates. An increase in expansion of cereal flours with extrusion temperature was also reported by other authors, while Della Vale, Vergnes, Colonna and Patria (1997), and Mendonça et al. (2000) observed the highest RER for the lowest feed moisture and extrusion temperature.

Extrusion temperature plays an important role in changing the rheological properties of extruded melts, which in turn affect the expansion volume. The product temperature increases with the extrusion temperature, decreasing the product viscosity. Low viscosity of the extruded cereal melt is detrimental to expansion, since it allows the matrix cells to collapse under the high vapor pressure (Moraru & Kokini, 2003).

Kokini, Chang and Lai (1992) found that there is a temperature range in which radial expansion of starch reaches a maximum; this optimal temperature range depends on the type of starch. Beyond a critical temperature, which depends both on the type of starch and moisture content, expansion decreases with temperature, most likely due to excessive softening and potential structural degradation of the starch melt, which becomes unable to withstand the high vapor pressure and, therefore, collapses (Moraru & Kokini, 2003).

Applying the optimization technique of desirability aiming at obtaining higher RER and average hardness led to optimal process conditions of 250g/kg moisture, 45g/kg inulin, and extrusion temperature of 130 ºC.

Third Step – Process Optimization

Results obtained in the second step (Figure 2B and optimization technique) demonstrated a tendency to better RER responses for extruder temperatures higher than 130 ºC in zones 3 and 4 (die). The results obtained when some of those temperatures were tested (Table 3) showed that the mean value of expansion was high and hardness was low at 160 ºC, being significantly different from the other conditions. On the other hand, the SV and SL were higher at 150 ºC.

Table 3 – Effect of zone-3 and -4 temperatures on RER, hardness, fracturability, SV, and SL

Parameters*	Means ± SD				
	130ºC	**140ºC**	**150ºC**	**160ºC**	**P**
RER	1.208 ± 0.010^{b}	1.179 ±0.030^{b}	1.191 ± 0.017^{b}	1.303 ±0.011^{a}	0.0142
Hardness	192.349 ± 4.334^{a}	140.761 ±4.736$^{b.c}$	142.207 ± 2.813^{b}	126.470 ± 1.570^{c}	0.0002
Fracturability	71.145 ± 3.444^{a}	57.890 ± 3.72$^{c.d}$	63.585 ± 4.349$^{a.d}$	59.540 ± 0.594$^{b.d}$	0.0300
SV	1.482 ± 0.086^{c}	1.700 ± 0.046^{c}	3.291 ± 0.016^{a}	2.420 ± 0.124^{b}	<0.0001
SL	239.770 ± 1.286$^{b.c}$	263. 495±7.122$^{b.c}$	323.380 ± 12.740^{a}	281.860 ±17.213$^{a.c}$	0.0083

RER – Radial expansion Ratio; SV – Specific Volume; SL – Specific Length

*Means in the same line with different letters are significantly different. Each treatment was measured 15 times.

In terms of texture, the barrel temperature had a significant effect on the crispness and chewability of corn snacks. The temperature increase resulted in an increase of the expansion, giving a product with low density that was crispier and more chewable (Chen,

Serafin, Pandyan & Daun, 1991; Fernandes et al., 2002). Usually, the best characteristics of extruded products are: high expansion (because of its relation with the crispness), low hardness, high specific volume (or low density). The conditions chosen to obtain the final product were: 160 ºC in the die and zones 3 and 4, 250g/kg of moisture, and 45g/kg of inulin.

Characterization of the Obtained Puffed Ingredient

The developed ingredient had hardness of 126 N and expansion ratio of 1.3. The chemical composition was: 212.6 g/kg dietary fiber [51.8 g/kg soluble and 160.9 g/kg insoluble], 281.0 g/kg protein, 42.0 g/kg lipids, and 422.2g/kg carbohydrate with caloric value of 319.1 kcal/100 g).

Acceptance Test

The mean acceptability values of the final extrudate formulation were 6.20 (±1.70) for general appearance, 6.74 (±1.66) for color, 6.08 (±1.29) for flavor, 6.8 (±1.68) for taste, and 7.8 (±1.62) for texture. Most of the panelists found the extrudate texture to be the best attribute (75%), and appearance (37.5%) and lack of flavor (37.5%) were the worst attributes. The lack of flavor was considered positive because a characteristic off-flavor in soy products is undesirable and appearance can be improved through product formulation.

Conclusion

This work demonstrated the feasibility of producing an extruded ingredient with blends of oat bran, soy flour, and corn starch in amounts of 375g/kg, 375g/kg and 250g/kg, respectively. In the evaluated experimental region, the best processing

conditions to obtain good physical and sensory characteristics were: 250g/kg moisture and 160 ºC extrusion temperature. The addition of 45g/kg of inulin to the mixture was essential to impart flow to mixtures during extrusion. The obtained extruded product can be used as a granola or cereal bar ingredient or as a substitute for rice crispy as a snack with addition of flavors.

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Artigo 2

"Snack bars with high soy protein and isoflavone content to use in diets for controlling dyslipidaemia"

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O objetivo inicial deste estudo era produzir quatro barras de cereais diferentes para serem utilizadas no ensaio clínico com pacientes para avaliação do efeito hipocolesterolêmicos do farelo de aveia, da farinha de soja e da combinação de seus componentes.

O planejamento previa a produção das seguintes barras:

1) Barra com alto teor de beta-glucanas – barras de cereais com adição de 3g de beta-glucanas/100g de produto;

2) Barra com alto teor de proteína de soja – barras com adição de 25 g de proteína de soja/100g de produto;

2) Barra com alto teor de beta-glucanas e proteína de soja no mesmo produto – 3g de beta-glucanas e 25 g de proteína de soja;

4) Barra de cereais controle, ou seja, sem nenhum desses ingredientes acima.

No entanto, não foi possível desenvolver barras contendo tão alto teor de beta-glucanas devido ao fato de o farelo de aveia ser um ingrediente de difícil adição em produtos devido ao seu caráter hidrofílico. Devido a essa característica não foi possível obter liga entre os ingredientes, então as barras se apresentavam quebradiças e sensorialmente não palatáveis. Esses problemas tecnológicos poderiam ser resolvidos com a utilização de farelo com maior teor de beta-glucanas ou de outros ingredientes responsáveis por conferir liga entre os ingredientes, como gomas, oligofrutose e inulina. Neste trabalho não foi possível utilizar esses ingredientes, pois a formulação foi padronizada de forma a utilizar como ingredientes com capacidades hipocolesterolêmicas apenas aqueles derivados de soja.

Por isso, foram desenvolvidas apenas as barras de soja.

O desenvolvimento das barras de soja compõem o artigo 2 desta tese, sendo apresentado a seguir.

Snack bars with high soy protein and isoflavone content to use in diets for controlling dyslipidaemia

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Abstract

The main objective of this study was to develop a cereal bar product rich in isoflavones and soy protein to use as an alternative for consuming more soy instead of animal protein and to be used in diets for controlling dyslipidaemia. A soy snack bar with 39.88g/100g dietary fibre, 34.25 g/100g protein, 100.39 mg/100 g of isoflavones and 245.47 Kcal/100 g was produced. Shelf life of product was tested over a period of six months. The hardness, water activity and darkness of the snack bars increased during the storage time. A randomised study, with one group consuming a hypocholesterolaemic diet as a control, was performed to evaluate the effectiveness of the soy snack bar on the lipid profiles of thirty-eight dyslipidaemic subjects for a period of forty-five days. Consumption of 3 soy snack bars a day decreased triglycerides (TG) (-24%) and increased high density lipoprotein cholesterol (HDL-C) (+12%).

Keywords: Functional foods, cereal bars, soy, hypercholesterolemia

1. Introduction

Cereal bars were introduced over two decades ago as a healthy alternative to high-sugar snacks at a time when consumers were beginning to show an increased interest in eating healthy. The trend towards healthy products that contain high fibre, high protein and low fat has expanded and introduced more competition in the health food market (Bower & Whitten, 2000). Cereal bars are a nutritional food with multiple components (including cereal, fruit, nuts and sugar).

There are several types of cereal bars, including high-protein, high-fibre and high-calorie bars. There are also fruit bars, crunchy bars, bars with filling, salty bars, low calorie or diet bars, bars with or without chocolate and bars with potentially functional additives such as prebiotics.

Nutrition, convenience, price and sensory attributes are important characteristics for determining the acceptability of a food product (Boustani & Mitchell, 1990; Bower & Whitten, 2000). Moreover, according to Roberfroid (1999), one of the major challenges is to provide busy consumers with healthy, ready-to-eat foods.

Cereal bars are practical, easy to manufacture, incorporate several ingredients and are usually sold at a low price. These products can be conveniently added to a packed lunch or eaten as a snack. Cereal bars can also be used to convince people to try more unusual grains and seeds, thereby adding new, healthy ingredients to their diets. Although the process for manufacturing cereal bars is relatively easy, incorporating high amounts of functional components can be very difficult due to the individual characteristics of the components and their interactions with syrup or other ingredients. Additionally, these functional components can be detrimental to sensory characteristics, such as texture or taste, and physical properties, such as water activity.

Diets for controlling excess body weight, hyperglycaemia and dyslipidaemia typically include low energy and low saturated fat diets, but these diets have limited efficacy due to the long-term commitment that is required. However, long-term health benefits can be gained from dietary proteins and bioactive non-nutrients called phytochemicals that are present in soy (Cederroth & Nef, 2009).

Recently, soy products and products with added soy are in demand due to the purported health benefits of soy consumption. Soybeans contain isoflavones and proteins, compounds that have been shown to improve lipid metabolism by activating receptors for low-density lipoprotein (LDL) (Anderson, 2003). Moreover, soy proteins have been connected to reductions in menopause symptoms and to a reduced risk for several chronic diseases, including cancer, heart disease and osteoporosis (Riaz, 1999).

Hypercholesterolemia is a major risk factor for cardiovascular disease. Some, but not all, studies have shown that soy protein intake decreased total and low-density lipoprotein cholesterol as well as triglycerides and increased high-density lipoprotein cholesterol (Reynolds et al., 2006). The mechanism(s) for the potential hypolipidaemic effect of soy protein appears to be multifactorial: some possible mechanisms include the inhibition of cholesterol absorption, enhanced bile acid excretion, increased receptor-mediated clearance of lipids from the blood, LDL receptor enhanced activity, or 7α-hydroxylase enhanced activity (Wang et al., 2004). Moreover, in 1999, the American Food and Drug Administration (FDA) allowed manufacturers of soy-containing foods to indicate on the product labels the beneficial role of soy protein in reducing the risk of coronary heart disease (CHD).

The addition of soybean ingredients to food products as cereal bars could be an alternative to increasing the consumption of soy beans themselves, especially because the consumption of soy and soy derivatives appears to be low in Western countries and is associated with a high rate of cardiovascular diseases compared with Asian countries whose a population are frequent consumers of soybeans and derivatives. The objective of this study was to develop a snack bar

with high isoflavone and soy protein content for use in diets to control dyslipidaemia and provide one more conventional option of soy products to consumers.

2. Materials and Methods

2.1. Materials

The ingredients used to manufacture the snack bars were: soy crisps (SUPRO Plus Nuggets 60, Solae Company, São Paulo, Brazil), soy protein isolate (SUPRO 780, Solae Company, São Paulo, Brazil), Toasted soy without salt (Só Soja do Brasil Ltda, Caldas Novas, Brazil), textured soy protein (TEXPRO, Exin Indústria e Comércio, Massaranduba, Brazil), glucose syrup (Glucogil 40/82, Cargill, Guarujá, Brazil), maltodextrin (Maltogil, Cargill, Uberlândia, Brazil) and palm oil (Vegetable fat 370B, Agropalma, Belém, Brazil), as well as lecithin, colouring, glycerine, demerara sugar and dried bananas, which were obtained at a local market.

2.2. Chemical composition of the ingredients and of the soy snack bar

The chemical composition of the individual soy ingredients and of the total bar formulation were determined. Moisture (air oven at 105ºC), fat (Soxhlet), ash (muffle 550ºC), protein (Kjeldahl) and dietary fibre (total, soluble and insoluble fibres were determined using an enzymatic method) were analysed by official AOAC methods (2000). The nitrogen-protein conversion factor used to calculate

total protein content was 6.25, whereas data regarding the carbohydrates content was estimated by mass remaining after protein, fat, ash and water contents were subtracted from the total mass. The caloric value was calculated by applying calorie conversion factors to carbohydrates (4 kcal/g), proteins (4 kcal/g) and lipids (9 kcal/g). Caloric values are expressed in kcal/100 g.

The chemical composition of ingredients was determined to characterise the soy ingredients and to determine the amount of protein in each ingredient for calculating the predicted amounts needed to achieve at least 25 g of vegetable protein in 100 g of final product using the following formula: $\%P_{bar} = \Sigma$ %Ing x $\%P_{ing}/100$, where $\%P_{bar}$ = the percentage of protein in a soy bar (predicted value); %Ing = the percentage of the ingredient in the mixture; $\%P_{ing}$ = the protein percentage of the ingredient.

2.3. Extraction, separation and quantification of isoflavones from soy ingredients and soy snack bars

The extraction of isoflavones was performed according to the methodology of Carrão-Panizzi et al. (2002). The separation and quantification of isoflavones was performed according to Berhow (2002) with a liquid chromatograph (model 2690, Waters & Associates, Milford, MA, USA) and an ODS-3 C_{18} reversed-phase HPLC column (250 mm of length × 0.4 mm internal diameter). A system of linear binary gradients with mobile phases was used to separate isoflavones. The system included methanol containing 0.025% trifluoroacetic acid (TFA) (Solvent A) and ultrapure deionised distilled water containing 0.025% TFA (Solvent B).

2.4. Soy snack bar formulation and production

Together, the soy ingredients in the soy bar formulation had sufficient amounts of soy protein and isoflavones to provide the health benefits described by previous studies (Cederroth & Nef, 2009) when three bars are consumed per day. The amounts of protein and isoflavones present in the bars (about 28 g each) were about 30 g and 80-100 mg, respectively. The ingredients were used in the amounts defined by the previously mentioned laboratory tests.

The bars were manufactured in batches of 3.0 kg. In the manufacturing process, syrup was heated at 100ºC and 78ºBrix and blended with the other ingredients to form crude dough. The dough was laminated and cut to form individual bars, which were packaged in aluminium foil, sealed in packs and stored at room temperature (21-25ºC) prior to analysis.

2.5. Shelf-life Study

The texture, colour, water activity and microbiological analyses of the cereal bars were performed after they were manufactured. Measurements were taken every thirty days for six months. Samples were taken randomly for analysis during the storage period. Ten soy bars were randomly selected to be tested for texture or water activity, and five bars were selected for microbiological and colour analyses.

2.5.1. Instrumental Texture

The hardness (peak force during first compression) of soy bars was determined by using a TA.XT2 Texture Analyzer (Stable Micro System, U.K.) with a HDP/BSK blade set, a knife probe and XTRAD software. Ten samples with similar sizes (10.0 cm x 2.7 cm x 1.4 cm) were cut with a knife using a crosshead speed of 0.5 mm/s, a distance of 15 mm and a force threshold of 0.05 N.

2.5.2. Colour

The colour measurement was performed as described by Oliveira et al. (2003) with some adaptations. One light source was used on the sample, and the distance between the camera and the samples was 18 cm. The images were captured with a digital camera (Sony Cyber-shot, 7.2 mega pixels). The digital images were converted to mean RGB values by a pixel-to-pixel application. RGB is the most basic and well-known color model based in “how we perceive color” and means Red, Green and Blue (Adobe Technical Guides, 2000). The data was converted to the CIELAB system by obtaining L*, a*, b* values (the L* value defines the lightness, a* defines the red-greenness and b* defines the blue-yellowness). CIELAB is a colour system adopted by CIE (Commission Internationale de l'Éclairage) with measures of “L”, “a” and “b” development based in distinctions of the optical nerve and the brain (Adobe Technical Guides, 2000).

2.5.3. Water Activity (a_w)

Water activity of the soy bars was measured by using a water activity meter (AquaLab models CX-2, Decagon Devices, Inc., Pullmann, WA) with controlled temperature.

2.5.4. Microbiological Analysis

The presence and amounts of yeasts, moulds, *Staphylococcus* aureus, Salmonella sp and coliforms were determined at 45ºC, and the amount of Bacillus cereus was determined according to AOAC official methods (1998) n. 2001.05, 967.25, 991.14 and 980.31.

2.6. Sensory Analysis

Sensory evaluation of the final product in terms of general appearance, colour, flavour, taste and texture was performed by 50 individuals who were faculty, staff and students at Universidade Estadual de Londrina, randomly invited to participate. A verbally anchored 9-point structured hedonic scale (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike, 5-neither like nor dislike, 6-like, 7-like moderately, 8-like very much and 9-like extremely) was used to evaluate overall acceptability. The panellists tasted the snack bar sample and chose the point on the scale that best matched their opinion of the product. The panellists were asked about purchase intent (yes or no) and if that intent would

change if they were informed that the product could help reduce the risk of developing diseases such as cardiovascular disease (yes or no). This evaluation was only carried out about three months after manufacture.

2.7. Clinical Trial

Thirty-eight dyslipidaemic subjects were selected at a cardiology clinic in Londrina, Paraná, Brazil, randomly distributed in two experimental groups to either consume three soy bars/day over a forty-five day period (soy bar group) or to follow a standard hypocholesterolaemic diet low in saturated fat and cholesterol as well as high in polyunsaturated fatty acids (control group). The patients, with a mean age of 43.7±10.3 years, were sedentary and instructed not to make changes in their daily lifestyle. The subjects answered questionnaires about lifestyle, socioeconomic status and food frequency and routine with the aim of profiling the subjects. Body weight, height and waist circumference were measured to calculate body mass index (BMI) and cardiologic risks. The inclusion criteria were patients ≥18 y old, with total cholesterol plasma levels ≥200 mg/dL and no other disease. All participants were informed of the procedures they would undergo and signed an informed consent document. This study and the consent terms were approved by the ethics in human research committee at Londrina University Hospital (nº099/09). Approximately 20 mL of blood was collected after 12 h-fast in vacuum tubes before the start of the clinical trial (day 0) and after forty-five days (day 45) for the following biochemical analyses: total cholesterol (CHOL), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and glucose. A biochemical autoanalyser (Dade AR, Newark,

NJ, USA) was used in conjunction with Dade Behring kits to quantify the levels of cholesterol, triglyceride and glucose in the patient samples. LDL-C was calculated by the Friedewald equation (Friedewald et al., 1972).

2.8. Statistical Analysis

To assess the significance ($p<0.05$) of the results, the dependent variable values (L*, a* and b*) for the snack bars were subjected to an analysis of variance (ANOVA) test, followed by Tukey's test. Linear models were adjusted to the hardness and water activity data. Data analysis was carried out using Statistica version 7.1 (Statistica, 2006). For the clinical trial, data sets were compared in Statistica 7.1 by using the non-parametric Wilcoxon matched pairs signed rank test for dependent samples and the Kruskal–Wallis test for independent samples with $p<0.05$. The data are presented as median.

3. Results and Discussion

3.1. Chemical Composition

Table 1 shows that all of the soy derivative ingredients had low amounts of carbohydrates no-fiber and high amounts of protein compared to the ingredients that are typically used in cereal bars. Adding certain ingredients to snack bars could reduce calories or increase the functional potential of the bars. For example, soy crisps could be a substitute for rice crisps, and toasted soy could be a substitute for nuts. Moreover, the cost of these ingredients for a soy snack bar is

relatively cheap (about $0.50 per bar in this study) considering the benefits these ingredients could provide to consumer health.

Table 1 - Proximate composition in dry basis (d.b.) and caloric value of soy ingredients in the soy snack bar

Analysis	Soy Crisps	Soy Protein Isolate	Toasted Soy	Textured Soy Protein	Soy Snack Bar
Carbohydrate (%)	15.21	---	17.09	18.07	14.30
Dietary Fiber (%)	17.74	14.56	35.79	23.47	39.88
Insoluble Dietary Fiber (%)	10.39	14.20	30.98	18.05	35.56
Soluble Dietary Fiber (%)	7.35	0.36	4.82	5.42	4.32
Lipids (soxleht) (%)	1.75	4.32	40.87	1.00	8.82
Protein (N x 6,25) (%)	61.57	84.86	38.67	50.67	34.25
Ash (%)	3.73	4.51	2.29	6.79	2.75
TOTAL	**100**	**108.25**	**134.71**	**100**	**100**
Caloric Value (Kcal/100 g)	300.19	348.86	572.95	260.68	245.57
Isoflavones (mg/100 g)	148.32	110.53	115.11	351.38	100.39

Several texture and sensorial tests were performed on bars containing different proportions of the soy ingredients to determine which proportions resulted in the desired amount of soy protein and acceptable sensory attributes. Other important parameters during the development of the bars were the “Brix” of the syrup and the ratio of syrup to solid components.

The formulation we developed is presented in Table 2. The proportion of syrup to the mixture of dry soy ingredients and fruit was 38.8:61.2. The chemical composition (Table 1) of the bars indicated high percentages of protein (34.25

g/100g) and dietary fibre (39.88 g/100g), indicating that the bars have health functional potential.

Table 2 – The ingredients and the proportions that were used in the soy bar formulation

	Ingredients	Proportion (%)
Dry Ingredients	Soy Crisps	24.0
	Soy Protein Isolate	6.3
	Toasted Soy without salt	13.0
	Textured Soy Protein	8.0
	Dried Banana	10.0
	Dry Ingredients total	**61.2**
Syrup Ingredients	Glucose Syrup	27.5
	Raw sugar	2.2
	Maltodextrin	1.8
	Water	4.3
	Palm oil	1.5
	Lecithin	0.5
	Colouring	0.7
	Glycerine	0.3
	Syrup Ingredients total	**38.8**
	Total	**100**

According to Loveday et al. (2009), high-protein commercial snack bars contain 15-35 g/100g protein, which consists almost exclusively of dairy or soy proteins because of their health benefits and low cost. Freitas & Moretti (2006) reported that bars that are not classified as high-protein have an average protein content of 4.4 and a 4.0 g/100g dietary fibre content. These authors developed a

high protein bar with added soy and obtained the following composition: 60.97 g/100g carbohydrate, 15.31 g/100g protein, 5.64 g/100g lipid and 5.17 g/100g dietary fibre.

The cereal bars currently in the Brazilian market have caloric values between 300 and 500 Kcal/100 g. The bar developed in the present study has only 245.47 Kcal/100 g due to the high amount of fibre. Due to its reduced caloric value, the bar can be classified as a “light food”. The term “light” can be used for foods that present a minimum reduction of 25% in a specific nutrient or total caloric value compared to the conventional food (Fennema, 2000).

3.2. Isoflavones

Isoflavones include the aglycones daidzein, genistein and glycitein; certain β-glucosides; conjugated malonyl-glucosides; and acetyl-glucosides (Góes-Favoni et al., 2004). According to Barnes et al. (1994), the bioavailability and the metabolism of different isoflavones depend on the chemical form of the isoflavone. Aglycones are the most active isoflavones in disease prevention. Among the twelve isoflavones that were analysed in the soy bar, genistin, acetyl-genistin, daidzin and genistein were detected in high quantities, both when the bars were produced and after six months of storage (Table 3). There was also no significant loss of phytoestrogens during storage (Table 3).

Table 3 – Isoflavone content of soy bars during storage

Isoflavones	**mg/100 g**	
	Day 0	**After 6 months**
Daidzin	14.10	14.52
Glycitin	0.00	0.00
Genistin	30.97	31.00
6”-O-Malonyl-Daidzin	5.81	5.31
6”-O-Malonyl-Glycitin	0.00	0.00
6” –O-Malonyl-Genistin	11.05	10.85
6”–O-Acetyl-Daidzin	2.92	2.98
6”-O-Acetyl-Glycitin	0.00	0.00
6”-O-Acetyl-Genistin	16.08	15.98
Daidzein	5.92	5.90
Glycitein	0.00	0.00
Genistein	13.53	12.93
Total	**100.39**	**99.47**

Genistin, which was present in high quantities (30.97 – 31.00 mg/100 g), is a β-glucoside that typically undergoes enzymatic hydrolysis in the small intestine and releases genistein, which is absorbed and prompts biological effects in health (Dixon, 2004). Studies have demonstrated that genistein exhibits a high affinity for oestrogenic receptors (Wober et al., 2002) and has a high amount of activity against several hormone-dependent cancers (Anderson et al., 1999).

The total isoflavone content was 100.39 mg/100 g (Table 3). This result is similar to values in the literature for soy-based foods (Song et al., 1998). The consumption of a single soy bar (30 g) per day would contribute approximately 10 g of soy protein and 30 mg of total isoflavones. According to Setchell (1998), the intake of 25 g of soy protein associated with about 30-50 mg of isoflavones daily

can reduce serum cholesterol. Moreover, it is important to consume isoflavones and soy protein together. Studies have shown that the consumption of purified isoflavones was not as effective for reducing disease risk compared to the consumption of isoflavones with soy protein (Badger et al., 2002).

3.3. Shelf-life Study

Shelf-life can be defined as the length of time that a product can be stored before the appearance of the first characteristic that consumers find unappealing (Loveday et al., 2009). Chemical and physical interactions amog the ingredients of the bar can occur over time and begin to affect the taste and texture of the product. The factors that limit shelf life are chemical reactions or physical modifications. The time from manufacture to consumption of the snack bars may be influenced by lack of the thermodynamic equilibrium that is common in the multicomponent heterogeneous systems of processed foods (Mezzenga, 2007). The thermodynamic incompatibility of certain biopolymers (Tolstoguzov, 2003) and the existence of chemically heterogeneous microenvironments within foods (Kou et al., 2002) can potentially drive physicochemical reactions during storage (Loveday et al., 2010).

3.3.1. Instrumental Texture (Hardness)

The soy snack bars that were formulated in this study had hardness values that ranged from 2.54 N±0.29 when the bar was first manufactured to 10.00 N±1.92 after six months of storage (Figure 1). A linear model for hardness (H) was

used: H = 2.414 + 1.059 x Month, with R= 0.81. Figure 1 shows an increase in the hardness of the soy snack bar over time. An increase in hardness can be very damaging to the sensorial aspects of bars by reducing the chewiness of the bars. Bower & Whitten (2000) reported that the majority of cereal bar consumers consider chewiness a desirable attribute.

According to Loveday et al. (2009), the shelf life of high-protein bars is often limited by the development of a “hard” or “tough” texture that consumers find unpalatable. The mechanisms that produce this texture have not been conclusively indentified, but it is possible that protein cross-linking, aggregation or network formation occurs during storage (Zhou et al., 2008). Baier et al. (2007) reported that bar hardening during storage occurs due to the ordered secondary structures in proteins as well as the low surface hydrophobicity of protein particles. These authors cited moisture migration as a driving force for the hardening of protein bars, but they did not specify the source and destination of the migration. It is also possible that Maillard reactions between reducing sugars and reactive lysine residues play a part in the hardening of protein bars (Loveday et al., 2009).

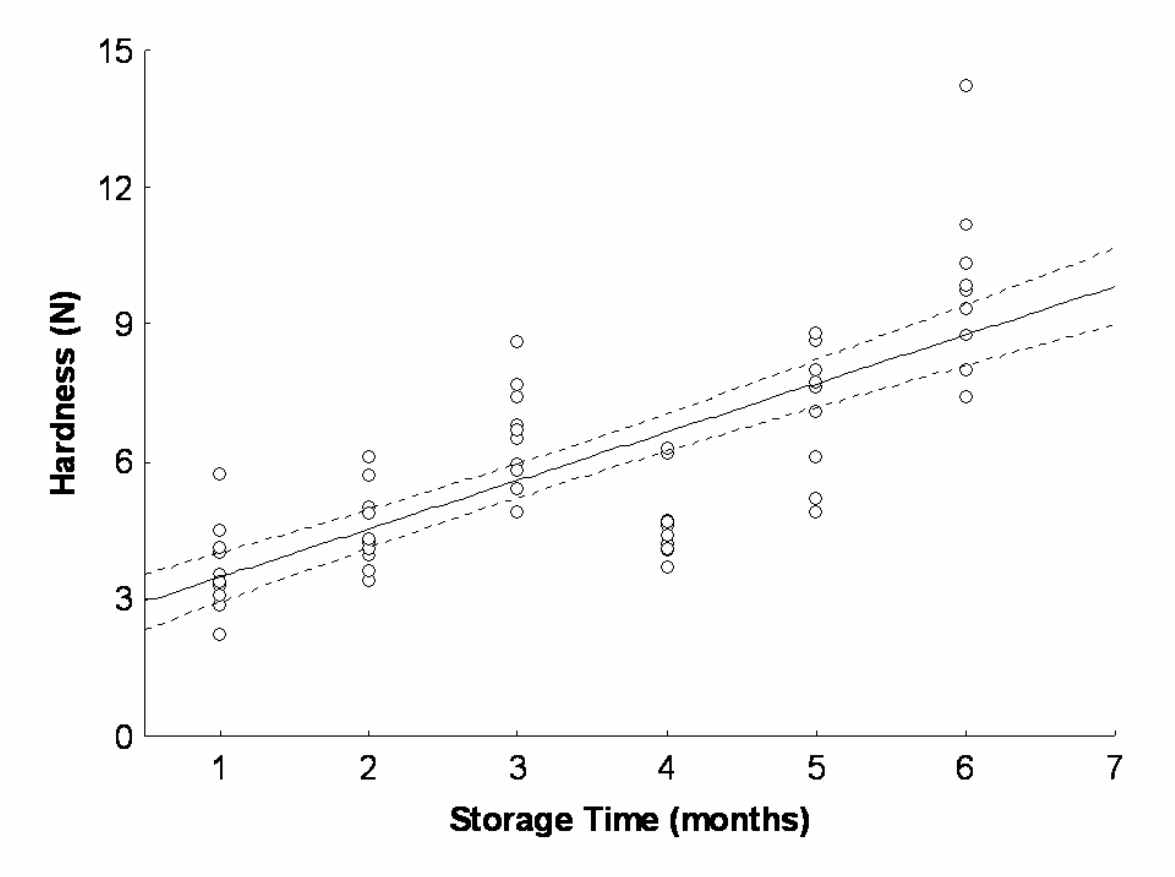

Figure 1 - Hardness of soy snack bars during six months of storage at room temperature (21-25ºC).

3.3.2. Colour

Images of soy snack bars that were taken directly after they were manufactured and during six months of storage are shown in Figure 2. RGB values obtained from the images were converted to CIELAB values to calculate the average colour of the bars. These photos show the various components of the bars, as well as changes in the colour palate and the colour lightness intensity of the samples during storage.

The L*, a* and b* values of soy bars evaluated during the six-month storage period are listed in Table 4. The L* and b* values decreased (from 52.92 to 42.46 and 53.69 to 47.06, respectively; $p<0.05$) and the a* values increased significantly

(from 11.96 to 18.32; $p<0.05$) during storage. This result suggests that the bars become darker, redder and less yellow over time (Figure 2). The colour of soy bars darkened slightly during storage, indicating the onset of final-stage Maillard reactions. In the initial and intermediate stages of this reaction, there is little or no colour change.

Theoretically, product darkening (developing a brown colour) occurs due to the reducing sugars (e.g., liquid honey or glucose syrup) or an increase in the amount of reducing sugars that are available to participate in the Maillard reaction (Yilmaz & Toledo, 2005). The shelf-life of intermediate-moisture foods is often limited by Maillard reactions between carbonyl groups of reducing carbohydrates and the exterior amine groups on proteins. The Maillard reaction can lead to an unappealing texture, flavour, nutritional value and colour of food products (Loveday et al., 2010). Moreover, Maillard reactions occur fastest at high temperatures and at a_w values between 0.65 and 0.75, similar to the a_w values determined for the bars in this study.

Table 4 – L*, a*, b* values for soy snack bars evaluated right after manufacturing and over a six month storage period

	Storage Time (months)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
L*	52.92±3.45^{a,c}	54.48±0.80^{a}	51.85±5.05^{a,c}	49.47±5.54^{a,b}	47.30±4.41^{a,b}	45.31±2.48^{b,c}	42.46±6.35^{b}
a*	11.96±1.86^{a}	11.56±0.96^{a}	10.91±1.67^{a}	12.38±1.70^{a}	12.82±1.45^{a}	11.93±1.97^{a}	18.32±2.25^{b}
b*	53.69±2.40^{a}	54.58±1.39^{a}	54.63±2.54^{a}	50.81±1.52^{a,b}	51.00±2.19^{a,b}	50.65±1.10^{a,b}	47.06±5.37^{b}

* Mean ±SD. Different letters in same line indicates a significant difference ($p<0.05$).

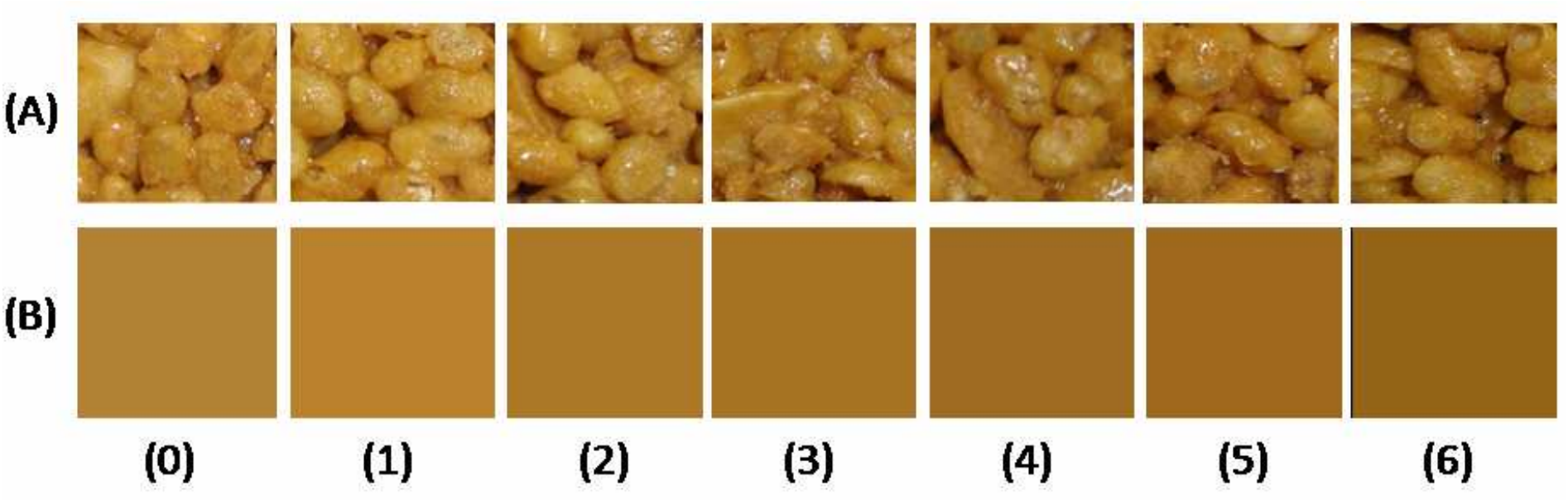

Figure 2 – Pictures of soy bars (A) where the average colour (B) of images captured with a digital camera was converted to a mean CIELAB system to obtain values for L*, a*, b* right after the bars were manufactured (Day 0) and over six months (1 to 6). The numbers below the images refer to months of storage.

3.3.3. Water activity (a_w) and microbiological analysis

Water activity (a_w) measurements help to predict the mechanical properties of foods as well as the stability and shelf-life of foods. The a_w value represents the availability of water in a material to development of chemistry reactions and microbiological modifications. This physical property influences microbial spoilage as well as chemical reactivity and enzymatic activity (Labuza, 2000). Cereal bars are generally formulated to have moisture between 10-15% w/w and a_w less than 0.65 (Loveday et al., 2009). More specifically, the bars are designed to maintain intermediate a_w values between 0.4 and 0.6 and to be stored at room temperature without significant microbial growth. At these a_w values, the products are susceptible to physical transformations such as lipid oxidation and non-enzymatic browning (Maillard reactions).

The a_w can be depressed by removing water from the formulation and/or by adding low molecular weight solutes (Loveday et al., 2009). Moreover, to obtain an intermediate a_w value, it is necessary to consider the ºBrix of th e syrup mix: the higher ºBrix, the harder the bars can be. Ordinarily, Brix in cereal bars is controlled to between 81 and 84ºBrix by heating the syrup from 100 to105ºC. The soy snack bars in this study were initially manufactured by heating syrup to 100ºC and 78ºBrix. The addition of soy protein isolates provided the bars with a dry appearance, so the heating temperature and ºBrix were decreased to minimise this effect, because the usual temperature and Brix were about 105℃ and 84ºBrix, respectively.

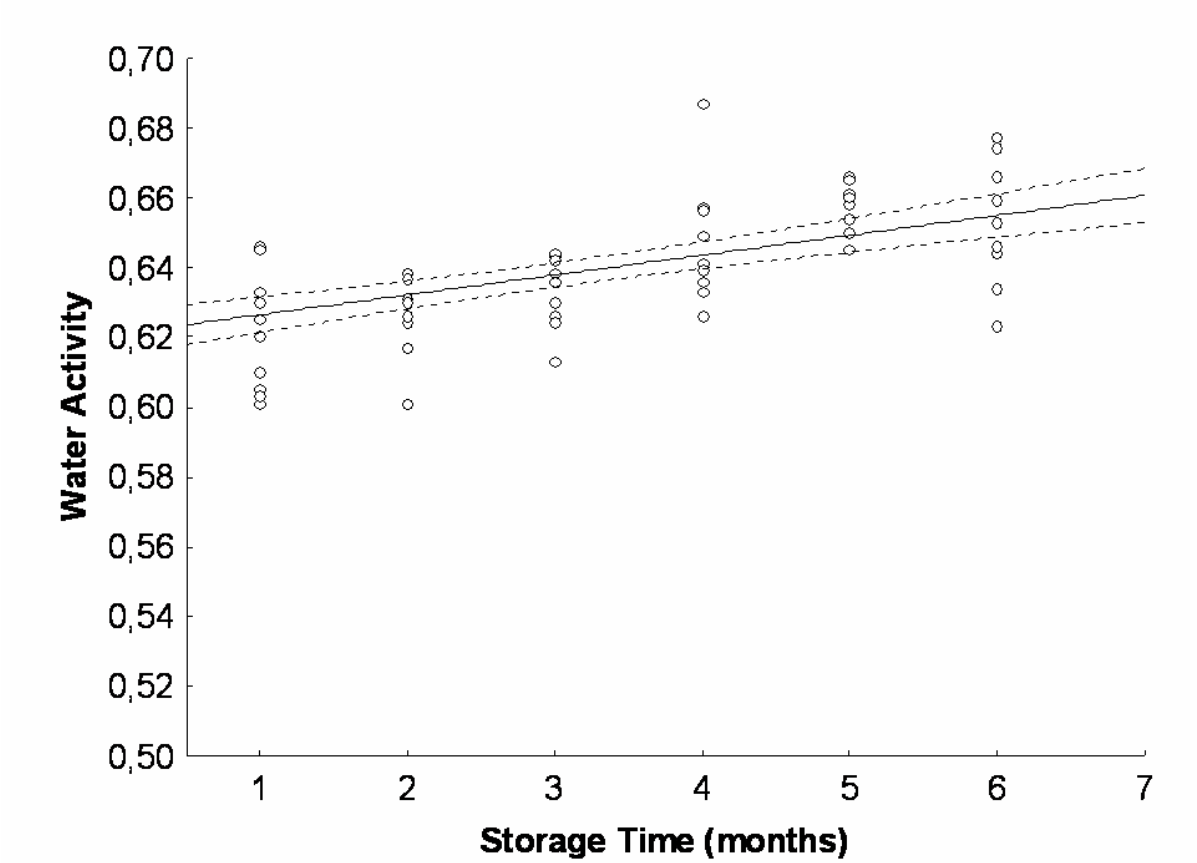

Figure 3 – Water Activity of soy snack bars during six months of storage at room temperature (21-25ºC).

The soy snack bars formulated for this study demonstrated a_w values ranging from 0.629±0.005 after the bars were manufactured to 0.651±0.006 after six months under room temperature (21-25ºC). A linear model was used to determine the water activity: a_w = 0.621 + 0.006 x Month, with R= 0.62 (Figure 3). There was a small increase or only a small oscillation in a_w during storage. This variation can be attributed to changes in humidity (which was not controlled) or to the lack of homogeneity in a multicomponent, heterogeneous product. The changes in a_w that are likely responsible for affecting the mechanical properties of snack bars may be associated with differences in the product's microstructure and chemical composition (Lewicki *et al.*, 2004). Freitas & Moretti (2006) manufactured

and characterised cereal bars with textured soy protein, wheat germ and oats that had a similar final a_w (0.637±0.017).

There was no significant effect of storage on the microbiological contamination of the product (data no shown). Over six months of storage, there were no major changes in any of the microbiological parameters. There were negative results for Coliforms, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella* sp., and there were fewer than 1.0×10^1 yeasts and moulds cells in each sample. The maintenance of a_w could have produced this microbiological stability.

3.4. Sensory Analysis

The mean sensory acceptability values of the soy snack bar were 7.1±1.3 with a 70% approval rating (scores ≥7). Among panellists who gave favorable scores for the bars, 54% indicated that flavour were the most influential attribute. Among panellists who gave unfavorable scores for the bars, 36% indicated that texture and 25% indicated that appearance was the most influential attribute. The fact that flavour was one of the best attributes was promising because a characteristic off-flavour in soy products is undesirable. The appearance and texture of the bars can be attributed to the reduced amount of syrup used to formulate these bars as well as the addition of soy protein isolate, which diminished the humidity of the product.

When panellists were asked if they would purchase this product, 83% of them responded that they would buy the product. When the question was repeated after stating that this product could help reduce the risk of developing diseases

such as cardiovascular disease, 94% of the panellists said that they would buy the product due to their concerns with maintaining and improving their health.

However, according Bower & Whitten (2000), the “healthiness” of the bars may not be as important with respect to consumer purchasing habits; features such as texture, appearance and price have been shown to be more relevant in consumers’ choices to purchase these products. Bars that are chewy, nutty or contain chocolate are generally more popular than bars that do not have these characteristics. The other desirable characteristics of cereal bars include a light colour and a moist appearance (Dutcosky et al., 2006).

A dry appearance, which was considered to be one of the worst attributes of the bars developed in this study, could be improved through modifications to the product formulation. Other ingredients could be used to improve the appearance of the bars. According to Niness (1999), oligofructose can be used as a humectant in a binder system to make the bars softer and more pliable. Brandt (2000) reported that inulin can act as a texture modifier by holding in moisture, which could help to keep the bars fresher for a longer period of time. Moreover, the addition of these soluble fibres or blends may even reduce the sugar and caloric content of the bars (Dutcosky et al., 2006). These ingredients were not included in the present bar formulation because these ingredients may interact with the soy ingredients or the other cholesterol lowering components to reduce their effect on patient lipid profiles, and the objective it was evaluate hipocholesterolemic effect only of soy product.

3.5. Clinical Trial

The bars developed in this study were used in a clinical trial with dyslipidaemic subjects to evaluate the effect of soy consumption on a patients' lipid profiles. In this study, the group that consumed soy snack bars had a diet with no limits in saturated fat or cholesterol ingestion. Moreover, the matrix of the bar was a multicomponent system. Wong et al. (1998) showed that the type of food or food matrix can influence absorption and the physiological effects of a food. The majority of previous clinical studies of soy products used products containing soy flour or soy protein isolate. However, these products are very difficult to incorporate into daily meals.

Snack bars have been used as a food matrix for incorporate health functional ingredients that have been evaluated in clinical trials (Kaufman et al., 1997; Polagruto et al., 2006; O'Neill et al., 2001; Hallund et al., 2006). The use of bars as a food matrix to present functional ingredients has produced good results in adherence to protocol due to the convenience of the bars for the study participants.

Several clinical studies have suggested that soy protein or isoflavones can produce significant reductions in plasma concentrations of CHOL and LDL-C in humans exposed to these compounds. However, a significant number of studies reported have not found beneficial effects of soy on body weight, serum lipid profiles, fat mass, blood glucose and insulin profiles (Cederroth & Nef, 2009).

Table 5 shows that there were no significant differences in glucose, total cholesterol and LDL-C levels after soy snack bars were consumed over a 45-day period. However, compared to patients in the control diet group, patients in the soy

bar group experienced a decrease in serum triglycerides 21% more than control group (Figure 4A), although it has been acknowledged that the higher the baseline TG level, the greater the TG-lowering effect. It was observed TG of baseline in soy group is higher than control group. This difference in baseline values are attributed to the randomization of the subjects was done based in baseline CHOL values. A small increase in HDL-C (+12%), while there was decrease in control group (-11%) (Figure 4B).

The consumption of three soy snack bars per day (about 30 g of protein and 100 mg isoflavone/100 g) decreased triglycerides (TG) by 24% ($p<0.006$) without a low-fat diet (Figure 4A). Clinically, this reduction is significant for reducing heart disease. Comparatively, statins, first line of hipocholesterolemic drug, reduce TG levels about 13% (LaRosa et al., 1999), but these values may be higher depending on the statin administered and TG levels of subjects in baseline.

According to Wang et al. (2004), the decrease in TG is associated with soy protein and not with isoflavones, and suggest that soy proteins reduce plasma TG levels through multiple mechanisms, including the suppression of *de novo* lipogenesis (newly synthesized fatty acids derived from carbohydrate metabolism, which are often used for triglycerides synthesis).

In a meta-analysis (Anderson et al., 1995), soy protein, but not casein, reduced TG (-11%) and LDL-C (-13%) levels, and soy protein supplementation obtained greater reductions of TG and LDL-c than did casein supplementation in subjects higher initial total cholesterol levels. Wang et al. (2004) also observed a reduction (12.4%) in TG when a soy protein diet was compared to an animal protein diet. Overall, most studies have reported no significant effects of soy protein on TG levels.

Table 5 – Baseline anthropometric characteristics, fasting blood lipids, and glucose concentrations of 38 subjects.

	Control (n= 16)			Soy Snack bar (n=22)		
Variables‡	**Baseline**	**After 45 days**	**p†**	**Baseline**	**After 45 days**	**p†**
CHOL (mg/dL)	235.0	218.5	0.028*	247.5	243.0	0.903
LDL-C (mg/dL)	145.0	144.5	0.535	147.5	163.5	0.330
HDL-C (mg/dL)	61.5	54.5	0.017*	54.5	61.0	0.026*
TG (mg/dL)	125.5	121.0	0.061	176.0	134.0	0.002*
Glucose (mg/dL)	85.5	82.0	0.023*	97.0	97.0	0.455
BMI (kg/m^2)	25.0	24.4	0.004*	28.5	28.4	0.178
Waist circ. (cm)§	83.3	85.5	0.638	95.9	96.5	0.653

BMI, body mass index; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides

‡ Values expressed as median; § Waist Circumference; † $p<0.05$; * Significant difference between baseline and after 45 days.

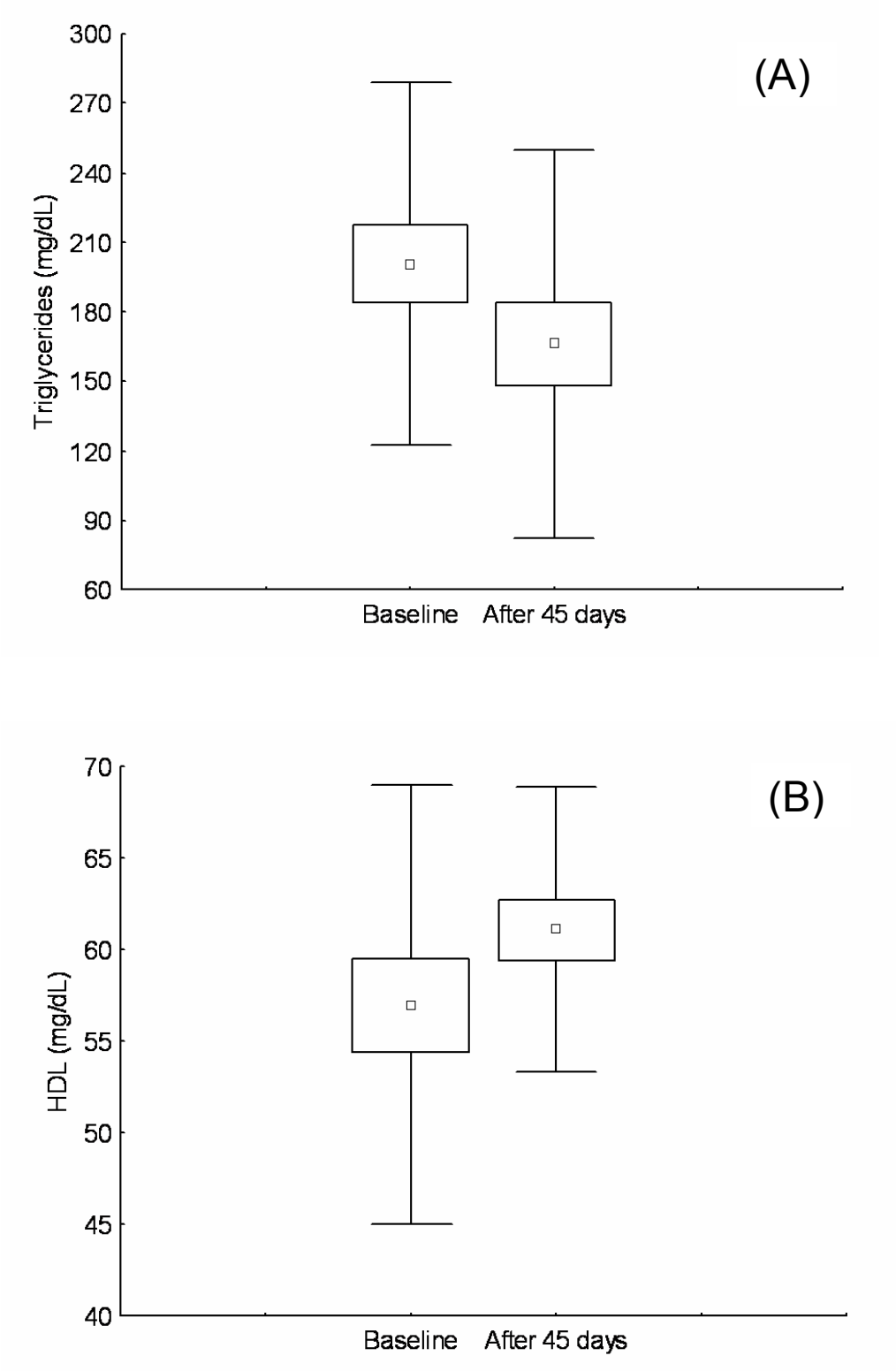

Figure 4 – Representation (Box & Whisker Plots) of profiles of Triglycerides (A) and the HDL (B) at baseline and after 45 days of soy snack bar consumption. □ Mean; I Mean ± SD; □ Mean ± SE.

In the present study, there was an increase in HDL-C (+12%) after the consumption of soy snack bars for 45 days (Figure 4B). In a meta-analysis, Reynolds et al. (2006) showed that the consumption of soy increased HDL-C levels in 25 of the 38 trials that were analysed. However, the increase was statistically significant in only two of these trials. Additionally, Reynolds et al. (2006) found a positive correlation between the amount of soy protein and isoflavones with net changes in HDL-C.

According to Anderson & Konz (2001) decrease in 1% HDL-C level increases 3% cardiologic risk. Scientific evidences indicate that HDL has an antiatherogenic properties and the capacity to promote cholesterol efflux from peripheral tissues by ATP-binding cassette transporter A1 (ABCA1) (Gotto & Brinton, 2004).

The control group in the present study followed a low-fat and low-cholesterol diet. In this group, CHOL (-7%) and glucose (-4%) decreased significantly ($p<0.05$) after 45 days (Table 5). However, there was also a decrease in HDL-C (-11%). The monitoring of the adherence of treatment was performed with a 3 meetings with the dieticians during the study in both experimental groups.

Body weight and body mass index (BMI) of the subjects that consumed soy snack bars remained relatively constant, and no significant changes were observed during the course of the study. The BMI of the subjects that followed the low-fat diet decreased by 3%. There were no observed changes in either group for waist circumference.

4. Conclusions

Elevated plasma cholesterol has been implicated as a primary risk factor for cardiovascular heart disease. As a result, several strategies have been investigated for controlling dyslipidaemia without medications. The development of functional foods could expand the options for controlling dyslipidaemia and for providing health benefits. The soy snack bars manufactured in this study were well accepted in sensory analysis. However, during storage, the bars developed a dry appearance and increased hardness. Additional modifications to the bar formulation could prevent these problems. In the clinical trial, consuming three soy snack bars a day, or about 30 g of protein and 100 mg/100 g of isoflavones, decreased TG (-24%) and prompted a small increase in HDL-C (+12%). We conclude that relatively high intakes of soy protein and isoflavones in moderately hypercholesterolaemic subjects (>200 mg/dL) may have a moderately beneficial effect on plasma lipid profile without additional diet restrictions.

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Artigo 3

“Combination of Soy And Oat Components In Diets for Dyslipidemic Subjects”

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Combination of Soy And Oat Components In Diets for Dyslipidemic Subjects

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Abstract

Objective: To evaluate the possibility of interaction between soy and oat components on lipid profile of the dyslipidemic subjects without following a diet low in fat and cholesterol. **Methods:** Seventy nine dyslipidemic subjects were randomized to one of four experimental groups (oat, soy, soy+oat, and control). Oat group consumed about 30g of the oat bran/d, soy group consumed three soy snack bars/d, containing about 30g of protein, soy+oat group ingested oat bran and soy bars/d in same amount asother groups, and thecontrol group followed a hypocholesterolemic diet. Evaluations were performed at baseline and after 45 days. The biochemical variables evaluated were CHOL (total cholesterol), LDL-C (Low density lipoprotein cholesterol), HDL-C (High density lipoprotein cholesterol), TG (Triglycerides), and glucose. Anthropometrics measures (weight and waist circumference) were performed to calculate the BMI (body mass index) and cardiologic risks. **Results:** After forty five days of consumption of oat bran decreased the CHOL, as well as, when soy bars were associated to oat bran and in the control group. All the experimental groups presented decrease in triglycerides, being less significant in the control group. Moreover, the association of oat and soy diminish the LDL-C. Subjects that follow a hypocholesterolemic diet present decrease in HDL-C while consumption of soy snack bars demonstrated to increase this parameter. **Conclusion:** Combination of the β-glucans and soy protein/isoflavones is effective in decrease the CHOL, LDL-C and TG in hypercholesterolemic subjects without had a hypocholesterolemic and low-fat diet.

Keywords: soluble fibers, β-glucans, vegetable protein, isoflavones, cholesterol

Introduction

Coronary heart disease (CHD) is a major health problem in several developed and emergent countries and many studies have shown that elevated concentrations of total or LDL-C in the blood are powerful risk factors for CHD (Law, 1999) whereas high concentrations of HDL-C or a low LDL-C (or total) to HDL-C ratio may protect against CHD (Castelli et al., 1992).

It is well established that reduction of saturated fat and cholesterol intake decrease the risk for cardiovascular disease. However, only diet has been considered by some to be ineffective in the management of hypercholesterolemia (Ramsay, Yeo, Jackson, 1991; Katan, Grundy, Willett, 1997). Usually dietary intervention to control excess body weight, hyperglycemia and dyslipidemia has included low energy and low fat diets, but these are of limited efficacy due to the strict and long-term commitment required (Cederroth & Nef, 2009). Most people have difficulty to follow a restrict diet due to busy lifestyles and the wealth of appeals of fast food, tasty and no healthy foods. However, long term health benefits can be gained from bioactive nutrients or non-nutrients found in foods.

There are several pharmacological and non-pharmacological strategies to diminish cholesterol. Among non-pharmacological possibilities there are the bioactive compounds which are “extra-nutritional” constituents that typically ocurr naturally in small quantities in plant products and lipid-rich foods (Kitts, 1994). In turn, the US Food and Drug Administration permits health claims for coronary heart disease (CHD) risk reduction, based on cholesterol lowering, for foods delivering adequate amounts of plant sterols (FDA, 2000), viscous fibers (oat β-glucan and psyllium) (FDA, 1998; FDA, 2001), and soy protein (FDA, 1999).

Many studies have demonstrated that intake of some bioactive compounds and the interactions between them together with the reduction of the saturated fat and cholesterol intake can decrease the total cholesterol and subfractions. However, there are fewer studies demonstrating the effects of these foods in cholesterolemia without the reduction of fats consume. Soluble fiber and soy protein have both been shown independently to decrease serum cholesterol. There are several proposals of action mechanisms of both bioactive compounds and still there is no scientific consensus of exact way that these compounds act to reduce serum cholesterol and subfractions.

One of these postulated that soluble fibers may increase the binding of bile acids in the intestinal lumen, which leads to a decreased enterohepatic circulation of the bile acids and a subsequent increase in the hepatic conversion of the cholesterol to bile acids (Glore et al., 1994; Bell et al., 1999). Another suggested mechanism is that the increased viscosity of the food mass in the

small intestine promoted by soluble fibers leads to the formation of a thick unstirred water layer, adjacent to the mucosa. This layer may act as a physical barrier to reduce the absorption of nutrients and bile acids (Beer et al., 1995).

The mechanism by which soy decreases serum lipids has not been defined, but may relate to the isoflavonoids associated with soy protein (Anderson, Johnstone, Cook-Newell, 1995) or the amino acid composition of soy (Kritchevsky, Tepper, Klurfeld, 1987; Carroll, 1991). Increased fecal bile acid losses have been proposed as part of the reason for the cholesterol decrease and have been noted in human studies, although few have assessed fecal bile acid losses (Jenkins et al., 1999). Others possible mechanisms of the hypocholesterolemic effect of soy protein include increases in LDL receptor activity, increases in the synthesis and fecal excretion of bile acids, and a suppression of cholesterol absorption (Kerckhoffs et al., 2002).

There are some studies in literature that investigate the association of soy protein and soluble fiber on lipid profile (Jenkins et al., 1999; Van Horn et al., 2001; Torres et al., 2009; Guevara-Cruz et al., 2010). Jenkins et al. (1999) investigated the effect of combination of soy protein and soluble fiber added to a standard cholesterol-lowering diet recommended by The National Cholesterol Education Program (NCEP). They related this combination appears to be additive in reducing LDL-C and has a further benefit in reducing the LDL-C:HDL-C ratio.

Both soy protein and soluble fibers seem to act on the intestinal and hepatic sites. In this case, their effects can be additional or opposite. Moreover, there are several chances of mechanisms for both compounds that can act together. The proposed mechanisms of action can be different, involving increased bile acid losses for viscous fiber (Kritchevsky & Story, 1974; Anderson et al., 1984; Jenkins et al., 1993) and reduced hepatic cholesterol synthesis and increased LDL receptor mediated cholesterol uptake for soy proteins (Kurowska & Carroll, 1992; Baum et al., 1998). In view of the differences in possible mechanisms of action it was assumed that their effects were likely to be additive or opposite the main of this study was to evaluate if cholesterol and subfractions reduction could be allowed by including a combination of soluble fibers and soy proteins in the diet without reduction in their saturated fat and cholesterol normal intake.

Materials and Methods

Subjects or casuistic

Seventy nine hypercholesterolemic patients, 30 male and 49 female, with a mean age of 45.3 ± 11.5 y were selected at a cardiologic clinic in Londrina, Paraná, Brazil to participate in the study. The patients were sedentary or had moderate physical activity and were oriented to make no changes in their habitual lifestyle. This was monitored by means of a questionnaire that was filled out every time the patients returned to the clinic for blood collection. Body weight was measured to the nearest 0.1 kg by using an electronic scale, with individuals wearing minimal clothing; height was measured to the nearest 0.1 cm by using a stadiometer and was used to calculate body mass index (BMI; kilograms per square meter). Subjects in each comparison consumed similar dietary fat. The inclusion criteria were patients ≥18 y old, with total cholesterol plasma levels ≥200 mg/dL. Individuals who presented diabetes mellitus, hypothyroidism, chronic diseases, or other causes of secondary dyslipidemia were excluded from the study. Women receiving hormone replacement therapy and patients who were using hypolipemic medication, isoflavones, soy, and/or oat for ≤30 d before entry into the protocol were also excluded. No modification occurred in the treatment of patients receiving hypertensive medication. All participants were informed regarding the procedures they would undergo and signed a term of free informed consent. This study and the term were approved by the ethics in human research committee of the Londrina University Hospital (nº99/09).

Study design: randomized controlled trial

Patients were randomized to one of four groups (oat, soy, soy/oat, and control) by nutritionists indicating treatment allocation. After orientation by a nutritionist, participants used the products offered for 45 d. The patients of the soy group (*n* = 22) ingested three soy snack bars a day their daily meals: breakfast, lunch, and dinner. The oat group (*n* = 20) ingested 30 g of oat bran

throughout the day. The soy/oat group (*n* = 21) ingested the same as the soy and oat groups. The batches and procedures of the products used by these groups were the same. The control group (*n* = 16) received a standard hypocholesterolemic diet low in saturated fat and cholesterol contents. All groups were evaluated by a blind assessor on two occasions at baseline, and after 45 d. There was an intermediate meeting nutritionist after 25 d to monitor the follow-up protocol for patients.

Preparation of soy snack bar and oat bran

The soy snack bar was developed and manufactured specially for this clinical study. Different soy ingredients were combined to reach soy protein and isoflavone amounts sufficient to induce to health benefits with three bars a day as described in scientific researches (Cederroth & Nef, 2009) and food organizations (FDA, 1999) as around 25 g protein and 80-100 mg isoflavones. More details of soy bar manufactured are found in our previous study Lobato et al. (2011). Each produced soy bars contained 28g each with 34.25% of protein and 100.39 mg/100g of isoflavones.

The oat bran was provided by SL Alimentos (Mauá da Serra, Brazil) and packed in individual aluminum bags containing 30 g of oat bran. The subjects consumed one bag of this bran a day during the study.

Chemical Composition and Caloric Values

Proximate composition and caloric values of oat bran and soy snack bar were performed. Moisture (air oven at 105ºC), fat (Soxhlet), ash (muffle 550ºC), protein (Kjeldahl) and dietary fiber (total, soluble, and insoluble) (enzymatic method), were analyzed by official AOAC methods (2000). The nitrogen-protein conversion factor used to calculate total protein content was 6.25, whereas the carbohydrates were obtained by difference. The β-glucans of oat bran were analyzed by method 995.16 (AOAC, 2005). The extraction of isoflavones was realized according to methodology by Carrão-Panizzi et al. (2002), separation and quantification was performed according to Berhow (2002) using a liquid chromatography (model 2690, Waters & Associates, Milford, MA, USA), with

ODS-3 C_{18} reversed-phase HPLC column (250mm×0.4 mm internal diameter). For the separation of isoflavones was adopted the system of linear binary gradient having as mobile phases: methanol containing 0.025% trifluoroacetic acid (TFA) (solvent A) and 2) ultrapure deionized distilled water containing 0.025% TFA (solvent B). The caloric value was calculated applying calorie conversion factors: Caloric value (Kcal/100g) = (% protein x 4) + (% crude fat x 9) + (% carbohydrate x 4).

Blood collection

Fasting blood samples were drawn after a 12-h overnight fast at baseline and after 45 d of intervention. Approximately 20 mL of blood was collected in vacuum tubes without anticoagulant for lipid profile and with sodium fluorite for glucose analyses. Immediately after collection, the samples were chilled on ice. To obtain the plasma, the tubes were centrifuged at 3000 x g and refrigerated centrifuge at 4℃.

Measurements of biochemical variables

Biochemical quantifications were done in a biochemical autoanalyzer (Dade AR, Newark, NJ, USA) using Dade Behring kits. Total cholesterol was quantified by the cholesterol oxidase technique. Whenever possible, LDL-C was calculated by the Friedewald equation (Friedewald, Levy, Fredrickson, 1972). HDL-C was quantified by the selective precipitation method using a buffered phosphotungstate reagent. Triacylglycerols were analyzed by means of the bichromatic enzymatic technique, using lipase and glycerol dehydrogenase. Analysis of plasma glucose was carried out using the glucose oxidase automatic GOD Trinder colorimetric assay (Glicose PAP Liquiform).

Food Record

The nutrient composition of the diets was calculated from dietary records (questionnaires) applied to the subjects in baseline and after 45 d. Food records were reviewed by nutritionists for completeness. Nutrient data analyses

were conducted using Nutrilife Software professional version (2007). The reference values were based in recommendations of US National Academy Press and Food and Nutrition Board.

Statistical analysis

Food records data were subjected to analysis of variance (ANOVA) to assess the significance ($p<0.05$) followed by Tukey's test. Data analysis was carried out using the Statistica program version 7.1 (Statistica, 2006). In clinical trial data sets were compared using the non-parametric Wilcoxon matched pairs signed ranks test to dependent samples and Kruskal–Wallis to independent samples, both $p<0.05$, using Statistica 7.1. The data were presented in median.

Results

Composition of soy bars, oat bran and caloric values

According to composition showed in Table 1 the individuals that consumed oat bran portions of 30 g a day ingested 3.12 g of this soluble fiber and 8.43 g of TDF daily. According to FDA (1998) consumption of the soluble fiber from foods such as oat bran, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. It is necessary to ingest at least 3 g or 0.75g by portion of soluble fiber per day to have this effect.

The results of soy snack bars composition showed 34.25 of protein, 39.88 of dietary fiber, 100.39 mg of isoflavones and the caloric value was 245.57 Kcal in 100 g of bars (Table1). The individuals from groups soy and soy+oat consumed 3 soy bars (28 g each) a day during the study. Thus, they ingested 84.33 mg of isoflavone and 28.77 g of protein a day. In a previous work (Lobato et al., 2011) it was shown the soy bars manufactured and the composition of isoflavones on this product: genistin (31.0 mg/100g), acetyl-genistin (16.0 mg/100g), daidzin (14.5 mg/100g) and genistein (13.0 mg/100g), and total of isoflavones in this soy product was 100.39 mg/100g.

Table 1 – Proximate composition in dry basis (d.b.) of soy snack bar and oat bran, total isoflavones and β-glucans concentration.

Analyzes	Soy snack bar	Oat Bran
Carbohydrate (%)	14.30	29.55
Dietary fiber (%)	39.88	28.09
Insoluble Fiber (%)	35.56	18.06
Soluble Fiber (%)	4.32	10.03
Lipids (%)	8.82	8.92
Proteins (N x 6,25) (%)	34.25	29.62
Ash (%)	2.75	3.82
Total	**100**	**100**
Caloric Value (Kcal/100g)	245.57	292.26
Isoflavones (mg/100g)	100.39	---
β-glucans (g/100g)	---	10.40

Mean ages of subjects that participate of the study was 38.7 ± 12.8 in control group (5 men and 11 women), 48.9 ± 7.9 in soy group (12 men and 10 women), 48.5 ± 13.2 in oat group (5 men and 15 women), and 45.2 ± 11.9 in soy + oat group (8 men and 13 women). In Table 2 are shown eating habits and lifestyles of subjects. There were few subjects with pos graduation participating in the study. Subjects from all groups demonstrated moderate practice of physical activity and were instructed to maintain it. The majority of subjects reported no smoking in all groups, any hypertension or controlled hypertension with medicament, with most hypertensive in group soy + oat (74%) and fewer in the control group (10%).

Eating habits were record related with soy, oat and their derivates, and cereal bars consumption. Soy products were reported being daily and consumed little in all groups. Besides, oat products were more consumed daily and sometimes. Cereal bars were consumed little in all groups. Little

consumption of soy products was expected due the majority of subjects being of Western origin (data no show) clearly not having the habit of consuming soy. It was expected more cereal bar consumption because of practice and convenience, but it was not observed in this population, probably due to less schooling, emphasizing other types of food. This fact also can be applied to soy ant oat consumption. Moreover, these products and derivates can be difficult to be insert in appropriate amounts to actual physiological effects in everyday people.

Table 2 – Eating habits and lifestyles of study subjects

	Control (n=16)	Oat (n=20)	Soy (n=22)	Soy+Oat (n=21)
Age (mean±SD)	38.7±12.8	48.5±13.2	48.9±7.9	45.2±11.9
Gender				
Men	5	5	12	8
Women	11	15	10	13
Physical Activity (%)				
Yes	52	64	52	52
No	48	36	48	48
Smoking (%)				
Yes	0	0	4	0
No	100	100	96	100
Hypertension (%)				
Yes	10	41	40	74
No	90	59	60	26
Soy products consumption (%)				
No	32	55	64	48
Sometimes	58	27	12	44
Daily	10	18	24	8
Oat products consumption (%)				
No	48	41	76	48
Sometimes	26	18	24	17
Daily	26	41	0	35
Cereal bars consumption (%)				
No	47	68	68	44
Sometimes	53	23	32	52
Daily	0	9	0	4

After 45 days treatment with oat there was a significant decrease in total CHOL (p=0.026) and a tendency in diminish the TG (p=0.062). The patients that consumed soy snack bars had a decrease in TG (p=0.026) and an increase in HDL-C (p=0.002). In that group of individuals that consumed soy and oat there were a decrease, statistically significant, in CHOL (p=0.004), in LDL-C (p=0.045), and in TG (p=0.024). In control group, which subjects follow a hypocholesterolemic diet, significant decreases in CHOL (p=0.028), HDL-C (p=0.017), glucose (p=0.023), BMI (p=0.004), and a tendency in TG (p=0.061) was observed (Table 3, Figures 1, 2, and 3).

Due to the no controlled diet during the study, allowing the subjects to eat a great range of foods, the variances were high and most of macronutrients did not showed differences in intake by day, when ingest in baseline and after forty five days were compared (Table 4). Yet, significant differences were observed in protein, fiber and monounsaturated fat intake.

In all experimental groups it was observed a tendency to increase fiber intake. In groups of that consumed of soy bar the ingestion of fiber after 45 days was higher and statistically different from control and oat groups. As presented before, the content of fiber in bars was very high (39.88g/100g). Besides, the raise in intake was attributed also to the fact the snack bars are a conventional food that resembles confectionary products, so it was not a substitute for other foods usually consumed, being added between meals. Oat bran has also high fiber content, but it is less convenient than soy bars and has to be included in yogurts, milk, and juices or as ingredient of meals. The increase in fiber intake in the oat group was 25% and in control group, 50%. Only soy+oat group there was expressive tendency to decrease in cholesterol ingest, 210.0 to 117.0 mg, reaching the recommended levels of daily intake (<200mg).

Table 3 – Blood biomarkers and anthropometric variables of the experimental groups‡

Variables	Control (n= 16)			Oat (n= 20)			Soy (n= 22)			Oat + Soy (n= 21)		
	Baseline	After 45 d	p†	Baseline	After 45 d	p†	Baseline	After 45 d	p†	Baseline	After 45 d	p†
CHOL (mg/dL)	235.0	218.5	0.028*	245.0	229.5	0.026*	247.5	243.0	0.903	232.5	227.0	0.004*
LDL-C (mg/dL)	145.0	144.5	0.535	149.0	145.0	0.108	147.5	163.5	0.330	149.0	140.0	0.045*
HDL-C (mg/dL)	61.5	54.5	0.017*	62.0	61.5	0.868	54.5	61.0	0.026*	54.5	55.0	0.888
TG (mg/dL)	125.5	121.0	0.061	158.5	112.0	0.062	176.0	134.0	0.002*	164.5	146.0	0.024*
Glucose (mg/dL)	85.5	82.0	0.023*	88.0	87.0	0.557	97.0	97.0	0.455	90.5	87.5	0.641
BMI (kg/m^2)	25.0	24.4	0.004*	24.5	24.7	0.681	28.5	28.4	0.178	25.3	25.0	0.571
Waist circumference (cm)	83.3	85.5	0.638	87.0	85.3	0.642	95.9	96.5	0.653	91.5	91.0	0.975

BMI, body mass index; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol, TG, triacylglycerol.

‡ Values expressed as median, †p<0,05; *Significant difference between baseline and after 45 days.

Table 4 – Calculated Macronutrient Intakes in baseline and after forty five days of study.

	Recommendation*	Control		Oat		Soy		Soy + Oat	
		Baseline	**After 45 d**	**Baseline**	**After 45 d**	**Baseline**	**After 45 d**	**Baseline**	**After 45 d**
Protein (g)	46-56g	69.5 ± 46.1	70.0 ± 45.3 [b,c]	59.1 ± 37.3	65.4 ± 21.4 [b,c]	69.1 ± 34.4	101.9 ± 46.5 [a]	68.9 ± 41.9	90.4 ± 36.1 [a,c]
Carbohydrate (g)	130g	208.4 ± 81.2	182.9 ± 78.1	182.8 ± 64.2	188.0 ± 72.0	197.6 ± 82.3	200.4 ± 73.7	199.4 ± 83.8	212.6 ± 107.1
Total lipids (g)	20-35g	56.8 ± 30.9	49.6 ± 37.0	37.4 ± 14.2	44.3 ± 19.1	51.4 ± 28.4	52.2 ± 22.6	55.3 ± 40.2	53.2 ± 23.3
Cholesterol (mg)	<200mg/d	158.9 ± 139.2	162.3 ± 141.3	151.0 ± 138.7	153.4 ± 109.4	221.8 ± 140.3	193.3 ± 147.9	210.0 ± 162.1	117.0 ± 76.6
Saturated (g)	<7%/d	15.7 ± 12.3	16.0 ± 15.0	9.5 ± 6.0	12.4 ± 6.6	15.1 ± 12.3	11.8 ± 7.3	16.1 ± 14.6	10.8 ± 5.0
Polyunsaturated (g)	<10%/d	9.3 ± 5.8	6.1 ± 4.3	5.3 ± 3.2	6.6 ± 4.5	8.7 ± 6.4	7.9 ± 3.1	7.3 ± 6.4	7.5 ± 3.11
Monounsaturated (g)	10-20%/d	14.2 ± 11.0	10.7 ± 10.1 [c]	9.0 ± 5.7	12.2 ± 7.0 [b]	15.0 ± 11.9	17.2 ± 8.5 [a,b]	15.9 ± 13.8	10.9 ± 6.9 [c]
Fiber (g)	20-30g/d	10.7 ± 5.9	16.1 ± 11.1 [c]	12.4 ± 5.2	15.6 ± 7.3 [c]	10.0 ± 5.9	47.0 ± 7.0 [b]	11.3 ± 6.5	61.2 ± 23.1 [a]
Kcal	± 1500.0	1623.1 ± 707.1	1414.7 ± 459.4	1304.6 ± 389.4	1416.2 ± 354.8	1529.2 ± 628.7	1598.0 ± 477.0	1498.1 ± 509.2	1692.0 ± 723.5

Values are expressed in mean ± SD. ***** Recommendation by day (US National Academy Press and Food and Nutrition Board). These values of the reference vary by sex and age. Different letters in same line indicates significant difference ($p<0,05$).

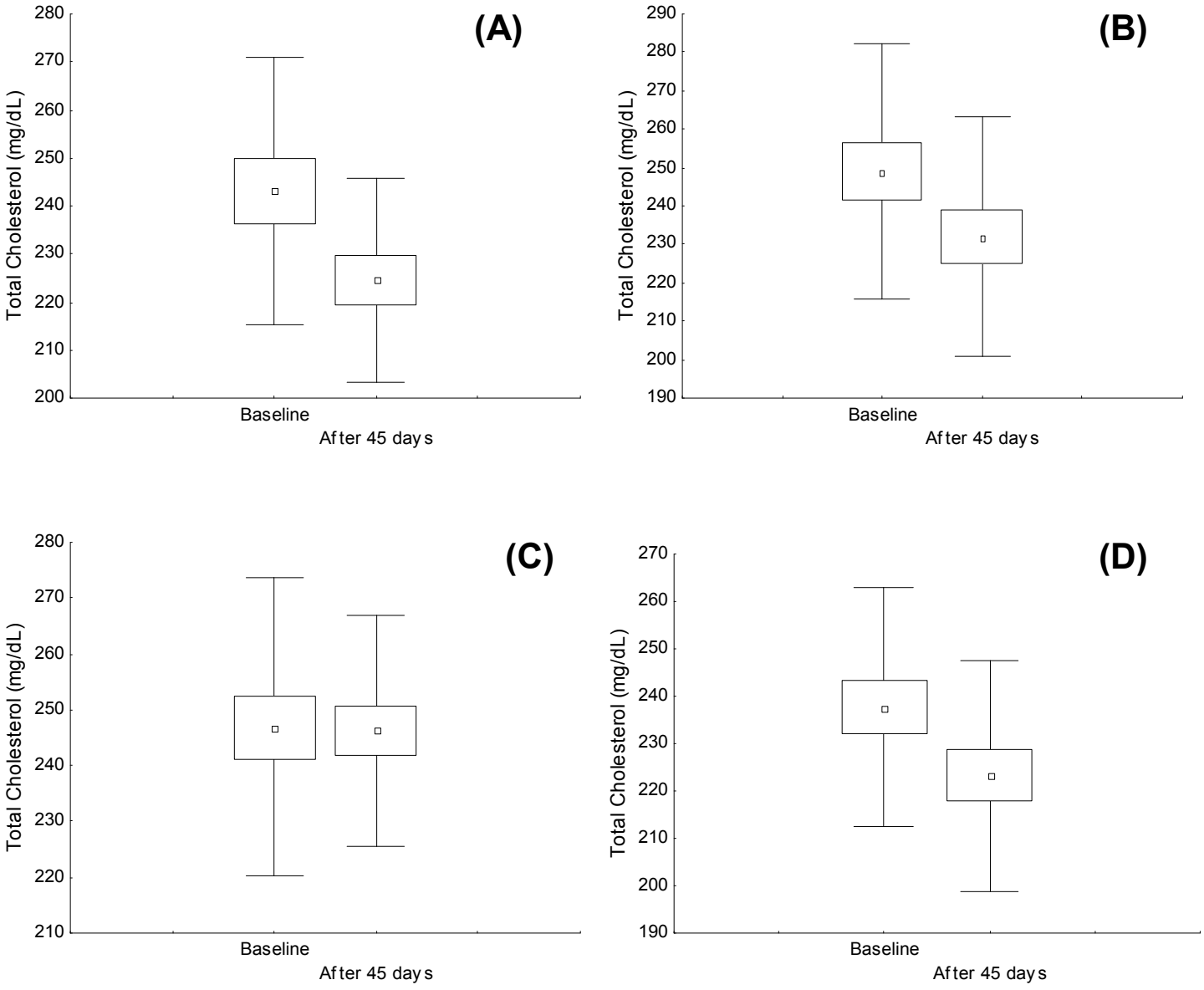

Figure 1 – Representations (Box & Whisker Plots) of profile of the Total Cholesterol of Control (A), Oat (B) Soy (C) Soy + Oat (D) in baseline and after 45 days. □ Mean; I Mean ± SD; □ Mean ± SE.

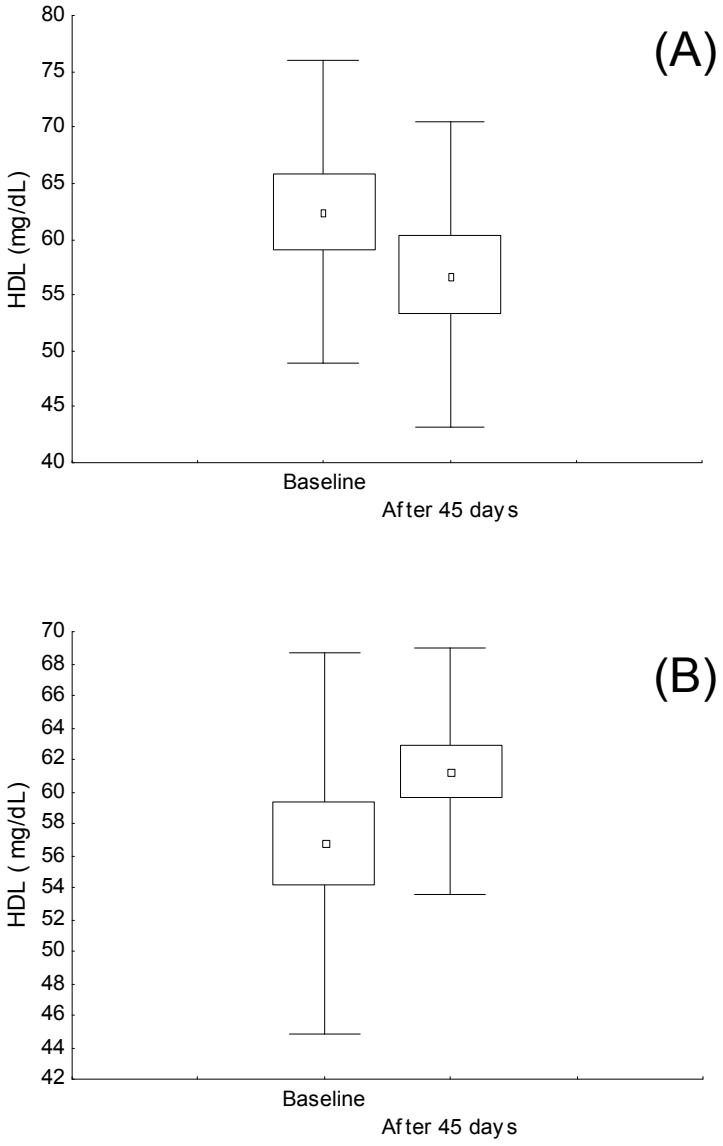

Figure 2 – Representations (Box & Whisker Plots) of profile of the HDL of Control (A), Soy (B) in baseline and after 45 days. □ Mean; I Mean ± SD; ☐ Mean ± SE.

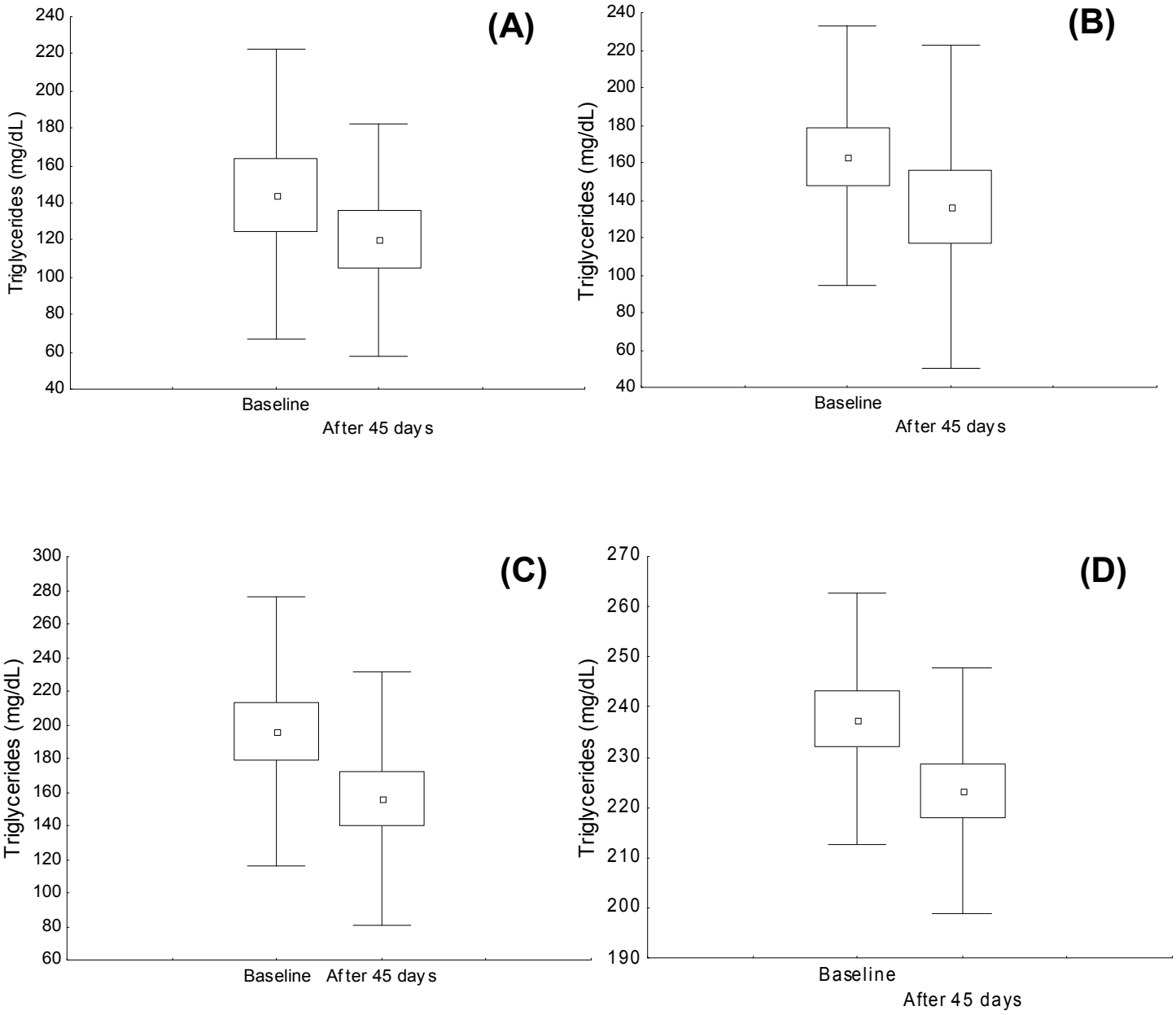

Figure 3 – Representations (Box & Whisker Plots) of profile of the Triglycerides of Control (A), Oat (B) Soy (C) Soy + Oat (D) in baseline and after 45 days. □ Mean; I Mean ± SD; ☐ Mean ± SE.

Discussion

Health disorders as obesity, diabetes, cardiovascular diseases, high blood pressure, dyslipidemia have recently become a major health problem reaching pandemic proportions (Engelgau et al., 2004). Although the source of metabolic disorders is often the diet itself, nutrition can also form part of solution, in fact providing health benefits. Dietary intervention to reverse these health events are limited efficacy due to the strict and long-term commitment required (Cederroth & Nef, 2009).

The increases in levels of plasma cholesterol, particularly LDL-C, are associated with high risk of atherosclerotic diseases. Correlation between cholesterol lowering and mortality and positive influence in lowering cholesterol reduction on the progression of cardiovascular disease (CVD) are well established. According to Anderson and Konz (2001) changes in lipid profile are associated with changes in the risk of cardiovascular disease in the following proportions: +1% CHOL, +2-3% risk; +1% LDL-C +1.2-2.0% risk; -1%HDL-C, 3% risk, while that for TG, the risk is not well appreciated. Scientific evidences indicate that HDL has an antiatherogenic properties and the capacity to promote cholesterol efflux from peripheral tissues by ATP-binding cassette transporter A1 (ABCA1) (Gotto & Brinton, 2004).

In our study it was observed an increase in HDL-C (+12%) (Figure 2B) when the subjects of the soy group consumed about 28g/d of protein and 84 mg/d of isoflavone during 45 d , but no changes in HDL-C were observed when the same ingestion of soy components was associated with oat bran (soy + oat group) (Table 2). Potter et al. (1998) showed that an intake of 40 g/d of isolated soy protein associated with 90 mg of isoflavones for 6 mo decreased LDL-C and increased HDL-C concentrations in hypercholesterolemic post-menopausal women. However, Kerckhoffs et al. (2002) observed the soy protein diets had no effect on HDL-C concentrations, whereas TG concentrations tended to decrease, especially in subjects with hypertrigliceridemia. The most of literature data did not register significant effects of soy and derivates on HDL-C.

Unlike these of soy group, the subjects that followed a diet (control group) demonstrated a decrease in HDL-C. There are studies that showed reduction in HDL-C when the subjects had a diet based in carbohydrates, with or not low-fat, usually associated with the increase in TG (Katan et al., 1997; Katan, 1998). Food records (Table 3) show not diets with high consumption of carbohydrate.

In meta-analysis study, Anderson et al. (1995) verified that soy protein reduced mean TG (-11%) and LDL-C (-13%) levels when compared to casein, with the greater reduction achieved in subjects with higher initial total cholesterol levels.

In our study it was observed a decrease in triglycerides (-24%) (Figure 3C). The highest decrease can be explained by higher consumption of carbohydrate and lipids in this work than other trial designs with diet low-fat restrict. Wang et al. (2004) observed reduction in TG (12,4%) comparing with the animal protein diet. Moreover, the authors say that in humans, most work has reported no significant effect of soy protein on TG levels, although reductions and increases have been identified. Cheng et al (2004) revealed that during fasting both glucose and insulin levels were significantly reduced by soy isoflavones (100 mg/day). In our study, consumption of soy (soy group) had not effects on glucose levels, BMI, and waist circumference (Table 3) and on CHOL (Figure 1C). It was observed only a tendency to increase the LDL-C.

Food organizations have recommended consumption of around 25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, as a way to reduce the risk of heart disease (FDA, 1999). There are no regulated recommendations for consumption of isoflavones. However, there are suggestions in scientific researches that to ingest daily of around 30-50 mg isoflavones associated with soy protein can reduce serum cholesterol (Setchell, 1998).

The effect of 3 soy bar a day consumption on TG and HDL-C may have been also influenced by the isoflavone intake, but it cannot be affirmed because the separate effects of soy protein and isoflavone was not evaluated in this research. The fact of isoflavones has been shown to exert estrogenic effects raises the possibility that this phytochemical may affected glucose and lipid metabolism. In fact, estradiol itself is a well known modulator of glucose homeostasis, which also affects

obesity development (Cederroth & Nef, 2009). Factors that might influence isoflavone bioavailability include intestinal microflora, food matrix, the administered dose, intestinal transit time and the chemical composition of the dietary isoflavones (Cederroth & Nef, 2009).

In contrast to the above mentioned trials, a significant number of studies reported an absence of beneficial effects of soy on classical metabolic parameters such as body weight, serum lipid profiles, fat mass, blood glucose and insulin profiles (Anderson and Hoie, 2005, Li et al., 2005; Hall et al., 2006, Ikeda et al., 2006; Anderson et al., 2007; Santana et al., 2008). Clearly, the effects of soy protein on blood cholesterol concentrations in humans have been variable and the explanation for this remain elusive. Variations in the age and genetics of human study subjects (normocholesterolemic, familial hypercholesterolemic), the capacity of individuals to produce equol, study design (free-living, metabolic ward), fatty acid profile and cholesterol content of the soy protein-based diet, nonprotein constituents in the diet (e.g., fiber or plant sterols), length of study period, amount of soy protein consumed (in absolute terms and relative to animal protein) and type of soy preparation likely all contribute to the range of responses reported, presence of variable amounts of biologically active components in soy preparations, such as isoflavones, which have an independent effect on blood cholesterol concentrations (Lichtenstein, 1998; Cederroth & Nef, 2009).

Foods containing soluble fiber have been shown to be efficacious in the lowering of CHOL and LDL-C levels (Brown et al., 1999; Schneeman, 1999). Oat bran and other products containing β-glucans, between that have been shown to lower LDL-C by 5% to 18% (Karmally et al., 2005), and serum CHOL from 13% to 26% (Anderson et al, 1995). According to Theuwissen & Mensink (2008) although many of these metabolic ward studies showed impressive lipid reductions, trials with free-living subjects reported considerably more variability in lipid responses. Usually, the studies evaluated the effect of soluble fibers in patients that follow a low-fat diet.

In our study, the group that consumed about 30 g/d of oat bran without restrict diet had significant reduction only for CHOL (-6%) and showed a tendency to reduce TG (-29%) (Table 2, Figures 1B and 3B). A tendency in diminish LDL-C was

observed (-3%), but, no effect occurred on glucose, HDL-C, BMI and waist circumference.

Inconsistencies in the reported effects of oat products, related to hypocholesterolemic properties of β-glucan, may be due several factors, such as mode of administration, differences in solubility or molecular weight, food matrix and/or the food processing. Kerckhoffs et al. (2003), for example, investigated the effects of β-glucan (5.9 g per day) in bread and cookies with slightly decrease by 3% in LDL-C, while when the fiber was included in orange juice the reduction was 6.7%.

The approach to dietary lowering of cholesterol would seem be to use these approaches in combination to achieve maximal effects. The consumption of about 30g of oat bran and 28g of soy protein was able to decrease significantly the CHOL (about -3%), LDL (-6%) and TG levels (-12%) (Table 3, Figure 1D and 3D). However, analyzing the results it is not possible to affirm that there was an additive or synergic effect with this association. No effects were observed in HDL-C, glucose, BMI and waist circumference. Van Horn et al. (2001) examined free-living, postmenopausal women with hypercholesterolemia to evaluate the same combination as adjuncts to a Step I diet (NCEP, 2001) testing the associations: oats+milk, wheats+soy, oats+soy and wheat+milk. According to these authors, possible adjunctive treatments combined with the Step I diet, oats but not soy yielded further improvements, with reduction of the CHOL in 3% and LDL-C in 5%.

The combination of soluble fiber of oats and moderate intake of soy protein foods reduce both LDL cholesterol (-6.7±1.7%) and the ratio of LDL:HDL cholesterol (-6.3%±2.0) (Jenkins et al., 1999). These reductions were achieved on diets that were already low in saturated fat and dietary cholesterol. The authors explain this combination appears to be additive effects on lipid profile regularization. However, they do not evaluated only soluble fiber or soy protein in different groups in the same design. Torres et al. (2009) evaluated the association of 25g of soy protein and 15 g of soluble fiber of oats on lipid profile and polymorphisms of genes following a low saturated fat diet and achieve goals of reducing TC, LDL-C and TG, independently of genotype.

Conclusion

It is evident that both mechanism of action of soy protein and soluble fiber to lower cholesterol are multifactorial. In this work it was observed a decrease in CHOL, LDL and TG of individuals that ingested together oat bran (containing 10.40 g/100g of soluble fiber) and soy snack bars (containing 34.25 g/100g of protein and 100.39 mg/100g of isoflavones) during 45 days without following a diet providing an alternative to drug therapy. However, was not observed a real additive or synergic effects, but should be more investigated in subjects that follow or not a low-fat diet. Addition of functional ingredients in cereal bar shown being a great alternative to increase the consumption of bioactive compounds at their convenience, practicality and it is related with health by consumers.

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Artigo 4

“Oat Bran And Soy Flour On Lipid Profile And Immune Response In Rats”

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OAT BRAN AND SOY FLOUR ON LIPID PROFILE AND IMMUNE RESPONSE IN RATS

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Abbreviations

CHOL – total cholesterol; LDL-C – Low Density Lipoprotein Cholesterol; HDL-C – High Density Lipoprotein Cholesterol; VLDL-C – Very Low Density Lipoprotein Cholesterol; TG – Triglycerides; FCE – Food conversion efficiency; Ig – Immunoglobulin; IgA – Immunoglobulin A; IgG1 – Immunoglobulin G1; IgG2a – Immunoglobulin G2a; IgM – Immunoglobulin M; IgE – Immunoglobulin E; TMBZ - 3,3′,5,5′-tetramethylbenzidine; AIN 93M – American Institute of Nutrition 93M – feed standard to rodents in maintenance diet; FI – Food Intake; WG – Weight gain; EDTA - Ethylenediamine tetraacetic acid; PBS - Phosphate Buffered Saline; IgY – Immunoglobulin Y; ELISA - Enzyme-Linked Immunosorbent Assay; LDLox – LDL oxidized.

Keywords: soy protein, isoflavones, β-glucan, immunoglobulins, Wistar rats

1. Introduction

There are several investigations that reported hypocholesterolemic effects of the soluble dietary fiber and soy protein (with or without isoflavones) consumed separately. Moreover, the combination of these compounds has been extensively investigated aiming to potentiate their effects. Jenkins et al. (1999) investigated the combined effect of soy protein and soluble fiber added to a standard cholesterol-lowering diet in rats and observed that this combination reduced both LDL cholesterol and the LDL: HDL cholesterol ratio.

The mechanisms by which soluble fibers or soy proteins lowers blood cholesterol and lipid levels have not yet been clearly established. To soluble fibers, one explanation is its

ability to form a gel that interferes with the absorption of lipids and cholesterol. Another possible mechanism is that soluble dietary fiber increases sterol excretion (Torres et al., 2006). The cholesterol-lowering activity from oat bran has consistently been associated with their subfractions, more specifically to the β-glucan component (Braaten et al., 1994). To soy protein, the mechanisms of its hypocholesterolemic action is related to the inhibition of cholesterol absorption, enhance of cholesterol bile acid excretion, increase of receptor mediated clearance, LDL receptor activity, or 7-α-hydroxylase activity (Wang et al., 2004). Soy protein has been thought to be responsible for the cholesterol lowering effect of soybean (FDA, 1999; Anderson, Johnstone, Cook-Newell, 1995). Furthermore, isoflavones could also be responsible for the cholesterol lowering (Ali et al., 2004).

Considering the effects on various metabolic pathways, not only about lipid fractions, it is possible that dietary fibers and soy proteins also influence immune function as direct or indirect consequence of physiological changes. Studies demonstrating a lower incidence of bacterial translocation across the intestine barrier with the administration of dietary fiber suggest that this dietary nutrient modulates immunity (Schley & Field, 2002). Moreover, dietary fiber can stimulate the proliferation of probiotics in gut, production of short-chain fatty acids from fiber fermentation (Schley & Field, 2002), as consequence more acidic environment in the intestine and this situation inhibits the conversion of primary bile acids to their secondary counterparts (Jacobs, 1988).

Adding fibre to the diet has yielded various other effects on immune function, including an increase in serum, mesenteric lymph node, and mucosal immunoglobulin production (Lim et al., 1997; Yamada et al., 1999), altered production of cytokine in mesenteric lymph node (Lim et al., 1997), among many other actions.

Bile acids enhance immunoglobulin (Ig) E production by mesenteric lymph node lymphocytes and suppress the production of IgA, IgG and IgM at relatively high serum concentrations (400-500 μmol/L), that can be encountered in disease states (Lim et al., 1994). Furthermore, it has been reported that the oral administration of such water-soluble dietary fibers elevated the serum IgA and IgG levels and enhanced the production of these Igs of the mesenteric lymph node lymphocytes in rats (Lim et al., 1997).

Soy protein and/or isoflavones have been studied about their possible beneficial effects on the immune system. Beer et al. (1989) showed there were no immunological significant responses to the long term ingest of soy protein concentrates. On other hand, isoflavones, phytochemical compounds found in soy, have been shown effects on immunity. These phytoestrogens include aglycones daidzein, genistein and glycitein, respective β-glucosides and conjugated malonyl-glucosides (Góes-Favoni et al., 2004) and it has been reported to have both estrogenic and antiestrogenic activity (Cassidy, Bingham & Setchell, 1995). Effect of genistein on humoral and cell-mediated immunity has been extensively examined, and various effects on immune responses have been found (Sakai et al., 2010), as the increase in the activity levels of cytotoxic T cells and NK cells, conferring cells resistance to tumor challenge (Guo et al., 2001; Guo et al., 2002). The effects of equol, a daidzein metabolite, enhanced Ag-specific IgE production (Sakai et al., 2010).

Atherosclerosis is a disease associated with an inflammatory response with accumulation of cholesterol in the artery wall. In individual with hypercholesterolemia, LDL accumulates and it is oxidized to proinflammatory compounds in the arterial intima, leading to activation of endothelial cells, macrophages and T lymphocytes (Zhou et al., 1998).

The immune response against oxidized LDL (oxLDL) results in antibody production (Ylã-Herttuala et al., 1994; Palinski et al., 1989). High titers of anti-oxLDL antibodies are found in atherosclerotic patients and in experimental animals with hypercholesterolemia. Both IgM and IgG antibodies are generated, suggesting that both T cell-dependent and independent pathways for B cell activation are involved in the immune response against oxLDL (Palinski et al., 1996). In rats, the production of IgG1 antibodies depends on help by Th2 CD4+ T cells, whereas switching from IgM to IgG2a requires Th1 CD4+ T cells. Both types of help involve secretion of specific T cell cytokines, as IFN- (Th1) and IL-4 (Th2) (Snapper & Finkelman, 1993).

The effects of hypercholesterolemia on the immune system can extend beyond promoting inflammation. It can increase the susceptibility to infectious diseases due to a high availability of lipids as a nutrient source for microbe, a reduced phagocytic capacity and an impaired cytotoxic-T-lymphocyte activation (Martens et al., 2008). Therefore, it is very important to control cholesterolemia. Food compounds have a potential to support this action. However, although specific nutrients are known to be important in the development and function of the immune system, the potential of dietary fibers, as β-glucan, and soy protein and isoflavones to impact on immune function should be more investigated.

Whereas soluble fibers, present in oats, and proteins, present in soy, separately or together have hypocholesterolemic and immunity effects, the aim of this study was to evaluate the effects of oat bran and soy flour on lipid profile and plasma immunoglobulin levels (IgM, IgG1 and IgG2a) in rats.

2. Materials and Methods

Oat bran was provided by SL Alimentos (Mauá da Serra, PR, Brazil) and defatted soy flour was purchased from Vitao Alimentos (Curitiba, PR, Brazil). The proximate composition (dry weight basis) of these ingredients were: defatted soy flour – 55.54% protein, 20.80% carbohydrate, 16.03% dietary fiber, 6.54% ash, 1.09% lipids, and 225.18 mg/100g isoflavones; oat bran – 26.66% protein, 39.85% carbohydrate, 22.45% dietary fiber, 3.55% ash, 7.49% lipids, and 10.36g/100g β-glucans.

2.1 Animals and diets

Male Wistar rats at age 5 wk, weighing 145.56 ± 16.94 g, were housed in plastic and stainless-steel cages and maintained at 22±2°C and with a 12-h light-and-dark cycle. For 7 d, the rats received a diet prepared according to the recommendation of the American Institute of Nutrition (AIN 93M) and water *ad libitum* for acclimation. After this period, they were randomly assigned to one of six groups, with five rats per group, and fed different diets (Table 1) for 45 d. Diets were prepared by Prag Soluções Biociências (Igarapava, SP, Brazil). Test diets were modified from AIN 93M (Reeves, Nielsen & Fahey, 1993) being all of them composed of similar amounts of protein, lipids, carbohydrate, vitamin, and mineral.

The composition of the diets is shown in Table 1. Diets A and B had the same composition, without cholesterol addition. Diet C was the control diet with addition of 1% cholesterol and 0.5% cholic acid only. Diets D, E and F had added cholesterol, cholic acid and test ingredients (oat bran and defatted soy flour). In diet D all protein (casein) was

replaced by protein of defatted soy flour; in diet E all fiber (cellulose) was replaced by fiber of oat bran, whereas in diet F all protein and fiber were replaced by mix of defatted soy flour and oat bran. Cholic acid was added to diets to improve cholesterol absorption by the intestine. Diets and water were *ad libitum* available.

Table 1 – Composition of experimental diets AIN 93M and modifications*

Ingredients	**Diet A**	**Diet B**	**Diet C**	**Diet D**	**Diet E**	**Diet F**
	Reference 1 (%)[§]	Reference 2 (%)[†]	Control (%)[‡]	Soy (%)	Oat (%)	[ϕ]Soy + Oat (%)
Oat Bran	-	-	-	-	24.307	27.515
Soy flour	-	-	-	22.686	-	
Corn Starch	46.57	46.57	46.57	46.57	43.063	46.450
Casein	14	14	14	0	7.52	0
Dextrinized starch	15.5	15.5	15.5	15.5	15.5	15.5
Sucrose	10	10	8.5	3.698	1	1
Soy oil	4	4	4	3.753	2.179	3.105
Celullose microc	5	5	5	1.363	0	0
L-cistina	0.18	0.18	0.18	0.18	0.18	0.18
B. colina	0.25	0.25	0.25	0.25	0.25	0.25
BHT	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008
Mineral mix G	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1	1	1
Cholesterol	---	---	1	1	1	1
Cholic acid	---	---	0.5	0.5	0.5	0.5

*Diets were isoenergetic and given in pellets form. They are presented in dry basis.

[§]Reference 1 - Group without inoculation of IgY; [†]Reference 2 - Group with inoculation of IgY; [‡]Control Group with cholesterol; [ϕ]Mix of Soy flour (S) and Oat bran (O) in a proportion to achieve total substitution of protein and fiber (2S x 2.65 + O x 1.35).

The study was performed in the Laboratory of Physiologic Sciences of Universidade Estadual de Londrina approved by the Animal Experimentation Ethics Committee of Universidade Estadual de Londrina (nº44/07) and used is in accordance with Ethical Principles in Animal Research.

2.2. Weight gain, food intake, and food conversion efficiency

Body weight and food intake (obtained by difference between weights of diet offered and residual) were recorded every week. Food conversion efficiency (FCE) was obtained by the rate of the mass of the food intake (FI) and total weight gain (WG) during experimental time (FCE = FI/WG).

2.3. Experimental protocol and blood collection

The experimental protocol is presented in Figure 1. For 7 d, the rats received an acclimation diet (-7 to 0 days). From 0 to 45 day the rats consumed experimental diets. The blood was collected by cardiac puncture in tubes with EDTA (2mg/ml) for lipid analysis at 0, 15 and 30 day and for immunological analysis at 14, 23 and 45 day. Immediately after collection the blood was centrifugated at 5.000 rpm during 5 min. Lipid profile was performed in the same day of collection, whereas the plasma was stored at -20°C for posterior immunological analysis. After the experimental time, rats were killed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia.

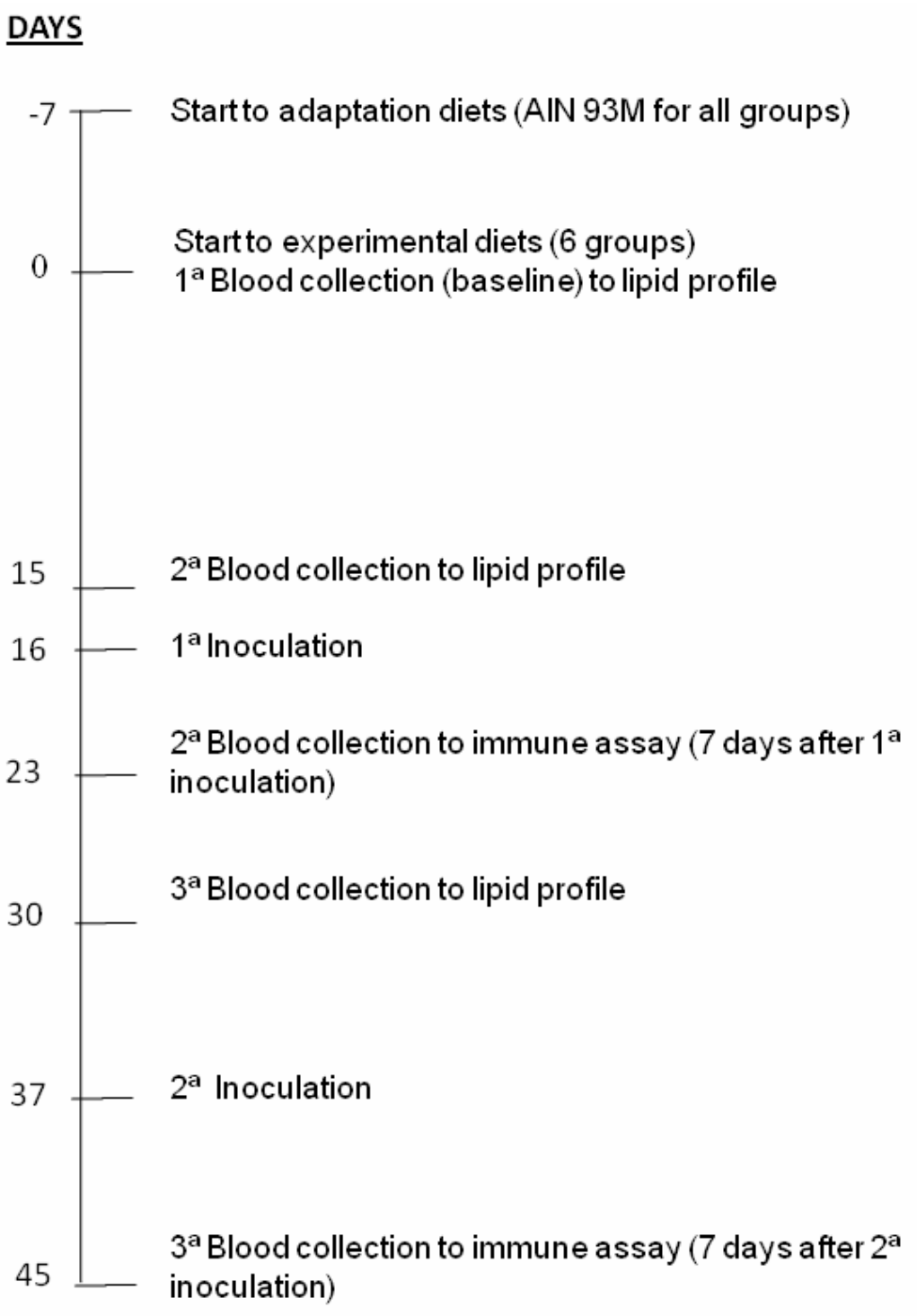

Figure 1 – Experimental Protocol followed during 45 days.

2.4. Immunizations

IgY antibodies extracted from egg yolk of laying hens (Akita & Nakai, 1992) were used as antigen (Walsh et al., 2000). Rats were immunized intramusculary (i.m.) in the right hind leg, with 100 µg of antigen in 100 µL of PBS / Freund's Complete Adjuvant (v/v). After three weeks, a second immunization i.m. was performed with an injection of 100 µL of PBS/ Freund's Incomplete Adjuvant (v/v) containing 100 µg of antigen IgY. In the reference group (Diet A), rats were inoculated whit the same volume of PBS without antigen in both inoculations.

2.5. Histological analysis

Immediately after the final blood collection, animals were sacrificed and their livers were collected and processed as previously described by Beçak & Paulete (1970). The pathological examinations of liver tissue was done with an optical microscope at 400 X by three experienced observers who assessed eight parameters for the integrity of hepatocytes and signs of tissue and cell injury in twenty randomly chosen fields, as described by Chaves (2006). The scores represent the average results of three observers and ranged from 0 (no lesion), 1-8 (mild injury), 9 to 16 (moderate injury) and 17 to 24 (severe injury).

2.6. Lipid profile and hematological analysis

Lipid profile of plasma samples was analyzed by enzymatic colorimetric procedures for CHOL (Cat. 460), HDL-C (Cat.: 413M) and TG (Cat.: 459) using kits from Gold Analisa Diagnóstica Ltda (Belo Horizonte, MG, Brazil), LDL-C and VLDL-C values were obtained with the following equations, respectively: LDL-C = CHOL – HDL-C – triglycerides / 5, and VLDL-C = Triglycerides / 5, as point out by manuals of kits. VLDL-C value was added to the value of LDL-C.

2.7. ELISA to anti-IgY (IgM, IgG1 and IgG2a antibodies)

Plasma levels of IgM, IgG1 and IgG2a were determined by ELISA (Engvall and Perlman, 1972). In brief, 100 µL of IgY antigen (10 ng/mL) in bicarbonate buffer was coated onto the wells of a micro plate (Corning, New York, USA) and incubated overnight at 4°C. The plate was washed with 0.05% PBS-Tween and blocked with 150 µL of 5% PBS-skim milk power (PBS-skim milk) for 60 min at 25°C. After washing, 100 µL of the plasma samples diluted to 1:500 in PBS-skim milk at 1% were added and incubated for 60 min at 25°C. After washing, 100 µL of peroxidase-conjugated mouse anti-rat IgM, IgG1 or IgG2a (Zymed, San Francisco, California, USA) diluted in PBS-skimmed milk 1% to a concentration of 1:10.000, 1:100.000 and 1:20.000, respectively was applied to each of the well and incubated for 60 min at 25°C. The plate was washed and a mixture of substrate TMBZ (3,3′,5,5′-tetramethylbenzidine) and H_2O_2 in substrate acetate buffer was added to the wells, incubated for 15 min at 25°C. Then after 50µL of H_2SO_4 1N was added to finish reactions. The absorbance was read at 450 nm with an ELISA reader (Spectra MAX 250,

Sunnyvale, California, USA). Antibody levels were expressed in ELISA units calculated from a standard curve.

2.8. Statistical analysis

Lipid profile data sets were compared using the non-parametric Wilcoxon matched pairs signed ranks test for dependent samples and Kruskal–Wallis for independent samples, both $p<0.05$. Data of histological analyses of liver was performed by Kruskal-Wallis following Dunn's test. The data were presented in medians. The dependent variables weight tissue/weight rat, weight gain, food intake, FCE and antibody levels were subjected to variance analysis (ANOVA) to assess the significance ($p<0.05$) followed by Tukey's test to compare of the means. Data analysis was carried out using the Statistica program version 7.1 (Statistica, 2006).

3. Results

3.1. Weight gain, food intake and food conversion efficiency (FCE)

Initial weights of rats were similar for all treatments (Table 2). At the end of the trial, the animals of control group (with cholesterol, diet C) and those fed with oat bran only (diet E) had higher weight gains (155.3g and 154.4g, respectively) than animals fed with the other diets. Intermediate values were observed in the reference diets without cholesterol addition

(diets A – 141.0g, and B – 142.8g). The lowest values of weight gain were observed for rats consuming soy, without (diet D – 134.4g) or with oat (diet F - 139.7g).

Food intake (Table 2) was greater for the rats of reference groups (without addition of cholesterol), without (diet A – 798.9g/45 days) and with inoculation (diet B – 789.7g/45 days). The rats that consumed diets with cholesterol ingested less food. In diets C (control), D (soy), and E (oat) the rats consumed 778.6g, 762.1g and 762.7g, respectively, feed in forty five days. In diet F, in which casein and cellulose were substituted by soy protein and oat fiber, the lower consumption (730.7g/45 days) was observed.

In diet C (control), and those diets with oat without (diet E) or with soy (diet F) rats required less food to increase one gram, 5.0, 4.9, and 5.2, respectively, compared to the rats fed with reference groups, diet A (5.7) and diet B (5.5), and diet D (soy; 5.7) (Table 2).

Table 2 – Weight gain, food intake and food conversion efficiency in the groups of rats submitted to different diets, after 45 days*

Groups	Code of groups	Initial Weight (g)	Weight gain (g)	Food Intake (g)	Food conversion
Reference 1[§]	A	183.0	141.0	798.9	5.7
Reference 2[†]	B	182.0	142.8	789.7	5.5
Control[‡]	C	188.0	155.3	778.6	5.0
Soy	D	181.2	134.4	762.1	5.7
Oat	E	181.7	154.4	762.7	4.9
Soy + Oat[ϕ]	F	185.3	139.7	730.7	5.2

*Data are presented as mean of the group.

[§]Reference 1 - Group without inoculation of IgY; [†]Reference 2 - Group with inoculation of IgY; [‡]Control Group with cholesterol; [ϕ]Mix of Soy flour (S) and Oat bran (O) in a proportion to achieve total substitution of protein and fiber (2S x 2.65 + O x 1.35).

3.2. Tissues weights

Table 3 shows the effects of different diets and inoculations on tissue weights. Rats fed diets without cholesterol had significantly lower ratio between tissue and body weight, for liver (31.1 mg/g to diet A and 27.8 mg/g to diet B) and spleen (1.9 mg/g to diet A and 1.8 mg/g to diet B) weights. The higher values to liver and spleen weight were observed in diets with oat (46.4 mg/g and 3.3 mg/g, respectively) and oat + soy (47.3 mg/g and 3.0 mg/g, respectively), but without significant difference. No difference was observed in the ratio tissue weight/weight body of the thymus and left kidney in all treatments and between groups A and B for all tissues. According this observation, there was not influence of the inoculation on tissue weights when analyzed without cholesterol in diet. The weights of Peyer's patches were not shown.

3.3. Lipid profile

No significant differences were observed in CHOL, LDL-C, HDL-C, and TG levels at 15º day of experiment (Table 4). However, increase from 97.2 mg/dL to 141.7 mg/dL in CHOL, 32.9 mg/dL to 106.2 mg/dL in LDL-C, and decrease from 61.3 mg/dL to 40.2 mg/dL in HDL-C was observed in group C achieved the objective to cause modification in lipid parameters not even reaching the hypercholesterolemia with addition of 1% cholesterol and 0.5% of cholic acid in diets, although Wistar rats are not the most suitable animal model for this purpose.

Table 3 – Tissue to body weight ratios of rats exposed to different experimental diets and inoculations for 45 days*

Tissue weight/ weight rat	Experimental Groups					
	Reference 1[§]	Reference 2[†]	Control[‡]	Soy	Oat	Soy + Oat
	A	B	C	D	E	F
Liver (mg/g)	31.1 ± 2.0[b,c]	27.8 ± 3.0 [b]	42.7 ± 8.0[a]	40.0 ± 4.0 [a,c]	46.4 ± 6.0[a]	47.3 ± 2.0[a]
Spleen(mg/g)	1.9 ± 0.2[c]	1.8 ± 0.2[c]	2.4 ± 0.5 [b,c]	2.4 ± 0.4 [b,c]	3.3 ± 0.8 [a,b]	3.0 ± 0.7 [a,b]
Thymus (mg/g)	1.3 ± 0.2	1.0 ± 0.1	1.3 ± 0.7	1.1 ± 0.3	0.9 ± 0.1	1.0 ± 0.1
Left Kidney (mg/g)	3.0 ± 0.3	3.0 ± 0.2	2.5 ± 0.7	2.9 ± 0.1	2.9 ± 0.2	2.7 ± 0.2

* Values expressed as mean ± SD and presented in tissue weight/weight rat; [§]Reference 1 - Group without inoculation of IgY and without cholesterol; [†]Reference 2 - Group with inoculation of IgY and without cholesterol; [‡]Control Group with 1% cholesterol; Diets with soy, oat and soy + oat also contain 1% cholesterol. Values in the same line followed by the different superscript letter are significantly different ($P < 0.05$).

Table 4 –Lipid profile of rats exposed to different experimental diets for 15 days*

Variables	**Time**	**Reference[§]**	**Control[†]**	**Soy**	**Oat**	**Soy + Oat**
		A	**C**	**D**	**E**	**F**
CHOL (mg/dL)	Baseline	91.2	97.2	100.3	101.5	100.9
	15 days	76.2	141.7	103.9	100.9	87.6
LDL-C (mg/dL)	Baseline	27.7	32.9	32.0	26.2	32.2
	15 days	17.1	106.2	92.8	76.1	51.7
HDL-C (mg/dL)	Baseline	69.3	61.3	67.6	75.9	57.5
	15 days	57.8	40.2	28.6	35.7	28.1
TG (mg/dL)	Baseline	87.5	90.8	81.9	91.4	92.1
	15 days	80.3	82.1	102.0	103.9	103.9
n		5	5	5	5	5

CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides. [§]Reference group is represented only by group Diet A (without antigen (IgY) inoculation and without cholesterol). No difference between groups Diet A and Diet B was verified in lipid profile (same diet composition). [†]Control Group with addition of 1% cholesterol only. Diets with soy, oat and soy + oat also contain 1% cholesterol.* Values expressed as median.

Machado et al. (2003) obtained similar results when added cholesterol and cholic acid in diet AIN-93G fed to Wistar rats to cause moderate hypercholesterolemy after 14 days. After 15 days it was observed that consumption of soy (diet D) or oat (diet E) avoided increasing in CHOL comparing with control group, but the same was not observed in LDL-C. The rats that consumed diet F, with oat and soy together, obtained a decrease in CHOL after 15 days from 100.9 mg/dL to 87.6 mg/dL (13%). The experimental diets D, E and F promoted increase in TG. In all diets it was observed a decrease in HDL-C values (Table 4).

Data on lipid profile after 30 days is not shown, because it seems that may have been interference with the primary IgY inoculation occurred 15 days before the third collection.

3.4. Histological Analysis

Figure 2 show the effect of treatments in the liver of rats. The histological analysis demonstrated all groups with cholesterol addition on the diet (C, D, E and F) had significant alterations in liver comparing with groups A and B (no cholesterol intake). In general, groups C, D, E and F had moderate injuries while groups A and B presented minor injuries. From this, it was verified that the experimental diets with soy, oat or soy+oat were unable to protect or reverse mechanisms that cause injuries.

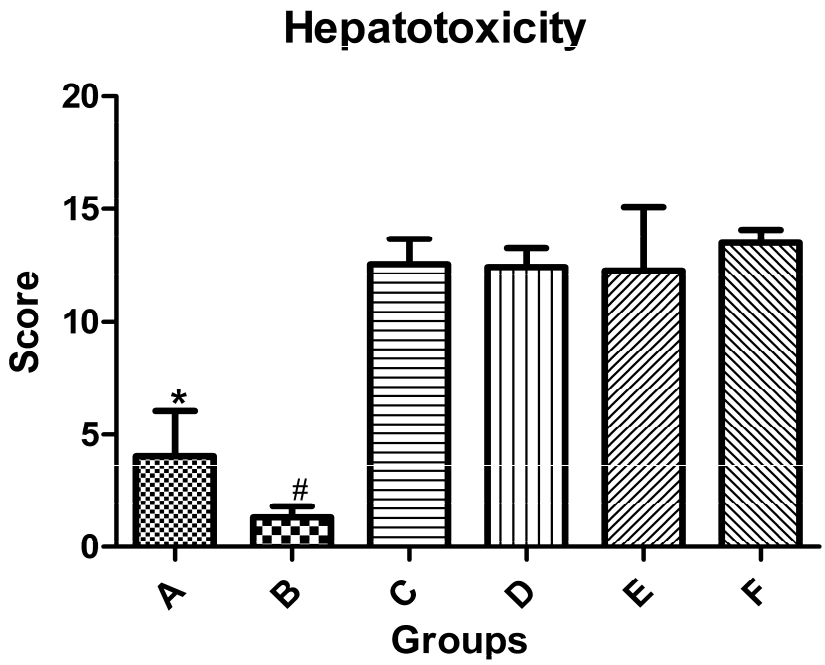

Figure 2 – Histological analysis of livers from rats that consumed experimental diets A (reference 1 – without cholesterol and without inoculation), B (reference 2 – without cholesterol and with inoculation), C (control – with cholesterol and with inoculation), D (soy – with cholesterol and with inoculation), E (oat – with cholesterol and with inoculation), F (soy+oat – with cholesterol and with inoculation). The scores represent the average results of three observers and ranged from 0 (no lesion), 1-8 (mild injury), 9 to 16 (moderate injury) and 17 to 24 (severe injury).

3.5. Plasma immunoglobulin levels

Serological analysis demonstrated an increase in antibodies level due antigenic stimulation after second antigen inoculation (45 days). It was not observed significant differences in IgM levels among experimental groups, regardless antigen inoculation (Figure 3). As expected, in animals of groups B, C, D, E e F there was an increase in anti-IgY antibodies (IgG1) in relation to animals from group A. Animals of group B (without cholesterol and inoculated) had lower levels of antibodies than group C (control – with addition cholesterol) and D (with addition soy flour and cholesterol) and values similar to groups E (with addition of oat bran and cholesterol) and F (with addition of soy flour, oat bran and cholesterol). Moreover, group C had values of IgG1 antibodies greater than groups D, E and F. From this, diet rich in cholesterol stimulate production of IgG1 and oat bran has the ability to reverse this effect.

In relation to IgG2a, the animals of groups B, E and F presented levels of antibodies significantly higher than animals no inoculated (group A). Group B showed higher level of antibodies IgG2a than groups C and D (Figure 3). Therefore, diet rich in cholesterol had an inhibitory effect on IgG2a antibodies production and this effect is partially reversed by oat bran consume. No significant protector effect was observed to soy flour in all antibodies production tested.

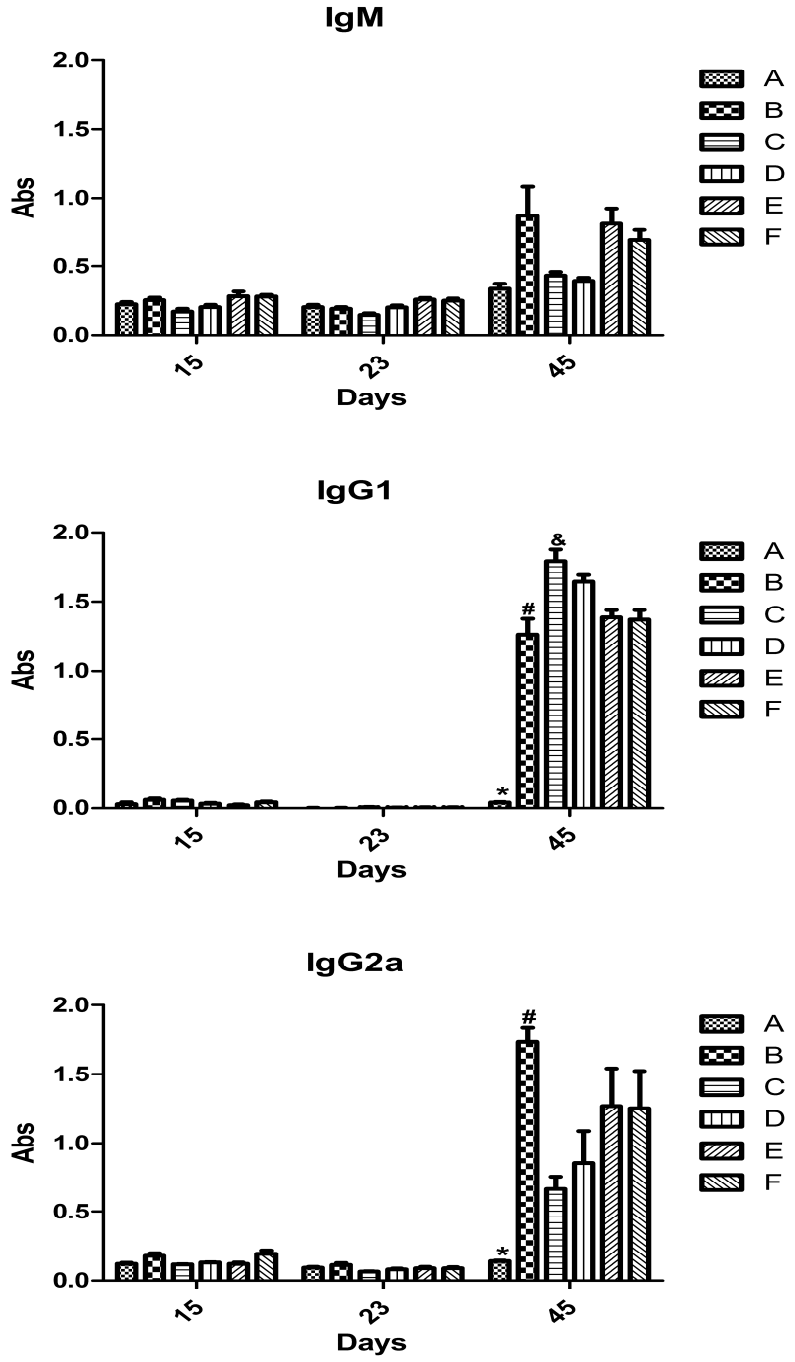

Figure 3 - Plasma immunoglobulins IgM, IgG1 and IgG2a of rats that consumed experimental diets A (reference 1 – without cholesterol and without inoculation), B (reference 2 – without cholesterol and with inoculation), C (control – with cholesterol and with inoculation), D (soy – with cholesterol and with inoculation), E (oat – with cholesterol and with inoculation), F (soy+oat – with cholesterol and with inoculation), in 15, 23 and 45 days.

4. Discussion

4.1. Weight gain and tissues weights

In the present study we observed an effect of defatted soy flour (containing 225.18 mg of isoflavone/100g of flour) intake on weight gain (Table 2). Diets D (soy) and F (soy + oat) showed the lowest gain of the weights (134.4g and 139.7g, respectively). In these diets soy proteins contained in soy flour substituted the casein present in diets A and B. It was observed that consumption of soy can prevent the weight gain provided by intake of cholesterol, comparing with gain of weight by rats from the group C (control diet; 155.3g).

This effect may be due to isoflavones content. Ali et al. (2004) demonstrated that dietary isoflavones (0.1%) significantly reduced body weights in obese rats. Naaz et al. (2003) demonstrated decrease in body weight only with high intake of genistein. Studies do not show difference in weight gain when rats consumed soy protein with or without cholesterol during two months indicating that the soybean protein diet and cholesterol (0,1%) supplementation had no effect on weight gain (Madani et al., 2004).

In diet F the rats consumed oat bran and defatted soy flour. The ingredients together did not show additive effect on weight gain, prevailing soy effect. In diet E, it was observed that consumption of oat bran (containing 10.36g/100g β-glucan) did not avoid the weight gain proportioned by cholesterol intake or by own oat (154.4g). It was also reported that β-glucan preparations from different cereals affected weight gain and feed efficiency of rats (Klopfenstein & Hoseney, 1987). Kalra and Jood (2000) showed that consumed of casein promoted higher gain of weight when compared with diet with barley flour during forty days with Wistar rats, but in this study no cholesterol was added in the diets.

Significant differences were show in liver and spleen ratios to body weights of rats feed with cholesterol diets (C, D, E, and F) compared with those without cholesterol (A and B) demonstrating the overload of cholesterol on this body organs (Table 3). Machado et al. (2003) and Beynen et al. (1986) related increase in weight of the liver in Wistar rats that consumed diets rich in cholesterol and cholic acid.

None of the tested foods was effective to avoid this increase in tissue weights, but it is observed that diets containing oat bran seems to have a tendency to higher values. Ali et al. (2004) demonstrated that dietary isoflavones (0.1%) significantly reduced weights of liver, kidney and spleen in obese rats, but there was not addition of cholesterol in the diets. This author suggested that isoflavone but not the type of protein affect the body weight or the tissue weights, and it is tissue specific and may depend on species and gender. Other studies have shown no significant differences in body weight or tissue weight between rat fed casein or soy protein concentrate (Bathena et al., 2002; Bathena et al.; 2003).

Delaney et al. (2003) evaluated the effect of different concentrations of β-glucan in organ weights, with diets without cholesterol addition. No significant difference was found in all organs evaluated, but there were an increase in spleen weights. Due this, the increase in liver and spleen weight was attributed to cholesterol ingested and no to oat bran intake.

4.2. Food intake and Food conversion efficiency

In our study it was observed that the diets added of soy, oat or soy + oat had a lower food intake comparing with diets control (with cholesterol) and references (without cholesterol). These data demonstrated that the consumption of fiber and/or vegetable protein could diminish the food intake, probably due the high satiety provided by these compounds. Kalra and Jood (2000) showed that consumption of casein by Wistar rats,

during forty days had highest food intake when compared with diet with barley flour (containing β-glucans), but without cholesterol.

Studies of Madani et al., (2004) did not show difference in food intake during two months, in diets containing soy protein with or without cholesterol, indicating that the soybean protein diet and cholesterol (0,1%) supplementation had no effect on growth.

Small difference between food intake in groups A and B was observed. The group of rats that were inoculated with antigen (diet B) had a less ingested of food than group A. However, it is not attributed to the inoculation the lower food intake of the other groups.

The food conversion efficiency (FCE) is a mean of an animal's efficiency in converting feed mass into increased body mass. If it have a FCE = 5, means that every 5 grams of food intake will gain one gram of body weight. So, the smaller food conversion is better to gain of body weight. The FCE shown is inversely proportional to weight gain. So, the lowest FCE was observed in rats that consumed only oat, soy + oat or control diet (only cholesterol added). This mean that it was necessary to ingest less fed to weight gain when these diets are consumed.

4.3. Lipid profile

Results showed no significant differences in lipid profile of the treatments with soy, oat and soy + oat between baseline and after 15 days. All discussion will be done with tendency of effects. However, the treatments with soy (diet D) and oat (diet E) separately were able to avoid the increase in CHOL provided by intake of cholesterol of the diet, comparing with diet C (control) which have an increase in cholesterol after 15 days (37%). When rats consumed the diet F, with soy and oat together there was a reduction of CHOL (13%), but no significant different.

Madani et al. (2004) analyzing changes in serum lipoprotein lipids in Wistar rats fed with soybean protein and casein (with or without cholesterol) showed no difference in CHOL and TG after 2 months of diet. However, a decrease in LDL was observed in rats that consumed soy in comparison with casein (both with cholesterol in the diet). Ali et al. (2004) shown that isoflavones also reduce total as well as LDL and HDL cholesterol in rats. However, in many studies a small increase in HDL cholesterol is observed after feeding soybean or isoflavones alone, whereas in others either no change or a small decrease in HDL cholesterol is observed (Ali et al., 2004). Yamada et al. (1999) related that oral administration of different source of dietary fiber reduced the serum cholesterol levels compared to cellulose fed in rats.

The divergence in results compared with some investigations of literature may be partially explained by the fact that in some studies are offered food isolates in contrast to our study, where they were offered oat bran and soy flour. These products have, in addition to bioactive compounds targeted in this study, other compounds that may influence physiological responses.

4.4. Histological Analysis

In this study all diets with addition of cholesterol resulted in moderate injuries in liver after 45 days of treatment (Figure 2). Soy, oat or soy+oat were not reversed or protect the organ against injuries. Machado et al. (2003) obtained similar results when added cholesterol and cholic acid in diet for Wistar rats, aiming to cause moderate hypercholesterolemy after 14 days. This lineage of rats is very resistant to the development of hypercholesterolemia and atherosclerosis due the high hepatic conversion of the cholesterol in bile acids. However, the consumption of the cholesterol may be reflected in

deposition in liver. Machado et al. (2003) and Beher et al. (1962) found accumulation of cholesterol in livers of rats even without raising serum cholesterol.

4.5. Plasma immunoglobulin level

After second inoculation (45 days) there was an increase in antibodies level. No significant differences were observed in IgM levels among groups (Figure 3B). Besides levels of IgG were significantly different of the control (Group B – without cholesterol and inoculated). In rats IgG1 levels depends on help by Th1-type, whereas IgG2a requires Th2 help. High antigen concentrations have been reported to induce Th2 responses (Constant & Bottomly, 1997), and it is possible that the increasing levels of oxLDL during hypercholesterolemia preferentially induced Th2 activation (Zhou et al., 1998).

Th2 immune response refers to the humoral immune response which by definition acts in the extracellular medium, producing antibodies to the body's defense. Analyzing figure 3C it is possible to observe that the induction of infection made in group B generated higher production of IgG2a. In group C, where the rats received hypercholesterolemic diet had lower production of immunoglobulin. This result may indicate that the intake of cholesterol and hypercholesterolemia, seen in this group (Table 4) can change / reduce the immune response. This fact is important when considering the immunizations done by vaccines. If these results observed in rats could be used in therapies to humans could be said that hypercholesterolemic individuals may respond to the vaccine less effectively than those normocholesterolemic. It can see in Figure 3C that oat bran (Groups E and F) could partially reverse this effect of cholesterol intake, acting as immunomodulator, enabling a better immune response in mice with altered lipid profile. Intake of soy flour did not show any immunomodulatory effects.

The Th1 immune response refers to the cellular imune response. Analyzing figure 3B it is apparent that those rats that received hypercholesterolemic diet (Group C) and soybean diet (Group D) had higher production of IgG1 than group B (reference group inoculated with IgY) and the groups with oats (E and F). Again, it appears that oats could act as an immunomodulator by decreasing the production of IgG stimulated by hypercholesterolemic diet. Moreover, there was no effect of intake of soy flour in the production of Ig.

According to Yamada et al. (1997) dietary fibers enhance the Ig productivity of lymphocytes in an indirect manner, and their effect on cytokine production by immune cells partly participates in this enhancement. Yamada et al. (1999) demonstrated that dietary fiber ingestion strongly affected the serum Ig levels, and different results were found to different sources of fiber. The serum IgA level of the rats fed guar gum, glucomanann or pectin was significantly higher than that of the rats fed cellulose. In the case of the IgG level, the glucomannan group was significantly higher than the group fed guar gum. In case of the IgM, the glucomanann group was significantly higher than the group fed cellulose.

In this work, several effects were evaluated together. So, it is necessary more investigation about the observed results aiming to clarify the mechanisms and effects. The effect of hypercholesterolemia on the immune system and the protective or reversal effects of food compounds on body defenses might be investigated in more detail.

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5 CONCLUSÃO GERAL

A partir dos resultados obtidos neste estudo é possível concluir que:

- É viável a produção de ingrediente extrusado expandido, contendo farelo de aveia, farinha de soja e amido de milho nas proporções de 37,5, 37,5 e 25%, respectivamente;
- A adição de inulina (4,5%) à mistura, como coadjuvante, foi essencial para conferir fluidez ao processo;
- O controle das condições de processo (25% de umidade e 160ºC de temperatura) também foi importante para obtenção de ingrediente expandido com caracterísiticas sensoriais desejáveis;
- Não foi possível produzir barras de cereais contendo 3g de beta-glucana/100g, devido ao caráter hidrofílico do farelo de aveia.
- Barras de soja com 34,25g de proteína/100g e 100,39 mg de isoflavona /100g apresentaram características sensoriais bem aceitas pelos provadores e pelos voluntários do ensaio clínico (grupo soja) ;
- Durante o armazenamento das barras houve mudança na cor, atividade de água e na textura. Nessa formulação foram utilizados apenas ingredientes de soja e outros que não tivessem potencial hipocolesterolêmico. Outros ingredientes devem ser testados nessa formulação para melhoramento da estabilidade durante armazenamento;
- Barras de cereais são ótimos veículos para inserção de ingredientes funcionais por sua conveniência e apelo de saúde verificada pela aderência dos voluntários ao protocolo;
- É possível alcançar melhoras no perfil lipídico consumindo farelo de aveia e proteína de soja, separadamente, mesmo sem seguir dieta restrita em colesterol e gordura saturada. No entanto, isso não é o recomendado por órgãos de vigilância;
- Não foi verificado efeito sinérgico ou aditivo quando farelo de aveia e farinha de soja foram consumidos juntos, sem dieta restrita em colesterol e gordura saturada, durante 45 dias, mas houve melhora no perfil lipídico;
- No estudo com animais foi verificado que a ingestão de colesterol ou hipercolesterolemia podem mudar/reduzir a resposta imune. No caso de

imunizações com vacinas, haveria a possibilidade de pessoas hipercolesterolêmicas responderem menos efetivamente a vacinas do que as pessoas normocolesterolêmicas. No entanto, isto deve ser melhor investigado;

- A ingestão do farelo de aveia pelos animais, por 45 dias, parece reverter parcialmente essa diminuição da resposta imune causada pela ingestão de colesterol ou hipercolesterolemia.

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APÊNDICES

APÊNDICE A - Questionário para Provadores da Análise Sensorial

Desejamos formar uma equipe de provadores para avaliar barras de cereais. Ser um provador não exigirá de você nenhuma habilidade excepcional, não tomará muito seu tempo e não envolverá nenhuma tarefa difícil. A prova será realizada no Laboratório de Análise Sensorial do DCTA, leva em torno de 15 minutos e você poderá fazê-la no horário que tiver maior disponibilidade. Se tiver qualquer dúvida, ou necessitar de informações adicionais, entre em contato com a doutoranda Luciana Pereira Lobato, 3304-1535 (e-mail: lucianaplobato@gmail.com) ou para Profª. Maria Victoria Eiras Grossmann, 3371-4080 (email: victoria@uel.br). O telefone do Comitê de Ética em Pesquisa com Humanos é 3371-2490.

Nome_____
Telefone para contato / email:_____

1- Faixa etária
( ) 15-25
( ) 25-35
( ) 35-50
( ) acima de 50 anos

2- Sexo
( ) masculino
( ) feminino

3- Ocupação
( ) aluno _____
( ) funcionário
( ) professor
( ) outro _____

4- Escolaridade
( ) 1ºgrau
( ) 2ºgrau
( ) 3º grau
( ) outro _____

5. Gosta de barras de cereais: ( ) Sim ( ) Não

6. Gosta do sabor soja: ( ) Sim ( ) Não

8- Freqüência de Consumo de barras de cereais:

( ) Nunca
( ) Ocasionalmente - _____ vezes por ano
( ) Moderadamente - _____ vezes por mês
( ) Freqüentemente - _____ vezes por semana

9- Experiência como provador:
Já participou de algum teste sensorial? ( ) Não ( ) Sim Aceitação ( )
Discriminativo ( )
Descritivo ( )

APÊNDICE B – Termo de Consentimento Livre e Esclarecido para Análise Sensorial

Gostaríamos de convidá-lo a participar de uma equipe de provadores para opinar sobre qualidades tecnológicas como aparência, aroma, sabor e textura de barras de soja contendo ingredientes. A sessão não tomará muito seu tempo, não haverá nenhum custo para os participantes e você poderá desistir da participação a qualquer momento no decorrer dos testes. As provas serão realizadas no Laboratório de Análise Sensorial do Departamento de Ciência e Tecnologia de Alimentos-UEL. Serão ralizadas sessões que durarão em torno de 15 minutos e você poderá fazê-la no horário que tiver disponibilidade a partir do agendamento. O consumo desse produto não deve provocar nenhum desconforto e não oferecem risco por serem elaboradas com ingredientes de consumo rotineiro pela maioria das pessoas. Os dados aqui coletados serão tratados com confidencialidade. Caso deseje participar da equipe de provadores desta pesquisa você responderá um questionário para recrutamento de provadores e deverá preencher esse Termo de consentimento Livre e Esclarecido. Quaisquer dúvidas procurar a responsável pelo projeto Praf. Maria Victoria Eiras Grossmann, DCTA/UEL, telefone (43) 3371-4080, victoria@uel.br. O Comitê de Ética da UEL pode ser contatado na PROPPG/UEL, telefone (43) 3371-4290, cep_uel@uel.br

Eu, _____, R.G. _____, aceito participar do Projeto “Efeito da interação dos componentes da aveia e da soja sobre a colesterolemia, imunpestimulação e viabilidade tecnológica no desenvolvimento de barras de cereais” Fui informado que não haverá custo para os participantes e também da disponibilidade de tempo necessária para participação. Estou ciente que estou colaborando no desenvolvimento de uma tese de doutorado e que posso desistir da participação a qualquer momento no decorrer dos testes. Fui informada pela coordenadora do projeto Profª. Maria Victoria Eiras Grossmann de seu endereço e telefone, bem como o do Comitê de Ética da UEL.

_____,____de_____de 2009.

Assinatura_____

Telefones:_____
Email:_____

APÊNDICE C - Ficha de Aceitação da Análise Sensorial

NOME: _____ DATA: ____/ ____/ ____

Você está recebendo uma amostra de barras de soja contendo diferentes ingredientes. Por favor, avalie a aparência, prove e use a escala abaixo para indicar o quanto você gostou ou desgostou.

Amostra _____

9- gostei muitíssimo

8-

7-

6-

5- nem gostei/nem desgostei

4-

3-

2-

1-desgostei muitíssimo

Cite o que você **mais gostou** na amostra: _____
_____

Cite o que você **menos gostou** na amostra: _____
_____

APÊNDICE D- Termo de Consentimento Livre e Esclarecido para Estudo Clínico

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

PROJETO DE PESQUISA: Efeito da interação dos componentes da aveia e da soja sobre a colesterolemia, imunoestimulação e viabilidade tecnológica no desenvolvimento de barras de cereais

Doutorandas: Luciana Pereira Lobato e Alissana Ester Iakmiu Camargo Pereira
Responsáveis pelo projeto: Maria Victoria Eiras Grossmann e Décio Sabbatini Barbosa

Eu,_____RG_____ _____, declaro que em _____/____/2010, concordei em participar como voluntário (a) no grupo de estudo do projeto acima nomeado.

O responsável pelo projeto explicou-me o seguinte:
1. O estudo implica em colher amostras de sangue para análises bioquímicas.
2. A minha participação neste estudo é voluntária, tendo a liberdade para recusar ou retirar meu consentimento a qualquer momento.
3. O sigilo da minha participação, assim como todas as informações obtidas serão preservadas.
4. Ao longo da pesquisa, que será realizada no período de 45 dias, deverei ingerir as barras de soja, ou as barras de soja e a aveia, ou apenas a aveia, ou ainda uma dieta. A escolha para um ou outro produto será realizada de forma aleatória pela orientadora da pesquisa e, após o fornecimento, a ingestão dos mesmos deverá ser diária.
5. Fui informado (a) da possibilidade de necessitar de wash-out, ou seja, retirada de medicamento de uso contínuo que possa interferir nas análises durante o estudo.

Contatos dos participantes do projeto:
- Alissana Ester Camargo Pereira, 3371-2294 (e-mail: alissanaester@hotmail.com)
- Luciana Pereira Lobato, 3304-1535 (e-mail: lucianaplobato@gmail.com)
- Yara Souza Mosmann, 3351-7427 (email: yara_mosmann@hotmail.com)
- Clísia M. Carreira, 3371-2475 (e-mail: clisiamc@hotmail.com).
- Profª. Maria Victoria Eiras Grossmann, 3371-4080 (email: victoria@uel.br).
- Profº. Décio Sabbatini Barbosa, 3371-2451 (e-mail: sabatini@sercomtel.com.br)

Médico responsável: Dr. Ricardo Rodrigues
Fone para contato com os médicos: 3324-6232

O telefone do Comitê de Ética em Pesquisa com Humanos é 3371-2490.

Londrina, _____ de_____ de 2010.

_____
Assinatura do Voluntário (a)

_____
Assinatura do (a) Pesquisador (a)

APÊNDICE E - Questionário Inicial para pacientes

Este questionário é direcionado aos voluntários do Projeto “Soja/Aveia” e contém 19 questões referentes a hábitos alimentares e pessoais e condições de saúde.

Ressaltamos que estas informações são sigilosas, ou seja, somente os pesquisadores responsáveis pelo desenvolvimento desta pesquisa terão acesso a elas, ficando totalmente inacessíveis a pessoas alheias à pesquisa, por questões éticas. Desta forma, solicitamos que todas estas informações sejam repassadas de forma transparente.

I IDENTIFICAÇÃO Grupo 1 ( ) Grupo 2 ( ) Grupo 3 ( ) Grupo IV ( )

Nome:				
Idade:	Data de Nascimento: ...../...../.....	Sexo: ( )M ( )F	Estado Civil:	
Endereço:			Nº:	Apto:
Bairro:		CEP:	Cidade:	
Fone Residencial:		Celular:		
E-mail:				
Profissão:		Pressão arterial:		
Médico:		N. prontuário::		

II ESCOLARIDADE

( ) Ensino Fundamental Incompleto ( ) Ensino Fundamental Completo

( ) Ensino Médio Incompleto ( ) Ensino Médio Completo

( ) Ensino Superior Incompleto ( ) Ensino Superior Completo

( ) Especialização ( ) Mestrado ( ) Doutorado

III CONSUMO DE ALIMENTOS ESPECÍFICOS

1. Alimentos fritos

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

2. Tipos de óleo/gordura/azeite que consome diariamente

( ) Óleo de soja

( ) Óleo de milho

( ) Óleo de girassol

( ) Gordura animal (banha de porco)

( ) Manteiga

( ) Gordura vegetal (margarina)

Outro (s): .....

3. Alimentos ‘Light’ (alimentos com teor reduzido de gordura)

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

4. Leite

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

Tipo(s) de leite que consome:

( ) Integral ( ) Semi-desnatado ( ) Desnatado

5. Barras de cereais

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

7. Verduras / Legumes

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

8. Alimentos de ou com Soja (Exceto óleo de soja)

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

Formas de consumo:

( ) Farelos, farinhas, grãos

( ) Alimentos preparados (“leite” de soja, pães, biscoitos, iogurtes, sucos, proteína texturizada de soja (PTS), tofu, natô, missô, etc...)

9. Alimentos de ou com Aveia

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

Formas de consumo:

( ) Farelo, farinha, flocos

( ) Alimentos preparados (pães, biscoitos, barras de cereais, etc..)

10. A respeito de “Alimentos Funcionais”, você:

( ) Desconhece, não sabe do que se trata.

( ) Conhece e consome com freqüência.

( ) Conhece e consome pouco.

( ) Conhece, porém não consome.

IV HÁBITOS PESSOAIS

11. Pratica Atividade Física?*

( ) Sim ( ) Não

*Obs.: Consideram-se atividade física, os exercícios extras que são praticados de forma regular e que não fazem parte da atividade diária, com tempo de duração mínimo de 30 minutos e a freqüência igual ou superior a duas vezes por semana.

12. Com relação ao Tabagismo, você:

( ) Nunca fumou

( ) Ex-fumante (fumou mas não fuma atualmente) Há quanto tempo deixou de fumar:.....

( ) Fumante (fuma atualmente) Quantidade diária de cigarro:.....

13. Bebida Alcoólica*

( ) Não consome

( ) Consumo diário (5 a 6 vezes ou mais por semana)

( ) Consumo semanal (3 vezes ou menos por semana)

( ) Consumo esporádico (1 vez ao mês)

*Obs: Independentemente do tipo de bebida e da quantidade de consumo.

V CONDIÇÕES DE SAÚDE

14. Você é hipertenso? (Tem pressão alta)

( ) Sim ( ) Não ( ) Não sei

Se for hipertenso, faz uso de medicação para hipertensão?

( ) Sim ( ) Não

15. Você tem o hábito de aferir a pressão arterial?

( ) sim ( ) não

Se tem, com que freqüência?

( ) semanalmente ( ) quinzenalmente ( ) mensalmente ( ) esporadicamente

16. Realiza exames laboratoriais periódicos?

( ) Sim ( ) Não

Nº de vezes: ( ) 1 vez/ano ( ) 2 vezes/ano ( ) 1 vez a cada 2 anos (bianual)

Quais exames:.....

17. Com relação a doenças cardiovasculares (DCV) citadas abaixo, indique em qual(is) membro(s) da sua família elas ocorrem:

Membro da família: (....) pai (....) mãe (....) irmão/irmã

18. Função Gástrica

( ) normal ( ) Alguma alteração:.....

19. Função Intestinal

( ) normal ( ) Alguma alteração:.....

Consistência das fezes:

( ) normais ( ) ressecadas ( ) amolecidas

20. DADOS ANTROPOMÉTRICOS

Peso atual (Kg): _____

Altura (m): _____

IMC (kg/m2): _____

Circunferência da cintura: _____

APÊNDICE F - Questionário Final para pacientes

Nome:_____

Grupo ( ) **Data: ____/____/2010**

1. Você sentiu alguma mudança em sua alimentação normal a partir do início do tratamento?

( ) Sim ( ) Não

Qual(is)?.....

2. Alguma mudança no consumo de água e/ou outros líquidos a partir do início do tratamento?

( ) Sim ( ) Não

Qual (is)?.....

3. Após a entrega do exame de sangue ou após o início do tratamento, houve alguma mudança com relação a prática de atividade física?

( ) Sim ( ) Não

Qual (is)?.....

4. A partir do início do tratamento, como se comportou sua função gástrica?
(.....) Normal (não houve alteração) (.....) houve alteração
Se houve alteração, o que ocorreu?.....

5. A partir do início do tratamento, como se comportou sua função intestinal?
(.....) Normal (não houve alteração) (.....) houve alteração
Se houve alteração, o que ocorreu?.....

Consistência das fezes:

( ) normais ( ) ressecadas ( ) amolecidas

6. Durante o estudo, você deixou de consumir algum produto?
(.....) sim (.....) não Quantos?_____

Quais?_____

7. Se a barra de soja que você consumiu estivesse à venda, você a compraria?

(.....) Sim (.....)Não

Por quê?.....

Dados Antropométricos

Peso atual (Kg): _____ IMC (kg/m2): _____

Altura (m): _____ Circunferência da cintura: _____

APÊNDICE G – Recordatório Alimentar de 24h para pacientes

Nome:_____
Data:_____Grupo:_____

Tipo de Refeição	Alimentos	Quantidade Medidas caseiras
Desjejum		
Lanche		
Almoço		
Lanche		
Jantar		
Ceia		

ANEXOS

ANEXO A - Aprovação do Comitê de Ética em Experimentação Animal

UNIVERSIDADE ESTADUAL DE LONDRINA

GOVERNO DO PARANÁ

COMITÊ DE ÉTICA EM EXPERIMENTAÇÃO ANIMAL

OF. CIRC. CEEA Nº 77/2007 Londrina, 19 de novembro de 2007.

Prezada Pesquisadora

O CEEA/UEL, reunido a 13 de novembro do ano corrente, avaliou o projeto de pesquisa intitulado **"Efeito da interação dos componentes da aveia e da soja sobre a colesterolemia, imunoestimulação e viabilidade tecnológica na panificação"**, registrado no CEEA sob o nº 44/07, projeto de Tese do Programa de Pós-graduação em Ciência de Alimentos, desenvolvido sob sua responsabilidade e orientação, julgando-o *aprovado* para execução por entender que os princípios éticos postulados pelo Colégio Brasileiro de Experimentação Animal estão respeitados.

Cumpre orientar que caso se pretendam quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação do CEEA/UEL anteriormente à execução das modificações.

Sem mais para o momento, subscrevo-me.

Cordialmente,

Prof. Dr. Júlio Augusto Naylor Lisbôa
Coordenador do CEEA/UEL

Ilma. Sra.
Profa. Dra. Maria Victoria Eiras Grossmann
Coordenadora e Orientadora do Projeto
Departamento de Ciência e Tecnologia de Alimentos
Centro de Ciências Agrárias

ANEXO B – Aprovação do Comitê de Ética em Pesquisa Envolvendo Seres Humanos

Universidade Estadual de Londrina
COMITÊ DE ÉTICA EM PESQUISA

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná
Registro CONEP 268

Parecer PF Nº 099/09 **CAAE Nº 0071.0.268.000-09** **FOLHA DE ROSTO Nº 256527**	Londrina, 24 de fevereiro de 2010.

PESQUISADORA: **MARIA VICTORIA EIRAS GROSSMANN**
CCA/DCTA

Prezada Senhora:

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná**" (Registro CONEP 268)– de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"EFEITO DA INTERAÇÃO DOS COMPONENTES A AVEIA E DA SOJA SOBRE A COLESTEROLEMIA, IMUNOESTIMULAÇÃO E VIABILIDADE TECNOLÓGICA NO DESENVOLVIMENTO DE BARRAS DE CEREAIS"

Situação do Projeto: **APROVADO**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL relatório final da pesquisa.

Atenciosamente,

Profª. Drª. Ester M.O. Dalla Costa
Coordenadora
Comitê de Ética em Pesquisa-CEP/UEL

Campus Universitário: Rodovia Celso Garcia Cid (PR 445), km 380 - Fone (043) 371-4000 PABX - Fax 328-4440 - Caixa Postal 6.001 - CEP 86051-990 – Internet http://www.uel.br
Hospital Universitário/Centro de Ciências da Saúde: Av. Robert Koch, 60 - Vila Operária – Fone (043) 381-2000 PABX – Fax 337-4041 e 337-7495 - Caixa Postal 791 - CEP 86038-440
LONDRINA - PARANÁ - BRASIL

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