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CARACTERÍSTICAS FÍSICO-QUÍMICAS DE CULTIVARES DE FEIJÃO (*Phaseolus vulgaris* L.), E EFEITOS BIOLÓGICOS DA FRAÇÃO FIBRA SOLÚVEL

DISSERTAÇÃO DE MESTRADO

Ivo Roberto Dorneles Prolla

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CARACTERÍSTICAS FÍSICO-QUÍMICAS DE CULTIVARES DE FEIJÃO (*Phaseolus vulgaris* L.), E EFEITOS BIOLÓGICOS DA FRAÇÃO FIBRA SOLÚVEL

por

Ivo Roberto Dorneles Prolla

Dissertação apresentada ao Curso de Mestrado
do Programa de Pós-Graduação em Bioquímica Toxicológica,
da Universidade Federal de Santa Maria (UFSM, RS),
como requisito parcial para obtenção do grau de
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A Comissão Examinadora, abaixo assinada,
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CARACTERÍSTICAS FÍSICO-QUÍMICAS
DE CULTIVARES DE FEIJÃO (*Phaseolus vulgaris* L.),
E EFEITOS BIOLÓGICOS DA FRAÇÃO FIBRA SOLÚVEL

elaborada por
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como requisito parcial para obtenção do grau de
Mestre em Bioquímica Toxicológica

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Santa Maria, 13 de dezembro de 2006.

DEDICATÓRIA

À minha mãe Idalina, que lutando contra as adversidades do destino criou-me, educou-me e me possibilitou ter um destino diferente do seu.

Aos meus irmãos Gladstone, Jorge, Carmem e Fernando que sempre me cuidaram, apoiaram e valorizaram minha vida profissional.

À minha esposa Alba e à minha filha Luíza, que tiveram paciência e resignação nos momentos em que não estive presente ou disponível como marido e pai.

Tenham certeza que vocês fizeram parte, de uma forma ou de outra, desta obra.

Muito obrigado!

Amo vocês!

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Obrigado a todos vocês.

CONSELHO

Quando te decidires: segue!
Não esperes que o vento
Cubra de flores o caminho.
Nem sequer esperes o caminho.
Cria-o. Faze-o tu mesmo
E parte... Sem lembrar
Que outros passos pararam,
Que outros olhos ficaram te olhando seguir.

(Prado Veppo)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Bioquímica Toxicológica
Universidade Federal de Santa Maria

CARACTERÍSTICAS FÍSICO-QUÍMICAS DE CULTIVARES DE FEIJÃO (*Phaseolus vulgaris* L.), E EFEITOS BIOLÓGICOS DA FRAÇÃO FIBRA SOLÚVEL

AUTOR: IVO ROBERTO DORNELES PROLLA
ORIENTADOR: TATIANA EMANUELLI
Data e Local da Defesa: Santa Maria, 13 de dezembro de 2006.

Foram analisadas as características físico-químicas de sementes cruas de dezesseis cultivares de feijão comum (*Phaseolus vulgaris* L.), ao longo de duas safras consecutivas (2001/2002 e 2002/2003), bem como os teores de amido e fibra alimentar nas sementes após cozimento e estocagem. Avaliaram-se, também, os lipídeos séricos e a glicose sanguínea de ratos normolipidêmicos e normoglicêmicos, alimentados com dietas contendo cultivares de feijão com diferentes relações fibra solúvel/fibra total (FS/FT): dieta Pérola (0,11), dieta Diamante Negro (0,19) e dieta Iraí (0,26); o grupo controle recebeu dieta padrão (com fibra insolúvel). Exceto pelos teores de matéria seca, umidade e fibra alimentar total, as cultivares estudadas mantiveram suas características físico-químicas constantes ao longo das safras. Conforme a similaridade nos teores de macronutrientes (proteína bruta-PB, fibra alimentar total, fibra alimentar insolúvel, fibra alimentar solúvel-FS, amido disponível-AD e amido resistente-AR) as sementes das safras 2001/2002 e 2002/2003 foram categorizadas em quatro grupos distintos; da mesma forma, em relação aos micronutrientes (Fe, Zn, Mn, Cu, Ca, Mg e P), quatro grupos puderam ser identificados. As cultivares Guateian 6662 e Rio Tibagi apresentaram o melhor perfil nutricional (maiores teores de PB, FS, AD, Fe e Zn). A armazenagem sob refrigeração e o congelamento não determinaram alterações nos teores de fibra dos grãos cozidos, mas redução do AD e aumento do AR, principalmente naqueles com AR mais baixo antes do cozimento. Em relação à resposta biológica, os ratos alimentados com dietas contendo feijão apresentaram valores para colesterol sérico e índice glicêmico menores que os do grupo controle ($p<0,05$). Foi observado, também, que apesar do ganho de peso dos animais ter sido semelhante entre os grupos, os ratos alimentados com as dietas contendo feijão apresentaram menor retenção de gordura corporal ($p<0,05$). Os efeitos das dietas sobre os animais experimentais foram mais expressivos no grupo alimentado com a dieta Iraí (FS/FT: 0,26).

Palavras-chave: fibra alimentar; fibra insolúvel; amido disponível; amido resistente; conteúdo mineral; colesterol; curva glicêmica; gordura epididimal.

ABSTRACT

Dissertação de Mestrado
Programa de Pós-Graduação em Bioquímica Toxicológica
Universidade Federal de Santa Maria

PHYSICOCHEMICAL CHARACTERISTICS OF BEAN CULTIVARS (*Phaseolus vulgaris* L.), AND BIOLOGICAL EFFECTS OF SOLUBLE FIBER FRACTION

AUTHOR: IVO ROBERTO DORNELES PROLLA
ADVISER: TATIANA EMANUELLI
Date and Place of the defense: Santa Maria, December 13th, 2006

Raw seeds of sixteen common bean (*Phaseolus vulgaris* L.) cultivars were evaluated along two consecutive harvests (2001/2002 and 2002/2003) concerning their physicochemical characteristics, as well as the effect of cooking and storage conditions on starch and dietary fiber contents. Serum lipids and blood glucose levels were also evaluated in normolipidemic-normoglycemic rats witch were fed diets containing bean cultivars with different soluble fiber/total fiber ratios (SF/TF): Pérola diet (0.11), Diamante Negro diet (0.19) and Iraí diet (0.26); control group was fed a standard diet (with insoluble fiber). Except for dry matter, moisture, and total dietary fiber, cultivars kept their chemical characteristics between harvests. Regarding similarity among macronutrient levels (crude protein-CP, total dietary fiber, insoluble dietary fiber, soluble dietary fiber-SF, digestible starch-DS, and resistant starch-RS) seeds from harvests 2001/2002 and 2002/2003 were categorized into four different groups; the same was done for micronutrients (Fe, Zn, Mn, Cu, Ca, Mg, and P), and four groups were also identified. Guateian 6662 and Rio Tibagi were considered the cultivars with the best nutritional profile (highest levels of CP, SF, DS, Fe, and Zn). Storage under refrigerated or freezing conditions did not change fiber content of cooked beans, but decreased their DS content and increased RS content, mainly in seeds with low RS levels before cooking. Concerning biological response, rats fed bean diets experienced lower values for serum cholesterol ($P<0.05$) and lower glycemic indexes ($P<0.05$). It was also observed a similar weight gain among groups, however animals fed bean based diets showed lower fat retention ($P<0.05$). The effects of bean diets on experimental groups were more remarkable in animals fed Iraí diet (SF/TF: 0.26).

Keywords: dietary fiber; insoluble fiber; digestible starch; resistant starch; mineral content; cholesterol; glycemic curve; epididymal fat.

LISTA DE ABREVIATURAS

ACG: área sob a curva glicêmica
AD: amido disponível
AR: amido resistente
Ca: cálcio
Cu: cobre
FB: fibra bruta
Fe: ferro
FI: fibra alimentar insolúvel
FS/FT: relação fibra solúvel/fibra total
FS: fibra alimentar solúvel
FT: fibra alimentar total
g N: gramas de nitrogênio
HC: carboidrato
HDL: "high-density-lipoprotein"
IG: índice glicêmico
LDL: "low density lipoprotein"
Mg: magnésio
Mn: manganês
MS: massa seca
P: fósforo
PB: proteína bruta
RS: Rio Grande do Sul
Zn: zinco

LISTA DE ANEXOS

SUMÁRIO

APRESENTAÇÃO

Os resultados que fazem parte desta dissertação são apresentados sob a forma de manuscritos, os quais se encontram no item RESULTADOS. As seções Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se nos próprios manuscritos e representam na íntegra este estudo.

Os itens DISCUSSÃO e CONCLUSÃO contêm interpretações e comentários gerais referentes aos manuscritos contidos neste estudo.

A BIBLIOGRAFIA refere-se às citações que aparecem nos itens INTRODUÇÃO, REVISÃO BIBLIOGRÁFICA e DISCUSSÃO desta dissertação.

1 INTRODUÇÃO

Nos últimos anos inúmeras pesquisas foram conduzidas com o objetivo de relacionar o tipo de dieta consumida pelos povos e as doenças como diabete, deslipidemias, obesidade e doenças cardiovasculares. Assim, muitos alimentos passaram a ser considerados na etiopatogenia destas enfermidades. Porém, outros se destacaram pelos efeitos protetores de seus nutrientes sobre o funcionamento de tecidos, órgãos e sistemas: os chamados alimentos funcionais. Estes, apresentam propriedades que podem influenciar na evolução e prognóstico de muitas destas doenças cujas taxas de morbidade e mortalidade são ainda elevadas em nosso meio.

Uma vez que os alimentos apresentam diferenças marcantes no perfil bioquímico de seus nutrientes, os efeitos funcionais a eles relacionados podem, também, diferir de forma significativa. Isto é vital em nutrição humana, principalmente em situações adversas de saúde. Nestas situações, a prescrição de alimentos funcionais dentro de um plano dietoterápico específico poderia auxiliar no controle e também na prevenção de muitas enfermidades.

Dos diversos alimentos utilizados pelo homem, as sementes da família das leguminosas desempenham importante papel na dieta da maioria das populações, sendo o feijão comum (*Phaseolus vulgaris* L.) um dos mais consumidos, inclusive no Brasil. É rico em proteínas (19,6 a 26% da massa seca - MS), carboidratos complexos de baixo índice glicêmico (64 a 71% da MS) e pobre em gordura (1 a 2% da MS) (VIEIRA, 1967); é fonte importante de fibras (HARO et al., 1995) e de amido resistente (SAURA-CALIXTO et al., 1992). Por isto, é um dos alimentos que contempla as recomendações dietéticas atuais.

Estudos já demonstraram que as leguminosas, dentre estas o feijão, possuem propriedades reguladoras dos níveis de glicemia e insulina (FOSTER-POWELL & MILLER, 1995). Como há estreita relação entre diabete, hiperinsulinemia e deposição de gordura, o consumo de feijões poderia, de forma preventiva, influenciar no surgimento da obesidade. Além disto, outros estudos demonstraram que o feijão também é capaz de reduzir os níveis séricos de colesterol e, assim, o risco de doenças cardiovasculares (ANDERSON et al., 1984; BAZZANO et al., 2001; HAN et al., 2003).

Dentre os nutrientes encontrados no grão de feijão, a fração fibra solúvel parece desempenhar um importante papel como alimento funcional. Isto pode ser observado em estudos que avaliaram os efeitos de dietas contendo feijão como fonte de fibra solúvel e o comportamento dos lipídeos e glicose sanguíneos após a sua ingestão (ANDERSON & GUSTAFSON, 1988)

As cultivares de feijão apresentam diferenças agronômicas e tecnológicas notáveis. Isto ocorre principalmente devido ao perfil genotípico da planta. Porém, o melhoramento genético e as adversidades climáticas impostas à planta também influenciam sobremaneira este perfil. Estas alterações acabam por afetar a composição química das sementes, com aperfeiçoamento de algumas características em detrimento de outras. Isto pode ser comprovado por estudos envolvendo cultivares de *Phaseolus vulgaris* plantadas em diferentes regiões do mundo, onde as sementes têm apresentado diferenças marcantes na sua composição química (VIEIRA, 1967; SOTELO et al., 1995; YANEZ et al., 1995; SAMMAN et al., 1999; CASTELLÓN et al., 2003).

No Rio Grande do Sul (RS), assim como em nosso país, a lista de cultivares de feijão indicadas para o plantio é extensa. Uma vez que o perfil nutricional dos grãos pode ser influenciado pelo ano de plantio, a variabilidade na composição química das cultivares aqui plantadas e consumidas também deve ser significativa. Além disto, o processamento doméstico habitual dos grãos (cozimento e refrigeração) poderia determinar outras alterações relevantes na sua composição química.

Se todas estas alterações realmente determinam mudanças bioquímicas profundas nos grãos de feijão, estas diferenças poderiam ser mais bem exploradas na nutrição humana. Assim, cultivares de feijão com teores elevados da fração fibra solúvel, por exemplo, poderiam ser preferidas dentro de um plano de orientação dietética para pacientes portadores de deslipidemias, diabete, obesidade e doenças cardiovasculares. Além disso, estas cultivares seriam fortemente recomendadas para o consumo geral, uma vez que deteriam propriedades preventivas em pessoas com risco aumentado de desenvolverem alguma daquelas enfermidades.

Este estudo objetiva avaliar a hipótese de que feijões apresentam variações importantes nos teores de fibra solúvel e que cultivares com teores elevados desta fração apresentam efeito redutor dos lipídeos séricos e da glicemia, mesmo em situações em que estes valores encontram-se dentro da normalidade. Assim, estas cultivares poderiam ser preferentemente indicadas na orientação dietética de pacientes com ou em risco de desenvolverem doenças relacionadas ao metabolismo dos lipídios e/ou da glicose. Estes resultados reforçariam a idéia de que tabelas de composição alimentar devem ser mais completas e detalhadas quanto aos nutrientes de relevância clínica. Ainda, o cruzamento de cultivares de feijão com características nutricionais peculiares poderia ser estimulado, a fim de que fossem obtidas novas cultivares com perfis bioquímicos mais adequados a outras enfermidades crônicas prevalentes.

Desta forma, o presente estudo foi delineado com base em questionamentos que nortearam o desenrolar desta pesquisa. Foram eles:

1. As cultivares de feijão (*Phaseolus vulgaris* L.) plantadas e consumidas em nosso meio diferem significativamente entre si quanto às características físico-químicas?
2. Uma mesma cultivar pode alterar significativamente suas características físico-químicas de uma safra para outra?
3. Processos de cozimento e armazenamento podem alterar os teores de amido e de fibra do feijão?
4. As diferenças genéticas e as alterações no perfil químico (secundárias ao processamento doméstico), se existirem, atingem de forma relevante a fração fibra solúvel?
5. Se a diferença nos teores de fibra solúvel for significativa, cultivares com teores mais elevados podem determinar redução nos níveis de lipídeos e glicose sanguíneos, mesmo em situações de normolipidemia e normoglicemia?

Para respondermos a estes questionamentos elaboramos os seguintes objetivos específicos:

1. Determinar e comparar as características físico-químicas de sementes cruas de dezesseis cultivares de feijão comum (*Phaseolus vulgaris* L.) recomendadas para plantio no RS;
2. Avaliar a persistência destas características ao longo de duas safras consecutivas (2001/2002 e 2002/2003);
3. Estudar os efeitos da estocagem das sementes após cozimento seguido de refrigeração ou congelamento sobre teores de amido disponível, amido resistente, fibra alimentar total, insolúvel e solúvel;
4. Avaliar os efeitos de dietas experimentais contendo cultivares de feijão com diferentes relações fibra solúvel/fibra total sobre os níveis de lipídeos séricos

(triglicerídeos, colesterol total, colesterol HDL), glicemia e outros parâmetros biológicos em ratos normolipidêmicos e normoglicêmicos.

2 REVISÃO DA LITERATURA

2.1 A cultura do feijão

Existem várias espécies de sementes que, maduras, servem como alimento a uma grande parte da população mundial. Algumas destas são produzidas por plantas chamadas leguminosas graníferas onde cerca de 20 espécies são utilizadas na alimentação em quantidades apreciáveis, numa ou noutra região do mundo.

As mais importantes espécies de leguminosas são o guandu, o caupi, a ervilha, a lentilha, o grão-de-bico, a fava, o feijão-fava, os feijões asiáticos do gênero *Vigna,* o amendoim, a soja e o feijão comum. Este último, segundo relatos na literatura, já fazia parte da dieta de muitos povos antepassados. No Peru, foram encontrados restos de feijão comum com 8.000 anos; em dois locais no México foram encontrados restos com 4.300 a 7.000 anos de idade. Mas, no norte da Argentina foram encontrados os restos mais antigos até agora descritos, com 6.700 a 9.600 anos de idade (VIEIRA et al., 2001).

No Brasil o cultivo do feijão é bastante difundido em todo o território nacional, principalmente como cultura de subsistência em pequenas propriedades. Nosso país é o segundo produtor mundial de feijão (*Phaseolus*), perdendo apenas para a Índia e seguido pela China; é o primeiro na espécie *vulgaris*, seguido pelo México. Apesar disto, a produção brasileira é insuficiente para abastecer o mercado interno, necessitando importar este produto de outros países da América Latina e dos Estados Unidos (YOKOYAMA, 2002).

O feijão comum ou simplesmente feijão pertence à família *Leguminosae*, sub-família *Papipilionoideae*, gênero *Phaseolus* cujo nome científico é *Phaseolus vulgaris* L.. É uma planta herbácea, trepadora ou não, levemente pubescente, cujo ciclo de vida varia de, aproximadamente, 65 a 120 dias, dependendo da cultivar e das condições e época de plantio. Pode apresentar quatro tipos de hábitos de crescimento sendo um tipo chamado determinado e outros três definidos como indeterminados. Com vagens retas ou ligeiramente curvas, achatadas ou arredondadas, com bico reto ou curvado, em geral com 9 a 12 cm de comprimento e com 3 a 7 sementes (ALMEIDA & CANÉCHIO FILHO, 1987; VIEIRA et al., 2001).

O gênero *Phaseolus* compreende aproximadamente 55 espécies das quais apenas cinco são cultivadas: o feijoeiro comum (*Phaseolus vulgaris*); o feijão de lima (*P. lunatus*); o feijão ayocote (*P. coccineus*); o feijão tepari (*P. acutifolius*); e o *P. polyanthus* (EMBRAPA ARROZ e FEIJÃO, 2004).

O feijão é uma planta muito exigente. Para plantar feijão com êxito comercial, a escolha da área onde será feita a cultura é de importância fundamental. Devem-se preferir áreas que tenham outono e primavera mais ou menos longos, suficientes para completar o ciclo do feijoeiro, não se prestando aquelas onde o verão e o inverno são muito rigorosos. A temperatura média ótima para a cultura varia de 10^{o} a 25^{o}C (ALMEIDA & CANÉCHIO FILHO, 1987). A alta temperatura (superior a 30^{o}C) pode ocasionar, dependendo da cultivar, diminuição da percentagem de flores que vingam e do número de sementes por vagem. Por outro lado, as baixas temperaturas são desfavoráveis ao crescimento, e as geadas podem causar enormes danos à cultura (VIEIRA et al., 2001).

O feijoeiro prospera em diferentes tipos de solos, exceto nos compactos, nos salinos e nos encharcados. Os solos preferidos são os de textura areno-argilosa, leves, soltos, ricos em matéria orgânica e em elementos fertilizantes, e com pH entre 6 a 7,5, considerado adequado para um crescimento ótimo (ALMEIDA & CANÉCHIO FILHO, 1987).

Tanto o excesso quanto a escassez de água são prejudiciais. A alta umidade pode favorecer as doenças, pois o feijoeiro não tolera água estagnada, mesmo que por curto espaço de tempo. A seca, também prejudicial, pode ser contornada pela irrigação, principalmente na época da floração e do vageamento (VIEIRA et al., 2001). A precipitação pluviométrica ideal na época do plantio situa-se na faixa dos 100 mm (ALMEIDA & CANÉCHIO FILHO, 1987). Já durante a colheita, condições de seca são essenciais para a obtenção de sementes de boa qualidade (VIEIRA et al., 2001).

No Brasil o feijão pode ser plantado mais de uma vez por ano sendo comum, entre os pequenos agricultores, a prática deste cultivo de permeio com mandioca, cana, café e outras culturas, principalmente com o milho. Desta forma, é possível explorar a cultura em três épocas diferentes no mesmo ano: a safra “das águas”, cujo plantio é feito de agosto a novembro, com predominância na região sul; o plantio “da seca”, realizado de janeiro a março, abrangendo a maioria dos estados produtores; e o plantio “de inverno”, de abril a julho, realizada nas regiões centro-oeste e sudeste. As duas primeiras safras são responsáveis por 90% da produção nacional e provém de lavouras de pequenos e médios produtores, com baixo nível tecnológico; os outros 10% provém da safra "de inverno", de lavouras com alto nível tecnológico principalmente quanto à irrigação, determinando uma alta produtividade por hectare plantado (EMBRAPA ARROZ e FEIJÃO, 2004).

As cultivares de feijão plantadas no Brasil terminam o ciclo de vida em 80 a 100 dias (alguns em menos tempo: 65 a 75 dias) e a colheita costuma ser feita manualmente, mecanicamente ou pela combinação de ambas.

O feijão recém colhido normalmente apresenta-se com umidade alta, em torno de 15%, e muitas vezes com as sementes abrigando ovos de carunchos. Por isto, logo após a colheita, deve-se proceder à secagem (com redução da sua umidade para 10 a 11%), beneficiamento, fumigação e estocagem das sementes em ambiente arejado, com baixa umidade, a temperatura de 21^{o}C mantendo-as protegidas de insetos, de carunchos e da luz. Para temperaturas mais elevadas (26,5^{o}C), a umidade das sementes deve ser rebaixada até 8% para uma conservação segura. Desta forma, as sementes podem ser estocadas por dois anos com manutenção adequada de seu poder germinativo (VIEIRA et al., 2001).

2.2 Características físico-químicas dos grãos de feijão

A semente do feijão pode apresentar as mais diferentes cores: branca, negra, vermelha, amarela, parda, rósea, creme, alaranjada, bege, roxa e outras, muitas vezes com estrias ou sarapintas de diferentes cores. Há também as bicolores e as que exibem uma segunda cor no hilo. Atualmente, em nosso meio, os tipos comerciais mais aceitos são: o carioca (bege com estrias pardacentas), o preto e o mulatinho (bege). As demais têm menor aceitação comercial ou aceitação limitada a certas regiões do país. A massa das sementes também é bastante variável, com pesos de 12 até 70 gramas por 100 unidades. Os feijões carioca, preto e mulatinho, por serem pequenos, em geral pesam de 15 a 25 g/100 unidades. Já os feijões do tipo manteigão, que são graúdos, apresentam pesos geralmente superiores a 35 g/100 unidades, alguns ultrapassando 50 g. Geralmente as formas silvestres de *P. vulgaris* produzem sementes menores que as formas cultivadas (VIEIRA et al., 2001)

VASQUEZ-CARRILLO & CARDENAS-RAMOS (1992) avaliaram algumas características físicas, tecnológicas e protéicas de 4 cultivares de feijão (*P. vulgaris*) desenvolvidas no México e 12 sementes de feijões selvagens, sendo 8 mexicanas e 4 sul-americanas. As sementes sul-americanas mostravam um tamanho similar entre si (em média

10,4 g/100 sementes), mas diferentes capacidades de absorver água (de 36 a 64%), percentagens de casca e sólidos, e teores de proteína (de 23,8 a 27,2%). As sementes selvagens mexicanas foram as menores do estudo (2,8 g/100 sementes) e com a menor capacidade de absorver água. Estas continham a maior quantidade de casca e durante o processo de cozimento perdiam pequena quantidade de solutos para a água. O conteúdo protéico variou de 21,3 a 24,6%. As sementes cultivadas apresentaram as melhores características físicas e tecnológicas, o menor tempo de cozimento, mas o menor teor protéico do estudo (22,3%).

No Brasil, a lista de cultivares indicadas para plantio é extensa. Cada estado tem a sua própria recomendação, às vezes com indicação por região. Os feijões dos tipos carioca e preto, seguidos pelo mulatinho são os mais indicados. No Rio Grande do Sul, as cultivares indicadas para as safras 2002/2003 e 2003 foram: Guapo Brilhante, Macotaço, Diamante Negro, Pérola, FTS Magnífico, FTS Soberano, TPS Bonito, TPS Bionobre e TPS Nobre (BRASIL, 2002 a; 2002 b).

É sabido que a composição química das sementes é bastante variável, dependendo da cultivar. VIEIRA (1967) avaliou a composição de 17 cultivares de feijão e encontrou, com base na matéria seca, as seguintes variações: proteína bruta, de 19,6 a 26,0%; extrato etéreo, de 1,1 a 1,9%; cinzas brutas, de 3,3 a 4,3%; fibra bruta, de 2,8 a 5,5%; e extrato não-nitrogenado, de 63,9 a 70,9%. A Embrapa, em seus comunicados técnicos, cita valores semelhantes (matérias protéicas 22%, matérias graxas 1,5%, cinzas 4%, fibras 4,5%, umidade 12% e carboidratos 56%).

As variações na composição química podem ser atribuídas às modificações genéticas impostas à planta ou às condições ambientais durante o plantio. Em Fortaleza, Ceará, CASTELLÓN et al. (2003) avaliaram a fração lipídica de 6 cultivares comerciais de feijão caupi, e encontraram diferenças importantes nos teores de ácido palmítico, ácido linoléico e ácidos graxos pentacosanóico e eicosanóico. Concluíram que o melhoramento genético pode determinar diferenças quantitativas e qualitativas na composição bioquímica. Concluíram, também, que mudanças na capacidade germinativa, resistência a predadores, etc. podem alterar a expressão de genes codificadores de síntese de moléculas relevantes, levando a diferentes composições químicas.

Em um estudo publicado em 1999, os autores avaliaram a composição de algumas variedades de feijão plantadas em 7 regiões da Argentina (SAMMAN et al., 1999). Estes autores encontraram valores variáveis para o amido (12 a 14%), para as proteínas (18 a 22%), para a gordura (0,7 a 1,2%) e também para os minerais (cobre: 0,8 a 1,2 mg/100 g; ferro: 9 a 18 mg/100 g; zinco: 2,5 a 4,0 mg/100 g; e fósforo: 295 a 542 mg/100 g) e concluíram que conforme a região, diferenças importantes na composição química do feijão são encontradas.

SOTELO et al. (1995), no México, avaliando a composição química de feijões selvagens e cultivados, observaram que estes últimos, apesar de apresentarem menores proporções de proteínas (21,7 vs. 25,5%), cinzas (4,2 vs. 5,2%) e fibra bruta (5 vs. 7,1%), maiores quantidades de carboidratos (68,1 vs. 61,6%) e melhor perfil de aminoácidos. Com isto, demonstraram que, sob o ponto de vista químico, a domesticação parece ter efeitos positivos.

O conteúdo protéico dos grãos dos cereais varia, aproximadamente, de 8 a 16%. Uma dieta de cereais em quantidade adequada pode satisfazer as necessidades de calorias do consumidor, mas não lhe fornecerá a quantidade de proteínas, especialmente quando se trata de crianças e mulheres grávidas. Nos países com melhores condições nutricionais, esse déficit protéico é compensado pelos alimentos de origem animal, mais caros que os de origem vegetal. Já nos países em desenvolvimento da África, da Ásia e da América Latina, as leguminosas graníferas são utilizadas como fonte muito mais barata de proteínas e, por este motivo, são chamadas de "carne do pobre". Contêm de 18 a 35% de proteínas, sendo que a soja pode alcançar mais de 40%.

Enquanto as sementes de leguminosas são ricas em lisina, os cereais são ricos em aminoácidos sulfurados (metionina e cistina). Desta forma, a proporção mais adequada entre cereal e leguminosa que equilibraria as deficiências parece ser de 2,6:1. Isto foi demonstrado por ABD-EL-SAMEI et al. (1984), que realizaram um estudo comparativo sobre a composição de aminoácidos em três variedades locais de sementes de *P. vulgaris*. Eles observaram que a composição dos aminoácidos foi semelhante, sendo que todos apresentavam deficiência de aminoácidos sulfurados, um teor elevado de lisina (8,1 a 8,6 g/16 g N), ausência de cistina, fenilalanina e tirosina nos extratos das três variedades. Verificaram, também, uma correlação negativa entre o conteúdo de aminoácidos sulfurados e percentagem de proteínas nas sementes.

Em 1995, YANEZ et al. publicaram um artigo onde avaliavam a composição química e a qualidade biológica da proteína de 5 cultivares de feijão comum recém lançadas para uso no Chile. O conteúdo protéico bruto encontrado variava de 22 a 27% e a resposta biológica em ratos mostrou que os animais alimentados com a cultivar com mais proteínas ganhavam mais peso e apresentavam maior captação/incorporação protéica.

Além dos aspectos inerentes às proteínas das leguminosas, as mesmas ainda influenciam a formação de outros compostos de relevantes propriedades nutricionais; dentre eles, o amido resistente (AR). SAURA-CALIXTO et al. (1992) estudaram a formação de AR em feijão desproteinizado e não desproteinizado, inteiro e moído, após cocção e congelamento. Os maiores teores de AR foram obtidos nas preparações com feijão inteiro (3,7 a 8,7%). O feijão moído e o moído e desproteinizado apresentaram os menores teores (1,6 e 0,9%, respectivamente). Concluíram que a presença de proteína e o tamanho das partículas são os principais fatores que afetam a formação de AR.

Em relação às fibras, as leguminosas são reconhecidamente fontes importantes deste polissacarídeo. Em um estudo que avaliou a composição química, fibra dietética e conteúdo mineral de 15 alimentos freqüentemente consumidos no noroeste do México, o feijão foi o alimento que apresentou os maiores teores de fibra (9,2 g/100 g) (HARO et al., 1995).

A literatura pertinente contém várias publicações envolvendo características físico-químicas de cultivares de feijão das mais diferentes regiões do globo. Este é o primeiro estudo brasileiro que caracteriza e avalia a persistência ao longo de 2 safras de parâmetros físico-químicos em um número expressivo de cultivares de *Phaseolus vulgaris* L. plantadas e consumidas em nossa região.

2.3 Alterações secundárias ao processamento doméstico e ao tempo de estocagem

Além das alterações nas características químicas das sementes devido à variedade genética e aos fatores ambientais, processos de cozimento e armazenamento também podem produzir alterações importantes na composição dos grãos, principalmente nos teores de amido e fibra (KUTOS et al., 2003; VARGAS-TORRES et al., 2004).

É dito que as condições e o tempo de estocagem podem alterar algumas propriedades físicas e químicas dos grãos. No entanto, em um estudo realizado por HERNANDEZ-UNZON & ORTEGA-DELGADO (1989), no México, avaliando alterações possíveis em sementes de feijão estocadas por até oito anos, não foram observadas diferenças significativas nas composições químicas. Porém, sementes com 5 a 6 anos de estocagem absorviam mais água que aquelas com menos tempo, mas custavam mais a cozinhar. A diferença realmente importante observada foi a redução entre 94 e 98% do ácido fítico durante a estocagem longa.

Além do tempo de armazenamento, os diferentes métodos de cocção podem, ou não, modificar química e fisicamente os grãos. Um estudo realizado na Espanha avaliou as propriedades nutricionais após o cozimento de feijões por extrusão (MARTIN-CABREJAS et al., 1999). Para isto, foram utilizados tanto feijões frescos quanto feijões armazenados por 6 semanas e por 1 ano. Observaram aumento do tempo de cozimento destes de 7,7 e de 12 vezes, respectivamente, devido à dureza que se instalou. Nas amostras extrusadas houve incremento na absorção de água devido à maior proporção de amido gelatinizado. A extrusão causou, também, redistribuição da fibra insolúvel para solúvel, embora a fibra dietética total permanecesse igual. Não observaram, porém, diferenças físicas e/ou químicas entre feijões estocados ou frescos após o processo de extrusão.

No Chile, ESTEVEZ et al. (1991), estudando os efeitos do cozimento e secagem em várias leguminosas, observaram que nos feijões das variedades Tortola e Coscorron, não havia mudanças significativas nas proteínas, mas sim o aumento da digestibilidade das mesmas.

Um estudo italiano avaliou de forma comparativa os efeitos do cozimento convencional e ao microondas de grão-de-bico e de feijão comum. Os feijões cozidos ao microondas apresentaram uma redução drástica no tempo de cozimento (de 55 para 9 min), uma menor perda sólida para a água de cozimento (de 7,5 para 4,5 g/100 g de semente seca de feijão) e um aumento da digestibilidade do amido, devido à redução do amido resistente (de 32,4%, na semente crua, para 10% após o cozimento) e à elevação do amido rapidamente digerível (de 27,5% para cerca de 80%). Houve, também, uma redistribuição dos polissacarídeos não amiláceos de insolúveis para solúveis, mas sem alteração nos teores totais (MARCONI et al., 2000).

Na Guatemala, ACEVEDO et al. (1994) avaliaram as alterações decorrentes do processo de cozimento e fritura de feijões pretos. Observaram aumento da fibra dietética insolúvel de 18,1% no feijão cozido para 22,4% no feijão frito; diminuição da fibra dietética solúvel de 8,4 para 6,6% nestas mesmas preparações, assim como redução nos teores de amido (34,5 para 31,3%). A digestibilidade e a qualidade protéica, no entanto, reduziram-se do feijão cozido para o frito, correlacionando-se com a fibra dietética insolúvel.

Os estudos envolvendo a fração fibra alimentar são, ainda, controversos sendo que tanto o aumento quanto a redução nos teores de fibra total, solúvel e insolúvel de feijões cozidos foram relatados (HUGHES & SWANSON, 1989; KUTOS et al., 2003). No entanto, estudos sobre os efeitos do cozimento seguido de armazenamento nos níveis destes carboidratos não foram encontrados em feijões.

Quanto à fração amido, alguns autores demonstraram redução nos níveis de amido disponível e aumento nos de amido resistente em feijões cozidos estocados a 4°C (VARGAS-TORRES et al., 2004); outros, ao contrário, encontraram aumento do amido disponível (OSORIO-DIAZ et al., 2003); porém LANDA-HABANA et al. (2004) não encontraram alterações.

Como visto, a composição química dos grãos de feijão pode ser profundamente alterada após processos de cozimento e armazenamento sob refrigeração. Estas mudanças podem, assim, afetar a digestibilidade e, conseqüentemente, o valor nutricional de dietas formuladas com esta leguminosa.

2.4 Propriedades nutritivas e funcionais do feijão

As propriedades nutritivas relacionadas ao feijão comum baseiam-se no fato de ser fonte rica de proteínas (19,6 a 26% da MS), de carboidratos complexos de baixo índice glicêmico (64 a 71% da MS) e pobre em gordura (1 a 2% da MS) (VIEIRA, 1967), o que o torna um alimento apropriado às recomendações dietéticas atuais. É considerado a “carne do pobre” em função de seu elevado teor protéico que, juntamente com o arroz, fornece os aminoácidos necessários à nutrição humana.

Além disto, apresenta muitos efeitos funcionais atribuídos a sua composição química peculiar, pois é também uma fonte importante de fibras (HARO et al., 1995) e de amido resistente (SAURA-CALIXTO et al., 1992).

Há muitos anos, pesquisas são desenvolvidas no intuito de relacionar os alimentos ingeridos pelos povos e o surgimento e a evolução de doenças crônicas. Estas, que tipicamente cursam por dez ou mais anos, incluem principalmente o diabete melito, as deslipidemias, a obesidade e as doenças cardiovasculares. Estudos em animais e em humanos já demonstraram efeitos benéficos das leguminosas sobre algumas destas doenças, inclusive efeitos protetores do feijão sobre o surgimento de câncer (CORREA, 1981; HUGHES et al.,

1997; SING & FRASER, 1998; HANGEN & ENNINK, 2003). O mecanismo pelo qual esta proteção ocorre e qual componente tem propriedades anticarcinogênicas ainda não são conhecidos. O que se sabe é que níveis elevados de insulina (GIOVANNUCCI, 1995; SANDHU et al., 2002) e/ou altos níveis de glicemia (MCKEOWN-EYSSEN, 1994) podem estimular o surgimento de alguns tipos de câncer como o dos cólons. Sabe-se, também, que o consumo de alimentos com alto índice glicêmico por períodos longos pode levar à hiperinsulinemia, resistência à insulina e diabete melito tipo dois. Baseado nisto, um mecanismo provável pelo qual feijões inibiriam o surgimento de câncer estaria relacionado à regulação da glicemia e da insulina, uma vez que é considerado um alimento de baixo índice glicêmico (IG) (FOSTER-POWELL & MILLER, 1995).

Na atualidade, interações existentes entre dieta de elevado IG, diabete e obesidade são muito estudadas. Alimentos com alto IG estimulam a fome e levam à seleção progressiva de alimentos de IG sempre elevados. Forma-se, assim, um ciclo vicioso com ingestão preferencial de alimentos densamente calóricos, hiperglicemia e hiperinsulinemia (LUDWIG, 2002). As duas últimas, juntamente com a obesidade desenvolvida, são reconhecidamente fatores de risco para o câncer e patologias cardiovasculares, respectivamente.

Além dos distúrbios metabólicos já mencionados, as deslipidemias constituem um risco importante para as doenças do coração. É sabido que uma redução de 1% nos níveis de colesterol total reduzem em 2% o risco de uma pessoa desenvolver esse tipo de afecção (RIFKIND, 1984). Os grãos de feijão comum têm demonstrado um importante efeito redutor dos níveis séricos de lipídios (FUKUSHIMA et al., 2001). Tanto em animais (HAN et al., 2003) quanto em humanos (ANDERSON et al., 1984; BAZZANO et al., 2001) estudos demonstram que o feijão é capaz de reduzir os níveis séricos de colesterol e, conseqüentemente, o risco de doenças cardiovasculares. Em pacientes hiperlipidêmicos, a fração solúvel da fibra de feijão reduziu o colesterol total (13 a 26%), cholesterol HDL (5 a 20%) e triglicerídeos (3 a 25%) (ANDERSON & GUSTAFSON, 1988). Embora alguns autores tenham atribuído este efeito à fração amido, presumivelmente ao conteúdo de saponinas (AMIGO et al., 1992), a fibra solúvel parece ser realmente a porção ativa do grão (ALLER et al., 2004).

Poucos estudos avaliaram os níveis de fibra dietética e de AR em feijões (KUTOS et al., 2003; VARGAS-TORRES et al., 2004; LANDA-HABANA et al., 2004), enquanto muitos estudos avaliaram o conteúdo de amido total e fibra bruta, os quais não fornecem dados esclarecedores sobre as implicações nutricionais destes componentes (VIEIRA, 1967; ANTUNES et al., 1995; CASTELLÓN et al., 2003; LEMOS et al., 2004; SHIMELIS & RAKSHIT, 2005). Os carboidratos são a maior fração encontrada nas sementes de feijão. O conhecimento atual sobre as propriedades do amido indica que uma fração deste carboidrato é resistente à digestão enzimática no intestino delgado. Esta porção é denominada amido resistente (AR). Este pode ser um substrato para o processo de fermentação nos cólons com efeitos benéficos para a saúde humana, semelhante àqueles determinados pela fibra dietética.

O feijão comum apresenta, também, um efeito redutor da glicose plasmática (PARI & VENKATESWARAN, 2003). Esta propriedade tem sido relacionada não só à digestibilidade do amido, mas aos conteúdos de proteínas e de fibras. A fração fibra parece reduzir a resposta glicêmica devido à redução do esvaziamento gástrico e das taxas de absorção intestinal (THORNE et al., 1983). Esta resposta glicêmica é avaliada pelos valores de glicemia ao longo do tempo após a ingestão do alimento que é conhecida como curva glicêmica. Porém, atualmente, tem sido expressa como índice glicêmico (IG) ou área sob a curva glicêmica (ACG). Dietas com elevado IG estão associadas a maiores respostas insulinêmicas e risco aumentado de obesidade (PAWLAK et al., 2001). O feijão comum é considerado um alimento de baixo IG e, por isso, esta leguminosa pode ter importante papel na nutrição humana.

É sabido que grãos de feijão são excelente fonte de fibra alimentar, fornecendo uma mistura de fibras solúveis e insolúveis (MESSINA, 1999) com todos os efeitos notáveis relacionados a elas. Entretanto, as cultivares de feijão podem diferir significativamente em suas composições químicas, incluindo a fração fibra alimentar. Desta forma, suas propriedades sobre os lipídios e a glicose sanguíneos também podem ser variáveis.

Até o momento, a maioria dos estudos relacionados aos efeitos de dietas baseadas em feijões foram realizados em ratos hipercolesterolêmicos (DABAI et al., 1996; ROSA et al., 1998 a; 1998 b) ou com diabete induzido (PARI & VENKATESWARAN, 2003). Nestes modelos, o consumo de feijões melhorou significativamente os níveis séricos de gordura e de glicose. No entanto, não há relatos na literatura pertinente de estudos que tenham testado dietas contendo teores diferenciados de fibra solúvel de feijão sobre lipídeos e glicemia sanguíneos em animais metabolicamente normais.

3 RESULTADOS

Os resultados desta dissertação estão apresentados sob a forma de manuscritos, os quais se encontram aqui organizados. As apresentações dos manuscritos estão baseadas na versão para submissão, sendo que os roteiros para os autores estão anexados.

3.1 Manuscrito 1

CULTIVAR, HARVEST YEAR, AND STORAGE CONDITIONS AFFECTING COMPOSITION OF COMMON BEANS *(Phaseolus vulgaris* L.*)*

Manuscrito submetido à revista

LWT-FOOD SCIENCE AND TECHNOLOGY

Cultivar, harvest year, and storage conditions affecting composition of common beans (*Phaseolus vulgaris* L.)

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Abstract

Sixteen common bean cultivars were compared concerning the physicochemical characteristics of their raw seeds along two consecutive harvests, as well as the effect of storage conditions on starch and dietary fiber content of cooked beans. It was possible to identify groups of cultivars with different nutritional features. Bean cultivars were categorized into four different groups according either to their macronutrient contents (crude protein-PROT, total dietary fiber-TDF, insoluble dietary fiber-IDF, soluble dietary fiber-SDF, digestible starch-DS, and resistant starch-RS) or to their micronutrient levels (Fe, Zn, Mn, Cu, Ca, Mg, and P). Guateian 6662 and Rio Tibagi appeared to be the most adequate cultivars to prevent nutritional deficiencies, because they had high PROT, DS, Fe, and Zn content. The high dietary fiber and RS content of cultivars Iraí, Minuano, and TPS Bonito suggests that they could have beneficial role in controlling blood lipid and glucose levels. Cooked beans had a decrease in DS and an increase in RS content after storage (4^{o}C or -20^{o}C), but these changes were more prominent in beans that had low RS content before cooking than in those of high RS content. TDF, IDF, and SDF did not change after storage.

Keywords: Phaseolus vulgaris; Dietary fiber; Digestible starch; Resistant starch; Mineral content.

1. Introduction

Common beans (*Phaseolus vulgaris* L.) are grown and consumed throughout the world. They play an important role in the nutrition of low-income people especially in developing countries where they are often the most important dietary source of protein, carbohydrate, dietary fiber, and minerals (Tharanathan & Mahadevamma, 2003). It is noteworthy that protein energy undernutrition and micronutrient deficiencies in childhood can be prevented if beans and cereals are appropriately combined. Besides the nutritional role, bean consumption has other potential benefits for human health like lowering the risk of colon cancer and of heart disease, reducing total and LDL cholesterol, and regulating blood glucose and insulin levels (The Michigan Bean Commission, 2006).

Different chemical characteristics can be found in beans according to the genetic variety (Barampama & Simard, 1993; Kigel, 1999). Genetic research concerning bean culture is mainly aimed at improving productivity and resistance to field pests and environmental stress. Although, there is evidence that it may also change physicochemical characteristics of seeds and affect their nutritional value, studies concerning these changes in beans are still rare and limited to few cultivars (Augustin, Beck, Kalbfleish, Kagal, & Mathews, 1981; Barampama & Simard, 1993; Nunez-Gonzalez et al., 2002).

Nutrient profile of beans may also be affected by environmental factors (Samman, Maldonado, Alfaro, Farfan, & Gutierrez, 1999), which could make it difficult to obtain standard nutritional values for seeds cultivated in different regions and from different harvests. However, some studies in cereal cultivars (oat, rice, and wheat) indicate that despite the changes in absolute values due to environmental factors, cultivars may keep a standard compositional pattern, i.e. higher or lower starch or protein content (Silva, 2002; Storck, Silva, & Fagundes, 2005).

Besides these changes in chemical characteristics due to genetic and environmental factors, certain processing techniques, such as cooking and storage conditions, can also produce important changes in starch, fiber, and other components of legume seeds (Kutos, Golob, Kač, & Plestenjak, 2003; Vargas-Torres, Osorio-Diaz, Islas-Hernández, Tovar, Paredes-Lopéz, & Bello-Pérez, 2004). These changes may affect the digestibility and nutritional value of foods containing common bean seeds. All this variability has remarkable importance for strict diets that are based on food composition tables, which usually do not show specific values for different bean cultivars, neither their possible changes along harvest years. Besides, bean seeds of better nutritional quality could be supplied for low-income populations around the world.

Carbohydrates are the major component of beans. Current knowledge on nutritional features of starch indicates that a fraction of this carbohydrate is resistant to enzyme digestion in the small intestine. This resistant starch can be a substrate for the fermentation process in the colon with important benefits for human health, similar to the dietary fiber. However, relatively few studies evaluated resistant starch and dietary fiber content of common beans (Kutos et al., 2003; Vargas-Torres et al., 2004; Landa-Habana, Piña-Hernández, Ágama-Acevedo, Tovar, & Bello-Pérez, 2004), while most studies evaluated total starch content and crude fiber content that do not give a real picture of the nutritional implications of these components (Vieira, 1967; Antunes, Bilhalva, Elias, & Soares, 1995; Castellón et al., 2003; Lemos, Oliveira, Palomino, & Silva, 2004).

The objectives of the present study were: (i) to compare the physicochemical characteristics of sixteen common bean cultivars; (ii) to investigate the persistence of the nutrient profile along two harvests; and (iii) to assess the effect of storage conditions on digestible starch, resistant starch, and dietary fiber contents of cooked beans. Results will be useful for dietitians to choose the most appropriate cultivars for specific nutritional purposes like public health programs (health policy) and strict dietary orientations.

2. Materials and methods

2.1. Samples

Common bean seeds (*Phaseolus vulgaris* L.) of sixteen cultivars (Carioca, Diamante Negro, FTS Magnífico, FTS Soberano, Guapo Brilhante, Guateian 6662, IAPAR 31, Iraí, Macanudo, Minuano, Pérola, Rio Tibagi, TPS Bionobre, TPS Bonito, TPS Nobre, and Valente) were obtained from the Department of Fitotecnia of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. They were grown during two consecutive years (2001/2002 and 2002/2003) at the campus, which is placed at an altitude of 95 m. The local climate, according to Köppen's classification, is Cfa – subtropical, with total average annual rainfalls and temperature around 1769 mm and 19.2°C, respectively. The soil is a typical dystrophic red (Bandinelli et al., 2005). In both harvest years, seeds were grown under similar field conditions and normal agronomic practices required for bean crops were followed. Each cultivar was obtained from three independent lots of land, each one containing four rows (4 m length and 50 cm row to row distance). After harvest, seeds from the three lots were pooled and a representative sample (200 g) of each cultivar was taken and kept at –20°C in sealed polyethylene bags until analysis.

2.2. Physical analysis

One thousand seed mass (g) was calculated as ten times the mass of 100 seeds. The evaluation was performed in unbroken raw seeds chosen at random.

2.3. Chemical analysis

2.3.1. Raw bean analysis

Raw seeds (50 g) were finely ground into flour and kept at –20°C in sealed polyethylene bags until analysis. Bean flours were analyzed as described by AOAC (1995) for moisture (method 925.10), ash (method 923.03), fat (method 945.39), crude protein (method 960.52, N x 6.25, microKjeldahl method), and crude fiber (method 962.09). Dry matter and organic matter were calculated as 100 minus the centesimal content of moisture and ash, respectively. Digestible and resistant starch were determined after enzymatic hydrolysis according to the method no. 996.11 AOAC (1995, reviewed in 1998) as modified by Walter (2005) that uses a higher amount of sample (300 mg instead of 100 mg), phosphate buffer pH 6.8 instead of MOPS (4-morpholinepropanesulfonic acid) buffer pH 7.0, and proteolysis during digestion process. Amylose value was determined by iodometric assay (blue value; Gilbert & Spragg, 1964). Total and insoluble dietary fiber contents were determined by enzymic-gravimetric methods no. 985.29 and no. 991.42 (AOAC, 1995) and soluble dietary fiber content was calculated by difference. The enzymes employed for dietary fiber and starch determination were: α-amylase (Termamyl 120L®), protease (Flavourzyme®), and amyloglucosidase (AMG 300L®) supplied by Novozymes Latin América Ltda. (Araucária, PR, Brazil). Activity and purity of the enzymes was controlled weekly in our laboratory. For starch determination, glucose was measured using glucose-oxidase-peroxidase test kit (Glucox 500) supplied by Doles (Goiânia, GO, Brazil). Calcium, iron, zinc, copper, manganese, and magnesium were determined by atomic absorption spectroscopy (Atomic Absorption Spectrometer AA 12/1475 - Intralab), and phosphorus content was determined colorimetrically (Tedesco, Gianello, Bissani, Bohnen, & Volkweiss, 1995).

All experiments were conducted at least in duplicate. Duplicates falling within 10% of their mean were accepted to be showing satisfactory agreement. The analysis was repeated if the agreement was outside 10% of the mean for the duplicates, and then recalculated on the basis of the concordant replicates. Reagent blanks were always run with samples and helped to monitor the purity of the reagents used. Any significant value for the blank determination was accounted for in the calculation of the results. Analytical quality control was monitored through the use of in-house control and participation in the interlaboratory proficiency testing scheme (INTERLAB) of the Science and Technology Foundation (CIENTEC, Porto Alegre, RS, Brazil) that follows ISO and IUPAC guidelines for interlaboratory proficiency testing.

2.3.2. Cooked bean analysis

Based on raw seed analysis, four cultivars of high resistant starch content (FTS Magnífico, IAPAR 31, Pérola, and TPS Bonito) and four cultivars of low resistant starch content (Diamante Negro, Iraí, Macanudo, and Valente) were chosen in order to determine the effect of storage conditions on starch and dietary fiber contents of cooked beans. Fifty grams of bean seeds were soaked overnight in tap distilled water (250 ml) at room temperature. Twelve hours later, they were cooked at 100°C with the soaking water in beakers covered by an aluminum foil until they became suitable for consumption (approximately 40 min). The cooked seeds were either immediately analyzed (control) or stored with the cooking water in sealed containers at 4°C for 4 days or at –20°C for 28 days. At the moment of the analysis, the seeds were drained off, dried on a paper towel, and ground into a paste.

Total, insoluble, and soluble dietary fiber, as well as the digestible and resistant starch contents were determined in cooked beans according to the same methods described above. Dry matter was determined after 48 h at 55°C in an assisted air circulation oven, followed by 8 h at 105°C. All experiments were conducted at least in duplicate.

2.4. Statistical analysis

For evaluation of year to year variability, data concerning physicochemical characteristics from the two consecutive harvests were compared using the Student's *t*-test for paired samples (*P*<0.05) considering each cultivar sample as an independent replicate (n=16). The correlations between the various physicochemical characteristics evaluated were assessed by Pearson's correlation in the whole population of cultivars studied (n=16) separately in each harvest year.

Bean cultivars were divided into groups with distinctive chemical characteristics by cluster analysis using the Ward's method as indicated by Hair Jr, Anderson, Tahman, & Black, 1998). This technique is a classification procedure that groups objects in clusters in terms of their nearness or similarity for a set of variables. The measurement of the similarity is based, among other ones, on the squared Euclidean distance. Data were standardized (Z score) before cluster analysis in order to eliminate the bias introduced by the differences in the scales of the several variables (chemical characteristics) used in the analysis. Since small differences were observed in the physicochemical characteristics along the two harvests evaluated, results from the two harvests were used as two independent replicates of each cultivar in the cluster analysis. Comparisons among groups obtained by cluster analysis were made separately for each chemical characteristic by one-way analysis of variance (ANOVA) using untransformed values. The effect of storage conditions on the levels of digestible starch, resistant starch, total dietary fiber, insoluble dietary fiber, and soluble dietary fiber of cooked beans were evaluated using two-way ANOVA (2 types of sample x 3 storage conditions, with 4 independent replicates per group). Post-hoc analysis was carried out using Duncan's test (*P*<0.05). The software used for the analysis was SPSS 8.0 for Windows.

3. Results and discussion

3.1. Influence of harvest year on physicochemical characteristics of bean cultivars

Seed characteristics are determined by cultivar genotype and environmental conditions during plant growth and seed development. Table 1 shows the average physicochemical characteristics of the sixteen bean cultivars along two consecutive harvests (2001/2002 and 2002/2003). Of the sixteen bean cultivars evaluated only Carioca, Iraí, and Pérola had been previously studied concerning some physicochemical characteristics before and after cooking procedure (Universidade de São Paulo, 2005). The average values were similar to those reported for other common bean cultivars (Vieira, 1967; Barampama et al, 1993; Antunes et al., 1995; Universidade de São Paulo, 2005). Most physicochemical characteristics evaluated concerning macro and micronutrients contents did not change significantly throughout the harvests, except for the slight moisture decrease ($P<0.05$), and dry matter and total dietary fiber increase ($P<0.05$; Table 1) from the first to the second harvest. On average, these results indicate that the various cultivars exhibited a constant profile for most nutrients along the two harvests.

Although no major differences were observed in the average chemical characteristics of seeds between the two harvests, remarkable ranges were observed in physical (1000 seeds mass) and chemical characteristics (ash, crude protein, crude fiber, dietary fiber, crude fat, amylose, and starch content) within each harvest (Table 1). Common beans are important dietary sources of protein, starch, and fiber. Therefore, the range in the content of protein (36%), insoluble dietary fiber (70%), soluble dietary fiber (106%), digestible starch (77%), and resistant starch (100%) could be used to categorize bean cultivars according to their nutritional potential.

We assessed the correlations between the various physicochemical characteristics shown in Table 1. Similar to the results of Lemos et al. (2004) we also found a significant negative correlation between protein (PROT) and 1000 seed mass (r = -0.65 for harvest 1 and

-0.55 for harvest 2), indicating that bigger seeds have lower PROT contents. An interesting finding is that crude fiber had no significant correlation with total, soluble or insoluble dietary fiber, which indicates that crude fiber assay is unsuitable to estimate the fiber content of bean.

3.2. Categorizing bean cultivars according to their macronutrient content

Previous studies discuss separately the variation of each nutrient among bean cultivars. However, this approach provides an incomplete analysis of the nutritional potential of different cultivars. Therefore, in the present study we used the multivariate cluster analysis to classify the set of cultivars into groups of different nutritional quality based on their similarities for a set of characteristics. Table 2 shows characteristics of groups formed by multivariate cluster analysis based on their similarity for the content of some macronutrients measured along two consecutive harvests (crude protein-PROT, total dietary fiber-TDF, insoluble dietary fiber-IDF, soluble dietary fiber-SDF, digestible starch-DS, and resistant starch-RS). These nutrients were chosen based on the relevance of common beans intake as a dietary source. According to cluster analysis cultivars were divided into four groups (Table 2). Group A that was formed by cultivars TPS Bonito, Iraí, and Minuano had low PROT, high TDF, IDF, DS, and RS, and intermediate levels of SDF. Group B (Carioca, FTS Magnífico, FTS Soberano, Guapo Brilhante, IAPAR 31, Pérola, TPS Bionobre, and TPS Nobre) had intermediate levels of PROT and IDF, low levels of TDF and SDF, but high levels of DS and RS. Group C (Diamante Negro, Macanudo, and Valente) also had intermediate PROT and IDF levels, and low TDF and SDF, but in contrast to group B, group C had low levels of DS and RS. Group D (Guateian 6662 and Rio Tibagi) had the highest PROT and SDF levels, high DS levels, low TDF and IDF, and intermediate RS levels.

It is known that we can have different biological effects depending on the levels of nutrients in the diet. The present data shows that the genetic variability among bean cultivars significantly influenced its chemical characteristics. Among these cultivars those that exhibited an interesting nutritional profile could be selected for specific dietary purposes, for example, cultivars from group D (high PROT and DS levels) could be used for undernutrition prevention. Some macronutrients like TDF, IDF, SDF and RS have been demonstrated to reduce serum lipid levels, and regulate blood insulin and glucose levels (Guillon & Champ, 2002; Higgins, 2004). Hence, cultivars from groups A (high TDF and IDF, intermediate SDF, and high RS) and D (high SDF) could be indicated for reduction of the risk of colon cancer and coronary disease, as well as for the prevention of diseases related to insulin resistance (Guillon & Champ, 2002; Lupton & Turner, 2003).

3.3. Categorizing bean cultivars according to their micronutrient content

The great range observed in the ash content (70%) of bean cultivars (Table 1) suggests that the content of some essential minerals may vary significantly among the studied cultivars. Since common beans are an important source of iron and other minerals these micronutrients were also determined in the common beans evaluated (Table 3). According to the literature common beans exhibit a great range in the content of minerals (mg/100g dry weight basis) like Fe (2–18), Zn (1–7), Mn (1–2), Cu (0.20–1.96), Ca (55–284), Mg (38–369), and P (295–570) (Augustin et al., 1981; Barampama & Simard, 1993; Vazquez-Blanco, Vazquez-Oderiz, Simal-Lozano & Lopez-Hernandez, 1997; Samman et al., 1999; Nunez-Gonzalez et al., 2002). In this study, Fe, Zn, Mn, Cu, Ca, and P contents were similar to those found in literature (Table 3). However, Mg content was slightly lower than previously reported values.

Considering the nutritional importance of beans as mineral source, cultivars evaluated in the present study were also classified based on their similarity for the content of some micronutrients along two consecutive harvests (Table 3). Bean cultivars were divided into four different groups. No significant differences were observed in P content among the groups formed. Group A that was formed by cultivars Iapar 31, Iraí, and Macanudo had low levels of all minerals evaluated (Fe, Zn, Mn, Cu, Mg) with the exception of Ca that was found at intermediate levels in this group. Group B (Carioca, Guapo Brilhante, Minuano) had low levels of Zn and Mn, intermediate levels of Fe, and high levels of Cu, Ca, and Mg. Group C (Diamante Negro, TPS Bonito, Valente) had low levels of Ca, intermediate levels of Mn and Mg, and high levels of Fe, Zn, and Cu. Group D (FTS Magnífico, FTS Soberano, Guateian 6662, Pérola, Rio Tibagi, TPS Bionobre, TPS Nobre) had the highest content for all minerals evaluated.

Changes in mineral profile of beans can be explained by a variety of factors, including genotypic variability in absorption of minerals from soil (Nunez-Gonzalez et al., 2001), effect of fertilizers on metallic composition of plants (Sadiq & Hussain, 1994), and salinity levels of soil (Carbonell-Barrachina, Burlo, & Mataix, 1998). In this study, the soil used for seeding and the fertilization practices were kept the same for all cultivars. Hence, the differences of mineral profile among the cultivars could be attributed to genotypic variability.

As observed for macronutrients, these changes in mineral levels have an important role in human nutrition, because different cultivars could be used for specific nutritional purposes as mineral deficiencies. Based on Table 3 we can select cultivars with the highest levels of Fe, Zn, (groups C and D), and Ca (groups B and D) for the prevention of these ordinary mineral deficiencies. The data presented here can also be useful for selecting cultivars for the development of new ones, with a more appropriate nutritional profile for populations with specific mineral dietary deficiencies.

3.4. Effect of storage conditions on cooked beans

The effect of storage conditions on DS and RS contents of cooked beans were evaluated using cultivars that had either low or high RS levels before cooking (Fig. 1). DS content of cooked beans significantly reduced after storage at 4°C both for low and high RS cultivars. However, storage at -20°C only reduced DS content of low RS cultivars (Fig. 1A). RS content of both low and high RS cultivars was significantly increased after storage of cooked beans at 4^{o}C, but at -20^{o}C only low RS cultivars had a significant increase in RS content (Fig. 1B). It is noteworthy that the increase in RS content during storage was much more pronounced for low than for high RS cultivars, in such a way that the RS profile of cultivars was inverted after storage at -20^{o}C (Fig. 1B). These results suggest that beans that have low RS content before cooking are more prone to retrogradation after cooking. Hence, we can propose that the behavior of bean starch during storage may vary among different cultivars. This observation may help to explain previous controversial results on the effects of cooking and storage conditions on starch levels of common beans. Some authors found a decrease of digestible starch and increase of resistant starch after storage at 4°C (Vargas-Torres et al., 2004), others found no changes (Landa-Habana et al., 2004), and others found an increase of digestible starch (Osorio-Diaz, Bello-Perez, Sayago-Ayerdi, Benitez-Reyes, Tovar, & Paredes-Lopez, 2003).

The effects of storage conditions on TDF, IDF, and SDF contents of cooked beans were also evaluated using cultivars that had either low or high RS levels before cooking (Fig.

2). TDF (Fig. 2A), IDF (Fig. 2B), and SDF (Fig. 2C) contents of cooked beans did not change during storage.

Previous studies on the dietary fiber of common beans focused on the effects of cooking and revealed variable results, since either increase or decrease of TDF, IDF, and SDF was observed by different authors (Hughes & Swanson, 1989; Kutos et al., 2003). No study was found on the effects of different storage conditions on dietary fiber (DF) levels of common beans. In the present study we demonstrated that DF content did not change during storage of cooked beans. RS is a component of IDF, and it did change during storage (Fig. 1). However, no change was observed in IDF fraction, probably because RS contributes to a small part of this fraction in cooked beans (10-20%).

4. Conclusions

It was possible to identify groups of cultivars with different nutritional features. Guateian 6662 and Rio Tibagi appeared to be the most adequate cultivars to prevent nutritional deficiencies, because they had high PROT, DS, Fe, and Zn content. Cultivars with a composition more adequate for prevention of coronary disease and diseases related to insulin resistance (Iraí, Minuano, and TPS Bonito) were also identified. The observed variability among bean cultivars reveal the need for bringing food composition tables up to date by including an expected range of nutrients for each cultivar.

Besides, it was shown that the increase in RS content and the decrease in DS during storage of cooked beans were higher for beans that had low RS content before cooking. The TDF, IDF and SDF contents did not change. These results may be useful in selecting cultivars for breeding and for specific uses in human nutrition.

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Figure captions

Fig. 1. Effect of storage on digestible (A) and resistant (B) starch content of cooked common beans that had either a low (open bars) or a high (hatched bars) resistant starch content before cooking. Results (g/100 g of dry weight) are mean±standard error (n=4). Bars within the same panel that have no common letters are significantly different ($P<0.05$).

Fig. 2. Effect of storage on total dietary fiber (A), insoluble fiber (B), and soluble fiber (C) of cooked common beans that had either a low (open bars) or a high (hatched bars) resistant starch content before cooking. Results (g/100 g of dry weight) are mean±standard error (n=4).

Table 1

Physicochemical characteristics of common bean seeds from 2 consecutive harvests (2001/2002 and 2002/2003) [&]

Characteristics	1 (2001/2002)	2 (2002/2003)
1000 seed mass (g)	223.19±9.74 (165.00-331.70)	230.59±10.48 (158.40-338.60)
Dry matter (g/100 g fresh weight)	86.67± 0.13 (85.92-87.57)	87.09±0.12* (86.09-87.79)
Organic matter (g/100 g fresh weight)	96.12±0.12 (95.50-97.07)	96.16±0.14 (95.46-97.33)
Moisture (g/100 g fresh weight)	13.33±0.13 (12.43-14.08)	12.91±0.12* (12.21-13.91)
Ash (g/100 g fresh weight)	3.90±0.12 (2.93-4.50)	3.84±0.14 (2.67-4.54)
Crude protein (g/100 g dry weight)	25.69±0.55 (21.24-29.06)	25.11±0.41 (22.50-27.96)
Crude fiber (g/100 g dry weight)	4.79±0.17 (3.64-5.72)	4.98±0.18 (3.54-6.05)
Total dietary fiber (g/100 g dry weight)	23.97±0.54 (20.79-28.79)	25.35±0.72* (21.02-31.91)
Insoluble dietary fiber (g/100 g dry weight)	18.81±0.65 (13.80-23.49)	19.50±0.50 (16.97-22.79)
Soluble dietary fiber (g/100 g dry weight)	5.17±0.48 (3.59-10.68)	5.90±0.60 (1.44-9.72)
Crude fat (g/100 g dry weight)	1.90±0.25 (0.98-5.49)	1.82±0.21 (0.92-4.43)
Amylose (g/100 g dry weight)	8.35±0.28 (6.16-10.08)	9.02±0.21 (7.54-10.34)
Digestible starch (g/100 g dry weight)	29.40±1.15 (20.02-35.61)	30.81±1.12 (23.66-39.41)
Resistant starch (g/100 g dry weight)	3.34±0.18 (2.35-4.70)	3.44±0.17 (2.46-4.58)

[&]Results are expressed as mean value±standard error (min-max) of sixteen bean cultivars.

*Significantly different from harvest 2001/2002 ($P< 0.05$, Student's *t*-test).

Table 2

Groups formed by bean cultivars considering the levels of macronutrients*

Group/Cultivars	PROT	TDF	IDF	SDF	DS	RS
A: Iraí; Minuano; TPS Bonito	22.93 [c]↓ (0.67)	28.83 [a]↑ (0.18)	22.31 [a]↑ (0.22)	6.51 [a,b]↕ (0.36)	31.73 [a]↑ (0.85)	3.81 [a]↑ (0.40)
B: Carioca; FTS Magnífico; FTS Soberano; Guapo Brilhante; Iapar 31; Pérola; TPS Bionobre; TPS Nobre	25.61 [b]↕ (0.41)	23.96 [b]↓ (0.38)	19.23 [b]↕ (0.46)	4.72 [b]↓ (0.44)	31.54 [a]↑ (1.40)	3.60 [a]↑ (0.19)
C: Diamante Negro; Macanudo; Valente	25.59 [b]↕ (0.85)	22.71 [b]↓ (0.50)	17.84 [b,c]↕ (0.57)	4.87 [b]↓ (0.57)	24.29 [b]↓ (1.68)	2.50 [b]↓ (0.02)
D: Guateian 6662; Rio Tibagi	27.96 [a]↑ (0.26)	24.15 [b]↓ (0.28)	16.04 [c]↓ (0.64)	8.11 [a]↑ (0.92)	30.60 [a]↑ (3.02)	3.24 [a,b]↕ (0.30)

*Results (g/100 g of dry weight) are mean (standard error) of the group of cultivars indicated in the first column, each one analyzed in two harvests (2001/2002 and 2002/2003). Values within the same column that have no common superscript are significantly different ($P < 0.05$, Duncan's test). PROT: crude protein; TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; DS: digestible starch; RS: resistant starch. ↑, ↕, and ↓ indicate high, intermediate, and low levels of each macronutrient.

Table 3

Groups formed by bean cultivars considering the levels of micronutrients*

Group / Cultivars	Fe	Zn	Mn	Cu	Ca	Mg	P
A: Iapar 31; Iraí; Macanudo	8.26 ^{c}↓	3.18 ^{b}↓	1.35 ^{b}↓	1.10 ^{b}↓	257.23 ^{b}↕	21.53 ^{b}↓	358.59 ^{ns}
	(0.33)	(0.08)	(0.05)	(0.12)	(46.09)	(1.17)	(11.12)
B: Carioca; Guapo Brilhante; Minuano	9.08 ^{b}↕	3.20 ^{b}↓	1.44 ^{b}↓	1.39 ^{a}↑	373.23 ^{a}↑	23.88 ^{a}↑	351.17 ^{ns}
	(0.15)	(0.09)	(0.02)	(0.06)	(22.15)	(0.45)	(14.17)
C: Diamante Negro; TPS Bonito; Valente	9.26 ^{a,b}↑	3.57 ^{a}↑	1.53 ^{a,b}↕	1.44 ^{a}↑	147.01 ^{c}↓	23.19 ^{a,b}↕	335.69 ^{ns}
	(0.11)	(0.05)	(0.04)	(0.03)	(15.32)	(0.17)	(15.14)
D: FTS Magnífico; FTS Soberano; Guateian 6662;	9.65 ^{a}↑	3.53 ^{a}↑	1.67 ^{a}↑	1.55 ^{a}↑	399.75 ^{a}↑	24.64 ^{a}↑	342.47 ^{ns}
Pérola; Rio Tibagi; TPS Bionobre; TPS Nobre	(0.11)	(0.09)	(0.05)	(0.05)	(11.57)	(0.38)	(9.61)

*Results (mg/100g of dry weight) are mean (standard error) of the group of cultivars indicated in the first column, each one analyzed in two harvests (2001/2002 and 2002/2003). Values within the same column that have no common superscript are significantly different ($P < 0.05$, Duncan's test); ns: not significant. ↑, ↕, and ↓ indicate high, intermediate, and low levels of each micronutrient

Fig. 1

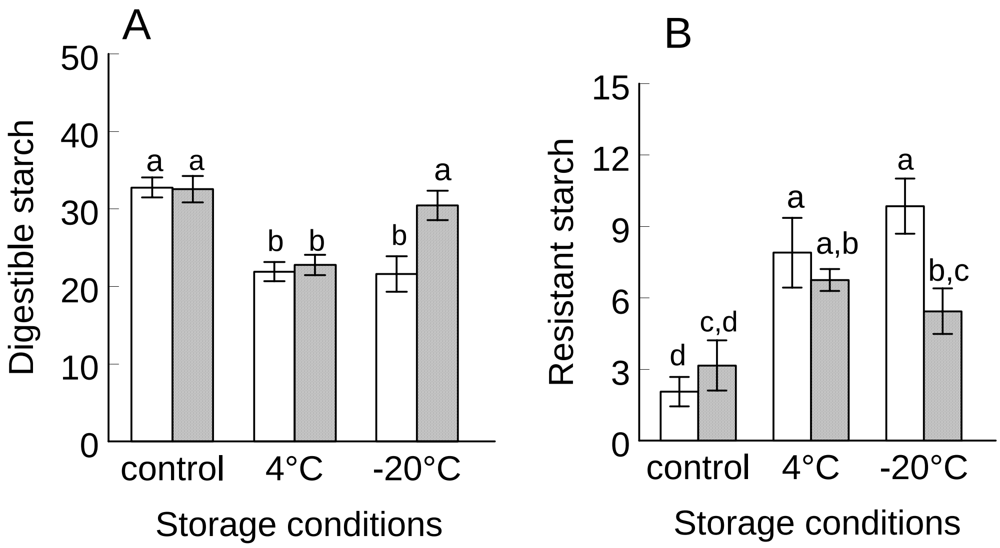
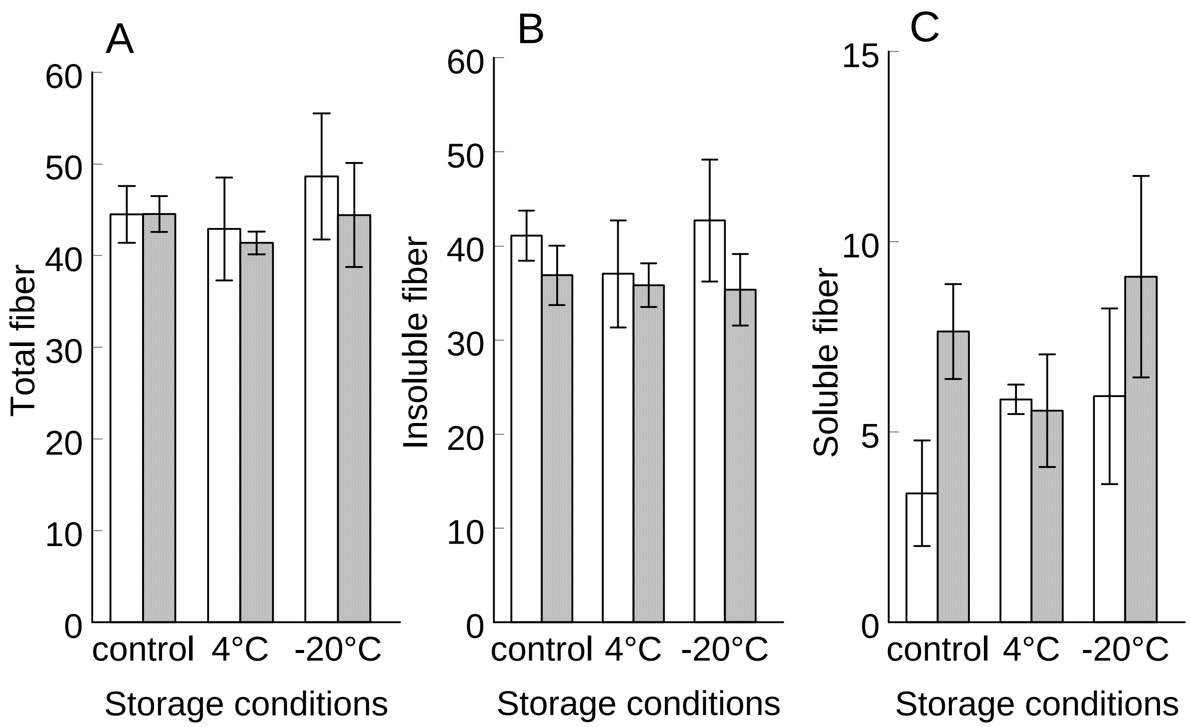

A
50
40
30
20
10
0
Digestible starch
a a
b b
b
a
control 4°C -20°C
Storage conditions
B
15
12
9
6
3
0
Resistant starch
d
c,d
a
a,b
a
b,c
control 4°C -20°C
Storage conditions

Fig. 2


A
B
C
Total fiber
Insoluble fiber
Soluble fiber
60
50
40
30
20
10
0
15
10
5
control
4°C
-20°C
Storage conditions

3.2 Manuscrito 2

EFFECTS OF COMMON BEAN (*Phaseolus vulgaris* L.) DIETS ON SERUM LIPIDS AND BLOOD GLUCOSE LEVELS IN NORMOLIPIDEMIC-NORMOGLYCAEMIC RATS

Manuscrito em fase final de revisão pelos autores para ser submetido à revista

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Effects of common bean (*Phaseolus vulgaris* L.) diets on serum lipids and blood glucose levels in normolipidemic-normoglycaemic rats

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Running title: Effect of beans on blood lipids and glucose

Abstract

Common beans (*Phaseolus vulgaris* L.) have different soluble fibre/total fibre ratios (SF/TF) according to the genetic variety. This study was aimed at determining the effects of three cultivars of common beans containing different SF/TF (Pérola diet, 0·11; Diamante Negro diet, 0·19; Iraí diet, 0·25) on serum lipids, blood glucose and others biological parameters in normolipidemic-normoglycaemic rats. Male Wistar rats (52·0±1·1 g) were fed isocaloric and isonitrogenous diets with 5% fibre content obtained from one of the three bean cultivars or from cellulose (100% insoluble fibre - Control) during an experimental period of 23 days. No differences were observed in food intake or body weight among groups. Animals fed Iraí diet had significantly higher food efficiency ratio (0·33 g/g) when compared to Diamante Negro and control (0·30 and 0·31 g/g, respectively), while those fed with Pérola diet had intermediate values (0·32 g/g). Iraí had higher dry matter, starch, and fibre digestibility (92·6, 94·6, and 86·8%, respectively) than the other bean diets (90·3-90·5, 91·9-92·3, and 77·1-79·5%; $P<0{\cdot}05$). All bean diets lead to higher faecal weight and faecal moisture (Pérola>Diamante Negro>Iraí), and lower faecal pH (Pérola<Diamante Negro<Iraí) than control ($P<0{\cdot}05$). All bean diets lead to lower epididymal fat weight and total blood cholesterol levels than control diet ($P<0{\cdot}05$). Fibre digestibility was negatively correlated to cholesterol levels (r=-0·84) and epididymal fat weight (r= -0·58; $P<0{\cdot}05$). The glycaemic peak occurred at 30 min for animals fed bean diets and at 60 min for those fed control diet ($P<0{\cdot}05$). Besides, animals fed bean diets had lower total area under the glycaemic curve than control animals ($P<0{\cdot}05$). We concluded that diets with cultivars of common beans containing higher SF/TF reduce serum cholesterol, blood glucose levels and fat retention in normolipidemic-normoglycaemic rats.

Keywords: digestibility; cholesterol; glycaemic curve; insoluble fibre; soluble fibre; faecal pH, epididymal fat.

1. Introduction

Common beans (*Phaseolus vulgaris* L.) are widely consumed throughout the world, mainly by low income populations. In addition to the nutritional properties, it is known that they have potential benefits on human health. Beans have been responsible for an important lowering effect on serum lipids (Fukushima *et al.* 2001). In hyperlipidemic subjects, bean water-soluble fibre was shown to decrease total cholesterol (13 to 26%), HDL cholesterol (5 to 20%) and triglyceride levels (3 to 25%) (Anderson & Gustafson, 1988). Although some authors have related the lipid-lowering effect to the starch fraction, presumably on its saponin content (Amígo *et al.* 1992), the soluble fibre seams to be really the active portion of the legume (Aller *et al.* 2004).

This legume has also a lowering effect on plasma glucose (Pari & Venkateswaran, 2003). This property has been related not only to starch digestibility, but also to fibre contents (Panlasigui *et al.* 1995). The fibre fraction seems to lower blood glucose responses due to a delaying in gastric emptying and due to a reduction in the rate of nutrient absorption from gastrointestinal tract (Thorne *et al.* 1983; Bjorck & Elmstahl, 2003). The blood glucose response can be expressed either as the area under the glycaemic curve or as the glycaemic index. High glycaemic index diets are associated with higher insulin response and increased risk of obesity (Pawlak *et al.* 2001). Common beans are considered a low glycaemic index food.

It is known that bean grains are excellent sources of dietary fibre, providing a mix of soluble and insoluble fibres (Messina, 1999) with all the remarkable effects already related to them. However, common bean cultivars have significant differences in chemical composition, including the fibre content. Thus, their properties can be also quite different.

The majority of the studies concerning the effects of common bean-based diets were performed in hypercholesterolemic (Dabai *et al.* 1996; Rosa *et al.* 1998 *a;* 1998 *b*) or hyperglycaemic rats (Pari & Venkateswaran, 2003). In these models, bean consumption improved lipids and glucose levels. However, studies on its effects in healthy animals are still lacking (Amigo *et al.* 1992). Besides, we did not find any study comparing the effect of bean cultivars with different levels of soluble fiber content on these parameters.

Therefore, this study was aimed at evaluating the effects of three cultivars of common beans containing different proportions of water-soluble fibre (Pérola: 11%; Diamante Negro: 19%; and Iraí: 25% of total dietary fibre levels, respectively) on serum lipids, blood glucose and other biological parameters in normolipidemic-normoglycaemic rats.

2. Materials and methods

2.1. Bean flour

Common bean (*Phaseolus vulgaris* L.) grains of three cultivars (Pérola, Diamante Negro and Iraí) were provided by Centro Nacional de Pesquisa de Arroz e Feijão – EMBRAPA (Santo Antônio de Goiás, Goiás, Brazil) and had been grown during the harvest year 2005.

Five kg of each bean grains were soaked overnight in tap water (1:3 wt/wt ratio) at room temperature. Twelve hours later, they were cooked separately at 100°C with the soaking water in covered pots until they became suitable for consumption (approximately 90 min).

The cooked grains were drained off, dried at 55°C in an assisted air circulation oven for 48 h and ground into fine flour, which was used for chemical analysis and for diet formulation.

2.2. Bean flour analysis

Bean flours were analyzed as described by AOAC (1995) for moisture (method 925.10), ash (method 923.03), fat (method 945.39), and crude protein (method 960.52, N x 6·25, microKjeldahl method). Dry matter was determined after 48 h at 55°C in an assisted air circulation oven, followed by 8 h at 105°C. Digestible starch was determined after enzymatic hydrolysis according to the method no. 996.11 (AOAC, 1995, reviewed in 1998) as modified by Walter (2005) that uses a higher amount of sample (300 mg instead of 100 mg), phosphate buffer pH 6·8 instead of MOPS (4-morpholinepropanesulfonic acid) buffer pH 7·0, and proteolysis during digestion process. Total and insoluble dietary fibre contents were determined by enzymic-gravimetric methods no. 985.29 and no. 991.42 (AOAC, 1995) and soluble dietary fibre content was calculated by difference. All the enzymes used in chemical analysis, Termamyl 120L® (amylase), AMG 200® (amyloglycosidase) and Alcalase 2·4L® (protease), were provided by Novozymes Latin America Ltda.

2.3. Animal experiment

This study was approved by the Ethics and Animal Welfare Committee of Universidade Federal de Santa Maria. Thirty-two male weanling Wistar rats (52·0±1·1 g) were randomly assigned into four dietary groups (8 animals each) and individually housed in cages placed in a room with controlled temperature (22±1°C), humidity (65±5%) and light (12-h light and 12-h dark periods). One group was fed a control diet (C-diet), according to AIN-93 rodent diet (Reeves *et al.* 1993), containing 5% fibre from crystalline cellulose (100% insoluble fibre).

The other three groups were fed experimental diets similar to AIN-93, except for the source and type of fibre. Each experimental diet was formulated with the bean flour of one cultivar: Pérola diet (P-diet), Diamante Negro diet (DN-diet), and Iraí diet (I-diet) (Table 1). The amount of flour was that to ensure the same percentage of total fibre present in C-diet (5%) (Tables 1 and 2). Protein and starch content of experimental diets were balanced in order to match the same levels found in AIN-93. All diets had the same amount of sucrose, soybean oil, mineral mix, vitamin mix, choline and methionine, ensuring isoproteic and isocaloric diets.

Rats were provided tap water and food *ad libitum* during the whole experiment. A period of 5 days was allowed for adaptation to the diets, followed by a 23-day experimental period with daily feed intake and weight gain record. On each day of the experimental period, faeces and spilled food for each rat were collected. The faeces were weighed and stored in plastic containers at 4°C until analysis. The spilled food were weighed and discharged.

Blood glucose levels were evaluated from the 17-day to the 20-day of the experimental period, using 8 randomly chosen animals in each day. After a 12 h fasting period, 2 µl blood was extracted from the tail of the animal and glucose concentration was immediately determined (time 0). Then, the rats were allowed to eat 2 g of their respective diets (control or experimental diets). After 20 minutes the pots of food were removed from the cages and new blood glucose determinations were performed at 15, 30, 45, 60 and 90 minutes later.

At the 23-day of the experimental period animals were fasted during 12 h and submitted to euthanasia by cardiac puncture under ether anesthesia. The blood was collected in a 5-ml tube and serum was obtained after centrifugation. Liver and epididymal fat pad were removed and weighed.

2.4. Faecal analysis

Dry matter, moisture, total dietary fibre and starch were analyzed following to the same methods described for bean flour. pH was evaluated directly in a solution made of 1 g of faeces and 10 ml of distilled water.

2.5. Blood analysis

Serum triglycerides, total cholesterol and HDL (high-density-lipoprotein) cholesterol were measured enzymatically using laboratory kits (Triglicérides 120, Colesterol 250 and Colesterol HDL, supplied by Doles Reagentes, Goiânia, GO, Brazil).

Blood glucose was assessed in blood samples extracted from the tail of the animal with reactive strips read in a portable glucosemeter (ACCU-CHEK® Advantage blood glucose meter). We determined the area under the curve (AUC) for each animal using the Data Master Program 2003, version 11·7. Total AUC was defined by the area under the 90 minutes blood glucose response curve following the ingestion of 2 g portion of the respective diet. We also calculated partial AUC for each period of time along the curve. The AUC of the testing food was divided by the AUC of the control diet and multiplied by 100. Thus, AUC for the control diet was expressed as 100, while for the experimental diets it was a percentage of this value (Thorne *et al.* 1983).

2.6. Statistical analysis

Results are presented as the mean ± standard error of the mean. Data were analysed by one-way analysis of variance (ANOVA). Data of glycaemic curve was analysed by ANOVA with time considered as repeated measure. Post-hoc analysis was carried out using Duncan's test ($P<0{\cdot}05$). The software used for the analysis was SPSS 8·0 for Windows.

3. Results and discussion

Male Wistar rats were fed isocaloric and isoproteic diets with 5% fibre content obtained from one of three bean cultivars or from cellulose (100% insoluble fibre - Control) during the whole experimental period (23 days). The only difference among diets was the soluble fibre/total fibre ratio (Table 2). Control (C-diet) and experimental diets (Pérola diet, P-diet; Diamante Negro diet, DN-diet; and Iraí diet, I-diet) were well tolerated by all animals. Final body weight, feed intake, and weight gain were similar among all groups (Table 3).

C-diet and I-diet groups showed higher dry matter and starch digestibility than DN-diet and P-diet groups (Table 4). The highest fibre digestibility was observed in I-diet that had the greatest soluble fibre/total fibre proportion (SF/TF) (Table 2). On the contrary, the C-diet that had only insoluble fibre, showed the lowest fibre digestibility, while DN-diet and P-diet had intermediate values (Table 4). As expected fibre digestibility was positively correlated to the SF/TF in the diet (r= 0·91; $P<0{\cdot}05$). We also found positive correlation between fibre digestibility and feed efficiency ratio (FER) (r= 0·46; $P<0{\cdot}05$), and positive correlation between SF/TF and FER (r= 0·55; $P<0{\cdot}05$).

Despite showing similar feed intake and similar values for digestibility of starch (Table 4), animals fed I-diet and C-diet had different FER (I-diet higher than C-diet) (Table 3). However, I- and C-diet had different SF/TF ratio (Table 2) and fibre digestibility (Table 4). On the other hand, animals fed P-diet (lower SF/TF) showed higher FER than animals fed DN-diet ($P<0{\cdot}05$) and similar to I-diet group (higher SF/TF) (Table 3). We noted that both feed intake and fibre digestibility in P-diet group were slightly higher than in DN-diet group ($P>0{\cdot}05$) (Table 3 and 4). As we found a positive correlation between SF/TF and FER (r= 0·55; $P<0{\cdot}05$), we considered that these differences may have influenced FER in these animals. Besides, we did not find any correlation between starch digestibility and FER. Jamroz *et al.* (2002) studied the digestibility and energy value of non-starch polysaccharides (NSP) in birds and showed that the degradation of NSP in the gut (39 to 42%) resulted in short chain fatty acids and contributed with 3·5% of the metabolisable energy. Robertson *et al.* (1987) studied the digestibility and rate of degradation of water-insoluble dietary fibre in pigs and showed that cellulose did not appear to be degraded, while vegetable fibre can have its fibre cell wall matrix completely destroyed by the microbial digestion during gut transit. Kienzle *et al.* (2001) studied the effect of cellulose on the digestibility of fat, protein and energy in dogs. They found that cellulose did not decrease the digestibility of fat, but decreased the digestibility of protein and energy. Besides, starch also decreased protein digestibility and the effects of cellulose and starch appeared to be additive. Thus, these papers support our findings, where experimental animals in I-diet (highest digestibility) and P-diet (intermediate digestibility but high feed intake) showed higher feed efficiency ratio.

Concerning starch digestibility, C-diet and I-diet groups showed the highest but similar levels, while DN-diet and P-diet showed the lowest values. It does not seam to have any relation to the type neither to the percentage of starch in the diet because C-diet had 50·1% of corn starch, while I-diet, DN-diet, and P-diet had 50·3%, 48·4%, and 51·2 %, respectively, of bean starch on dry matter basis. In addition, both digestible starch and resistant starch of these bean varieties were previously evaluated by the authors in raw grains (unpublished data). The levels, on dry matter basis, ranged from 28·4 to 31·5% and from 3·0 to 4·4%, respectively, but were not statistically different among them. So, as both digestible starch and resistant starch were similar among the bean varieties used in experimental diets, we do not believe that they could have caused any effect on the differences of starch digestibility in these groups.

Comparing experimental groups, there was positive correlation between faecal moisture and faecal weight (r= 0·77; *P*<0·01), and they were higher in P-diet than in I-diet groups (Table 5). We can observe that animals fed diets with low SF/TF (consequently higher levels of insoluble fibre) produced faeces with higher levels of these two parameters. Surprisingly, animals fed control diet (only insoluble fibre) showed the lowest levels for both moisture and weight. Concerning faecal pH, experimental groups showed similar values, despite the different SF/TF proportion in the diets. However, control animals showed higher faecal pH, even when compared to P-diet with the lowest SF/TF proportion. Burrows *et al.* (1982) showed that faecal weight and water content increased linearly, while digestibility of dry matter decreased, when cellulose was added in dog´s diets. Unexpectedly, we did not find any correlation between fibre digestibility and faecal moisture, faecal weight, or faecal pH. On the other hand, we found a negative correlation between starch digestibility and faecal moisture (r= -0·64, *P*<0·05), and between starch digestibility and faecal weight (r= -0·86, *P*<0·05), but a positive correlation between starch digestibility and faecal pH (r= 0·51, *P*<0·05). These data were important to explain why animals fed diets with high starch digestibility (C-diet and I-diet) showed lower values for faecal moisture, and faecal weight, but higher values for faecal pH. We assume that C-diet and I-diet groups, with a remarkable difference in SF/TF proportion (0 and 0·25, respectively), and fibre digestibility (68 and 86%, respectively), showed similar values for faecal weight because I-diet group produced faeces with higher faecal moisture, compensating the weight of the insoluble fibre in faeces of C-diet animals. Thus, we presume that in this study starch digestibility had more prominent influence on faecal weight and moisture than even insoluble fibre fraction that has low digestibility.

We found a positive correlation between final animal body weight and liver weight of animals (r= 0·76, *P*<0·05). So, liver/body weight ratio was used to compare the groups.

Groups fed experimental diets had liver/body weight ratio similar to control (Table 6). Based on this data, we can say that all the animals showed a well distributed weight gain. To evaluate fat retention, we measured epididymal fat weight. We also found a positive correlation between final animal body weight and epididymal fat weight (r= 0·52, *P*<0·05). C-diet animals showed the highest epididymal fat/body weight ratio (*P*<0·05; Table 6), and no significant difference was observed in epididymal fat/body weight ratios among experimental diets. However, we found a negative correlation between epididymal fat/body weight ratio and fibre digestibility (r= -0·58; *P*<0·05). This indicates that the intake of bean-based diets that had higher fibre digestibility was associated with a lower fat retention. As the weight gain among all groups were similar, it is possible to presume that animals fed bean-based diets increased their lean body mass instead of fat body mass. These data are supported by studies that related type and fibre content to digestibility of nutrients. For example, Burrows *et al.* (1982) showed that high cellulose level diets decrease digestibility of both dry matter (from 90 to 70%) and ash (from 43 to 32%). On the other hand, Larsen *et al.* (1994) showed that high viscosity diets delayed passage rate and, consequently, increased the absorption of amino acids in rats. Thus, as experimental diets had lower insoluble fibre level than control diet, it is easy to understand that experimental animals would have greater digestibility of the nutrients, mainly amino acids, showing a similar weight gain but lower fat retention with low epididymal fat weight.

Experimental diets yielded lower total cholesterol levels compared to control (Table 6). Although no statistically significant difference was observed among experimental groups, animals fed I-diet that had the highest SF/TF proportion, showed the lowest cholesterol levels (*P*>0·05). In addition, I-diet and DN-diet yielded lower HDL cholesterol levels compared to control, while P-diet showed intermediate values. Some authors have used the HDL/total cholesterol ratio to express plasma lipid levels (Shutler et al., 1989). In our study this parameter was slightly higher in experimental animals, but was not significantly different among all groups.

Cholesterol and HDL levels were found to be positively correlated to epididymal fat/body weight ratio (r=0·59 and 0·57; $P<0·05$). So, animals with higher fat retention had a tendency to show upper blood lipid levels. In addition, total cholesterol level was negatively correlated to fibre digestibility (r= -0·84; $P<0·05$) and positively correlated to faecal pH (r=0·76; $P<0·05$). These data explain the findings that the animals fed I-diet, with high fibre digestibility, showed lower cholesterol levels in comparison to C-diet (low fibre digestibility).

There was a reduction around 19·34 to 28·58% in serum cholesterol levels when we compare any of experimental diets to C-diet. We can see that significant reduction in serum cholesterol levels occurred when SF/TF proportion was equal to or higher than 0·11 (as in P-diet). Although no statistically significant difference was observed among bean-diets, animals fed I-diet (highest SF/TF proportion) had the lowest cholesterol level.

Although animals fed I-diet showed lower serum triglyceride values (around 10%) than controls, this difference was not statistically significant (Table 6). So, animals fed bean-based diets, even with high SF/TF proportion, did not show any important lowering effect on serum triglycerides. Shutler *et al.* (1989) also found no changes in plasma triacylglycerol levels of normocholesterolemic men after daily consumption of *Phaseolus vulgaris,* despite significant reductions in total plasma cholesterol levels. There is evidence that, at least in humans, the lipid-lowering effect of beans seems to be more remarkable on cholesterol fraction. For example, Birketvedt *et al.* (2002) studied the effects of bean fibre on serum lipids and fat excretion in faeces in overweight and obese volunteers. After a 3 months period, the supplemented group, but not the placebo-group, showed reduction in serum cholesterol and increased in fat excretion in faeces. However, triacylglycerol and HDL did not change in either group. Hunninghake *et al.* (1994) showed that hypercholesterolemic subjects receiving a dietary fibre supplementation of 10 g/d or 20 g/d showed significantly reduction in total cholesterol and LDL/HDL ratio, but no reduction in serum HDL or triglycerides. In our study, bean diet had lipid-lowering effect on both total cholesterol and HDL fraction. It is known that around 50% of cholesterol in rats is transported as HDL (Olson & Schneeman, 1998; Simunek & Bartonova, 2005). In our animals, these levels ranged from 37·70 to 44·21%. Hence, it is understandable that a reduction in cholesterol levels would be accompanied by a reduction in HDL values. This can be seen through HDL/total cholesterol ratios which were not different among all groups. It shows that the percentage of total cholesterol which was transported as HDL cholesterol did not change in any group. Thus, both total cholesterol and HDL cholesterol levels were equally decreased by the diets.

Amígo *et al.* (1992) studied the effects of each constituent of beans on serum and biliary lipids in rats. They found that both whole bean and bean starch-based diets had lowering effects on serum cholesterol and triglyceride. Neither bean protein, bean lipid or bean fibre alone had this effect. Thus, they presumed that the active component of beans was linked to starch fraction and saponins. However, they could not discard a role of soluble fibres, since this fraction was not evaluated. In our study all the experimental diets had the same starch content. Moreover, the most significant changes in serum lipids have occurred between Iraí and control group, which had no difference in their starch digestibility. In addition, the percentage of bean starch in experimental diets ranged from 18 to 25·6%. P-diet had 1·9% and 4·2% more bean starch than I-diet and DN-diet, respectively. However, P-diet did not cause any lowering effect on cholesterol levels. Thus, in the present study bean starch content seems not to be responsible for this lowering effect. Our results suggest that the lipid lowering effect was linked to the soluble fraction of the bean fibre.

There was no difference in fasting levels of blood glucose among groups (Figure 1). Compared to C-diet animals, blood glucose levels of DN-diet and P-diet were significantly higher at 30 min, while glucose levels of I-diet and P-diet were significantly lower at 60 min. The moment for the rise in blood glucose (glycaemia peak) occurred at 60 min for animals fed C-diet, but at 30 min for those fed experimental bean diets.

Aller *et al.* (2004) compared the effect of a high soluble fibre content diet (82·2% of total dietary fibre) against a control one (18·9% of total dietary fibre) in healthy humans. They found 12·3% reduction on fasting glucose levels, but no significant changes on insulin levels. In the present study, the experimental bean diets had no effect on fasting blood glucose, despite the difference in SF/TF proportions. This could be related to the range of SF/TF ratio evaluated that was much lower than that used by Aller *et al.* (2004).

Based only on blood glucose values, the SF effects on plasma glycaemia did not seem to be of remarkable importance. However, an assessment of the areas under the glycaemic curve provided noteworthy information. The total areas under the curve (AUC) for all experimental bean diets were lower than for control group (P<0·05) (Table 7). When AUC were calculated separately for each period of time, the I-diet showed more stable values during the whole 90 minutes than the other two bean cultivars. Comparing P-diet (lowest SF/TF) to I-diet (highest SF/TF), P-diet showed higher areas over the first 15 min and lower areas during the 60-90 min interval. Based on this finding, animals fed I-diet kept postprandial plasma glucose values more stable along the time. Dilawari *et al.* (1981) compared the effects of different carbohydrate sources on plasma glucose. They found that the postprandial rise in plasma glucose and AUC were lower when carbohydrates were taken as beans (*Phaseolus vulgaris*) when compared to glucose. This can be explained because pulses and legumes are rich sources of fibre in the form of galactomannans (polysaccharides) which are not hydrolyzed by the digestive enzymes of man and are hence called unabsorbable carbohydrate (Leeds *et al*. 1979). Moreover, galactomannans present in pulses are more viscous than fibre present in others cereals and viscosity of the dietary fibre has been shown to correlate positively with the reduction in postprandial plasma glucose levels (Jenkins *et al.* 1978). Thus, besides the desirable effect of soluble fibre viscosity on blood glucose, we observed that SF/TF ratio can also influence the variability in AUC values.

Animals from all groups had similar weight gain and final body weight. However, control animals showed higher values for epididymal fat/body weight ratio than those fed experimental bean diets. This may be related to the lower area under the curve (AUC) in animals fed experimental bean diets when compared to controls. Pawlak *et al.* (2001) observed that rats fed a high glycaemic index (GI) diet showed higher epididymal fat mass than low GI group, despite comparable body weights. They also observed that high GI diet resulted in higher insulin response, which may underlie the increased fat deposition. In addition, we found a negative correlation between epididymal fat/body weight ratio and fibre digestibility. Thus, diets with higher SF/TF reduced tendency to fat retention.

5. Conclusions

According to this study, even normolipidemic-normoglycaemic rats fed bean diets with high soluble fibre to total fibre proportion (SF/TF: equal to or higher than 0·11) can have lowering effects on serum cholesterol levels (22·3 to 33%), lower glycaemic responses (glycaemix index), and lower fat retention. These results point out that consumption of bean cultivars with high SF/TF would help people, not only during nutritional treatment, but as an important tool against hypercholesterolemia, diabetes, and obesity. For this reason, food composition tables should bring data concerning SF/TF proportion, because bean varieties with higher soluble to insoluble fibre ratio could be preferred for nutritional and clinical purposes.

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Figure caption

Figure 1. Glycaemic curves according to diet type. C-diet: control diet; I-diet: Iraí diet; P-diet: Pérola diet; DN-diet: Diamante Negro diet. * Significantly different from control at the same time ($P<0\cdot05$).

Fig. 1

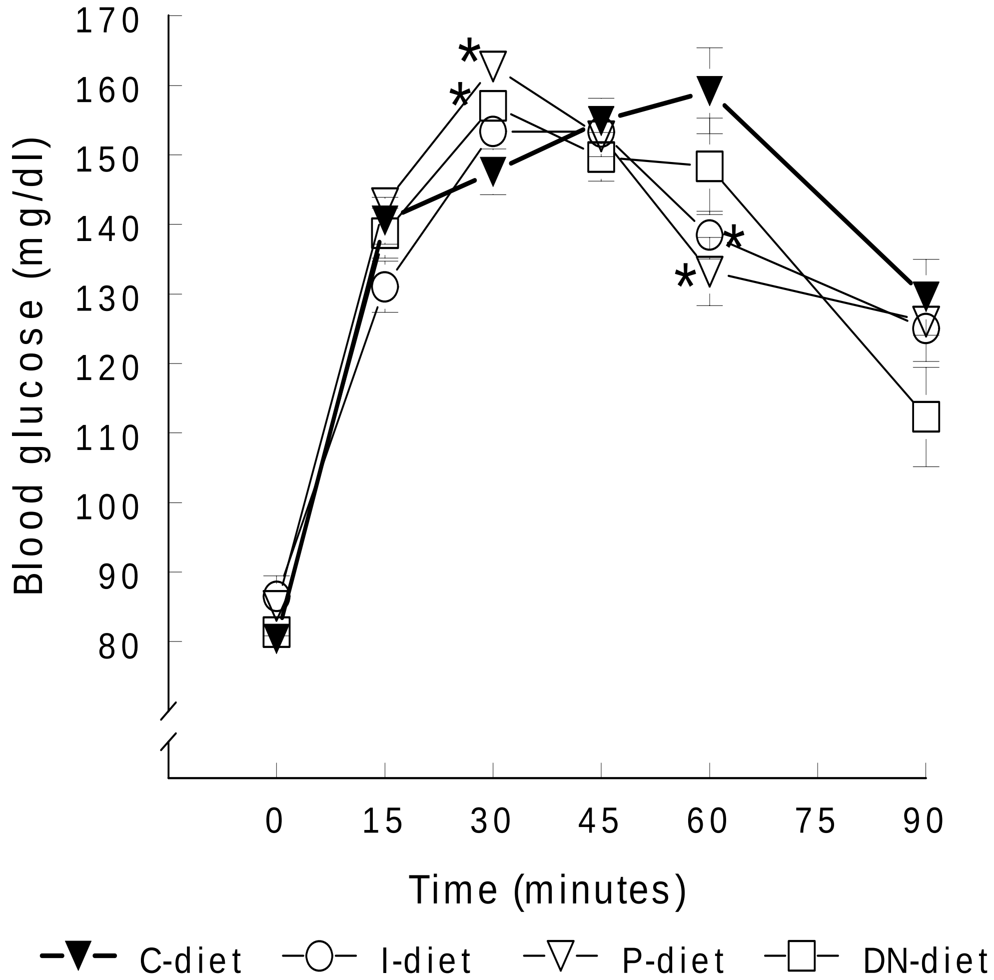
Blood glucose (mg/dl)
170
160
150
140
130
120
110
100
90
80
0
15
30
45
60
75
90
Time (minutes)
*
*
*
*
C-diet
I-diet
P-diet
DN-diet

Table 1. Content of diets (g/100 g diet)

Nutrients	Diets			
	Control	Pérola	Diamante Negro	Iraí
Bean flour	0	19·30	22·83	20·62
Fibre (cellulose)	5	0	0	0
Caseine	20	15·33	13·63	14·84
Corn starch	52·90	43·31	41·49	42·49

Table 2. Fibre content of diets (g/100 g diet)

Nutrients	Diets			
	Control	Pérola	Diamante Negro	Iraí
Total fibre (TF)	5	5	5	5
Soluble fibre (SF)	0	0·55	0·97	1·28
Insoluble fibre	5	4·45	4·03	3·72
SF/TF proportion	0	0·11	0·19	0·25

Table 3. Effect of bean-containing diet on biological parameters of rats

	Diets			
	Control	Pérola	Diamante Negro	Iraí
Final body weight (g)	188·51±5·28	192·68±4·25	194·23±4·19	197·41±5·85
Feed intake (g/day)	16·72±0·17	17·34±0·27	16·21±0·35	16·48±0·31
Body weight gain (g)	117·02±3·24	121·90±3·92	115·03±3·58	127·78±3·33
Feed efficiency ratio (g/g)	0·310±0·002^{bc}	0·320±0·003^{ab}	0·300±0·003^{c}	0·330±0·005^{a}

Results are expressed as mean value±standard error of the mean (n=8).

Parameters were measured during growth period (26–44 days of age).

Values within the same horizontal row with no common superscript differ significantly (P < 0·05, Duncan's test).

Table 4. Digestibility (g/100 g of dry weight) of dry matter, starch, and fibre of the diets

Digestibility (%)	Diets			
	Control	Pérola	Diamante Negro	Iraí
Dry matter	92·84±0·08[a]	90·47±0·35[b]	90·29±0·17[b]	92·58±0·16[a]
Starch	94·24±0·14[a]	92·30±0·44[b]	91·93±0·48[b]	94·61±0·32[a]
Fibre	68·22±1·31[c]	79·47±1·03[b]	77·13±1·66[b]	86·82±0·41[a]

Results are expressed as mean value±standard error of the mean (n=8).

Values within the same horizontal row with no common superscript differ significantly ($P < 0·05$, Duncan's test).

Table 5. Effect of diets on faecal parameters

	Diets			
	Control	Pérola	Diamante Negro	Iraí
Faecal weight (g/day)	1·11±0·04[b]	1·69±0·08[a]	1·62±0·03[a]	1·22±0·05[b]
Faecal pH	6·19±0·07[a]	5·68±0·05[c]	5·86±0·04[bc]	5·93±0·06[b]
Faecal moisture (g/100 g of fresh weight)	27·34±0·68[c]	37·47±1·12[a]	32·22±0·72[b]	30·63±1·19[b]

Results are expressed as mean value±standard error of the mean (n=8).

Values within the same horizontal row with no common superscript differ significantly ($P < 0·05$, Duncan's test).

Table 6. Effect of diets on liver and epididymal fat weight and on serum lipids concentration

	Diets			
	Control	Pérola	Diamante Negro	Irai
Liver/body weight ratio (mg/g)	37·53±0·48[ab]	37·03±0·42[b]	37·11±0·31[b]	38·95±0·70[a]
Epididymal fat/body weight ratio (mg/g)	16·86±0·62[a]	13·51±0·65[b]	12·85±0·36[b]	12·61±0·92[b]
Total cholesterol (mg/dL)	115·43±4·18[a]	92·82±3·11[b]	93·11±3·45[b]	82·44±3·98[b]
HDL cholesterol (mg/dL)	50·93±2·96[a]	43·99±2·59[ab]	36·.61±2·52[bc]	33·.67±2·33[c]
HDL/total cholesterol ratio	37·70±0·67	42·95±1·11	44·21±2·03	39·35±3·64
Triglyceride (mg/dL)	87·72±1·95	97·18±6·06	82·46±3·20	78·21±6·76

Results are expressed as mean value±standard error of the mean (n=8).

Values within the same horizontal row with no common superscript differ significantly (P < 0·05, Duncan's test).

Table 7. Area under the glycaemic curve (% of control value)

Diet	Interval of time (min)					
	0 – 15	15 - 30	30 - 45	45 - 60	60 - 90	0 - 90
Control	100[b]	100	100	100[a]	100[a]	100[a]
Pérola	104·75±1·61[a]	104·79±2·43	102·74±1·76	90·16±2·11[b]	87·26±1·86[c]	95·75±0·44[b]
Diamante Negro	100·45±0·83[b]	103·65±0·80	99·56±2·08	94·29±1·86[b]	89·40±1·44[bc]	95·93±1·20[b]
Iraí	98·66±1·55[b]	98·51±1·70	100·99±1·98	95·24±1·20[ab]	92·11±1·49[b]	96·18±0·71[b]

Results are expressed as mean value±standard error of the mean (n=8).

Values within the same column with no common superscript differ significantly (P < 0·05, Duncan's test).

4 DISCUSSÃO

O feijão comum é um alimento amplamente consumido pelos povos ao redor do mundo, principalmente por aqueles economicamente menos favorecidos. Apresenta propriedades nutritivas e funcionais inquestionáveis, principalmente em função de seu perfil bioquímico peculiar. No entanto, as cultivares plantadas nas mais variadas regiões do globo apresentam diferenças significativas na sua composição, tanto de macro quanto de micronutrientes e podem, com o passar dos anos, alterar seu perfil bioquímico e perder suas características genéticas originais.

Em nosso estudo comparamos o perfil bioquímico de 16 diferentes cultivares de feijão recomendadas para plantio no RS, e que foram colhidas em 2 anos consecutivos. Observamos que as cultivares não mudaram significativamente suas composições químicas entre as safras, exceto pela pequena variação nos teores de umidade, matéria seca e fibra total. A despeito do aumento nos teores de fibra alimentar total (FT) na safra 2002/2003 ter sido significativo, os teores de fibra alimentar insolúvel (FI) e de fibra alimentar solúvel (FS) não aumentaram significativamente, demonstrando que este incremento foi realmente discreto e se diluiu quando as frações foram analisadas separadamente. É sabido que os feijões podem variar a sua composição química conforme o local de plantio (SAMMAN et al., 1999). No entanto, não encontramos estudos que avaliassem as possíveis alterações no perfil de macro e micronutrientes entre cultivares de feijão de uma mesma variedade genética, mas oriundos de safras distintas. Nosso estudo demonstrou que feijões plantados em um mesmo local, sob as mesmas condições de cultivo tendem a manter suas características ao longo de pelo menos 2 safras. Resultados semelhantes já haviam sido observados em cultivares de aveia, trigo e arroz (SILVA, 2002; STORCK, SILVA, & FAGUNDES, 2005).

Entre as cultivares estudadas observamos uma grande variação nos teores de macronutrientes, principalmente de fibra alimentar solúvel (106%) e amido resistente (100%), seguidos por amido digestível (77%) e fibra alimentar insolúvel (70%). Já o teor de proteína foi o parâmetro de menor variação (36%) entre as sementes. Baseado nisto podemos concluir que as diferentes variedades genéticas de feijão estudadas mantiveram esta característica peculiar do alto conteúdo protéico (21,24-29,06 g/100 g MS) e sem diferenças importantes entre elas.

Utilizando análise de agrupamento para categorizar as cultivares com características químicas semelhantes obtivemos 4 grupos distintos quanto ao perfil de macronutrientes. As cultivares Guateian 6662 e Rio Tibagi compuseram o grupo com os maiores teores de proteínas, de fibra solúvel e de amido disponível. Este perfil peculiar torna estas cultivares mais adequadas às recomendações para prevenção e tratamento de doenças como diabete, deslipidemias, obesidade e doenças cardiovasculares. Além disto, são úteis como fonte alimentar para populações de risco nutricional. As cultivares Iraí, Minuano e TPS Bonito compuseram o grupo que apresentou os maiores teores de fibra insolúvel e de amido resistente. Porém, foram as com menor teor de proteínas. Assim, poderiam ser indicadas em situações onde uma oferta maior de fibras insolúveis fosse necessária (ex.: constipação) ou onde uma restrição protéica fosse indicada (ex.: insuficiência renal crônica).

Da mesma forma que para os macronutrientes, os minerais também foram agrupados pela semelhança em seus teores. Obtivemos 4 grupos distintos. O grupo formado pelas cultivares FTS Magnífico, FTS Soberano, Guateian 6662, Pérola, Rio Tibagi, TPS Bionobre e TPS Nobre foi o que apresentou os maiores teores de todos os minerais avaliados. Novamente as cultivares Guateian 6662 e Rio Tibagi estão presentes, reforçando o valor nutricional destas variedades no contexto deste estudo.

Por outro lado, as cultivares Diamante Negro, Macanudo e Valente compuseram o grupo com os menores valores para os macronutrientes, exceto pelo valor intermediário de proteína; as cultivares Iapar 31, Iraí e Macanudo compuseram o grupo com os menores valores para os micronutrientes. Assim, estas cultivares foram as de mais baixo valor nutricional quanto aos macro e micronutrientes, respectivamente.

Este estudo demonstrou, ainda, uma correlação negativa entre o tamanho da semente e o teor de proteína bruta (PB). Desta forma, sementes grandes, muitas vezes preferidas para o consumo, são as de menor valor nutricional para este nutriente. LEMOS et al. (2004) avaliaram as características agronômicas (produtividade de grãos, número de vagens/planta, número de grãos/vagem e massa de 100 grãos) e tecnológicas (teor de proteína bruta, tempo de cozimento, capacidade de hidratação e presença de grãos de casca dura que não hidratam) de 29 genótipos de feijão. Observaram que genótipos com os maiores teores protéicos nem sempre se sobressaíram nas outras características avaliadas, especialmente em relação à produtividade. Já genótipos com as maiores produtividades apresentaram teores de PB abaixo da média. Assim, concluíram que o teor de PB é inversamente proporcional à produtividade de grãos. Desta forma, se o objetivo do melhoramento genético for apenas gerar cultivares altamente produtivas ou cultivares mais atraentes ao consumidor (grãos grandes), o teor protéico certamente declinará com o passar do tempo, com surgimento de cultivares de menor valor nutricional.

Outra observação feita foi a não existência de correlação entre os teores de fibra bruta (FB) e fibra alimentar (total, solúvel ou insolúvel). Assim, médicos e nutricionistas que utilizam as informações contidas em rótulos de produtos ou em tabelas de composição alimentar devem ficar atentos pois, algumas vezes, apenas os valores referentes à FB estão explicitados. Como visto, estes valores não traduzem as reais quantidades de fibra dietética, que detém importância funcional.

A literatura relata de forma contraditória algumas modificações na composição química dos grãos de feijão cozidos após o armazenamento, tanto em relação aos teores de amido disponível quanto de amido resistente (OSORIO-DIAZ et al., 2003; VARGAS-TORRES et al., 2004; LANDA-HABANA et al., 2004). Decidimos, então, estudar os efeitos do armazenamento (refrigeração a 4^{o}C por 4 dias ou congelamento a -20^{o}C por 28 dias) sobre os teores de amido disponível (AD), amido resistente (AR), fibra total (FT), fibra insolúvel (FI) e fibra solúvel (FS) nestas sementes. O tipo de armazenamento escolhido baseou-se nas formas domésticas habitualmente utilizadas para conservar feijões após seu cozimento. Nossos resultados demonstraram que os grãos cozidos e armazenados apresentaram redução dos níveis do AD e elevação dos níveis de AR, tanto após refrigeração a 4^{o}C por 4 dias quanto após congelamento a -20^{o}C por 28 dias. Estas alterações nos teores de AD e de AR foram menos intensas após congelamento e foram influenciadas pelos teores de AR encontrados nas sementes cruas, pois o congelamento só alterou o AD e o AR em feijões cujos níveis de AR eram baixos antes do cozimento. Assim, em situações onde uma dieta com baixo teor de AD e alto teor de AR estiver indicada, como no tratamento e controle do diabete e da obesidade, feijões cozidos e refrigerados por apenas 4 dias podem ter um efeito metabólico desejável.

Quanto às fibras, tanto a refrigeração quanto o congelamento pelos períodos estudados não determinaram mudanças em relação aos níveis encontrados logo após o cozimento. Desta forma grãos de feijão ao serem cozidos e refrigerados passam a apresentar uma redução nos teores de AD e aumento nos teores de AR, sem alterar os teores de fibra. Assim, o perfil nutricional destes grãos seria modificado, tornando-os mais adequados ao controle e prevenção de doenças que cursam com alterações no metabolismo dos lipídeos e/ou da glicose, bem como da obesidade.

A literatura pertinente enfoca, cada vez mais, as recomendações sobre o perfil da dieta que a população humana deveria ingerir para recuperar ou manter sua saúde. O feijão, pela sua composição química peculiar, enquadra-se nestas recomendações dietéticas. Além disto, sua fração fibra solúvel possui propriedades redutoras dos lipídeos séricos (ANDERSON & GUSTAFSON, 1988) e dos níveis glicêmicos (THORNE et al., 1983; BJORCK & ELMSTAHL, 2003).

Desta forma, o consumo de feijões por pessoas com distúrbios do controle das gorduras e/ou da glicose sanguíneas poderia auxiliá-las a manter seus níveis de lipídeos e glicemia mais estáveis e próximos da normalidade. Assim, complicações inerentes às deslipidemias e à hiperglicemia, bem como a evolução para situações mais críticas, poderiam ser amenizadas. Mas será que este tipo de alimento também poderia influenciar de forma benéfica os níveis de lipídeos e de glicose em pessoas saudáveis ou em risco de desenvolver estes distúrbios metabólicos? Nosso estudo demonstrou que mesmo animais normolipidêmicos, uma vez submetidos a dietas em que a fibra alimentar foi substituída por feijões apresentaram valores mais baixos de colesterol total (19,3 a 28,6% menores) que animais alimentados com a dieta padrão, sendo esta redução relacionada à porcentagem de fibra solúvel do feijão. No entanto, para os triglicerídeos séricos, apesar de os valores encontrados nos animais alimentados com as dietas Iraí e Diamante Negro serem 10,8 e 6% menores que nos controles, respectivamente, esta redução não alcançou significância estatística. A literatura relata que uma redução de 1% nos níveis de colesterol total reduzem em 2% o risco de uma pessoa desenvolver doenças do coração (RIFKIND, 1984). Assim, por dedução, os animais teriam de 38,6 a 57,2% menos risco de desenvolverem doença arterial coronariana. No entanto, não há relatos da percentagem de redução necessária nos níveis de triglicerídeos para que sejam observados efeitos benéficos sobre a saúde. Se as percentagens forem semelhantes, estas taxas de redução detectadas pelo nosso estudo, apesar de modestas, poderão ser de relevância clínica e nutricional.

ANDERSON & GUSTAFSON (1988) estudaram os efeitos de dietas contendo aveia ou feijões, os quais são ricos em fibra solúvel, sobre os níveis de lipídeos em homens hipercolesterolêmicos. Observaram redução nos níveis de colesterol total, LDL, triglicerídeos e glicemia de jejum. Juntamente com outros dados de literatura concluíram que uma ingestão diária de 100-200 g de feijões cozidos reduziria os níveis de colesterol séricos em 12% a curto prazo, mas de 20-25% a longo prazo. Isto levaria a uma redução estimada de 40-50% nos riscos para doença arterial coronariana. No entanto, CHEN et al. (2006) avaliaram os efeitos da fibra solúvel de aveia (8 g/dia) dada por um período de 3 meses a um grupo de voluntários sadios e normocolesterolêmicos. Ao final do estudo não observaram redução significativa nos níveis de colesterol total ou LDL no grupo estudado. HEIJNEN et al. (1996) estudaram os efeitos do amido resistente (AR) sobre os níveis séricos de colesterol total, colesterol HDL e LDL, e triglicerídeos em homens e mulheres sadios e normolipidêmicos. Para isto utilizaram AR tipo 2 (amido de milho cru) e AR tipo 3 (amido de milho retrogradado) sob a forma de suplementos diários de 30 g por 3 semanas. Concluíram que nenhuma das formas de AR foi capaz de reduzir os lipídeos séricos em relação ao grupo controle. Assim, pode-se sugerir que o teor de AR das dietas experimentais parece não ter influenciado os nossos resultados. Além disso, observou-se uma correlação negativa entre a digestibilidade da fibra e o teor de colesterol. Podemos concluir que feijões apresentam um efeito redutor nos níveis de lipídeos séricos, principalmente do colesterol, mesmo em situações de normolipidemia, e que este efeito está ligado a fração digerível da fibra.

A avaliação das respostas glicêmicas após a ingestão das dietas experimentais demonstrou valores inferiores que os determinados pela dieta controle. DILAWARI et al. (1981) avaliaram os efeitos de dietas contendo 50 g de carboidratos de diferentes fontes (incluindo feijão) sobre os níveis de glicose sanguínea pós-prandiais. Compararam estes valores com os de uma curva glicêmica após ingestão de dextrose. Para isto, 6 voluntários sadios ingeriram as dietas experimentais em dias sucessivos e de forma randomizada. Em relação à curva controle observaram que a dieta contendo feijão determinou pico glicêmico mais tardio e de menor valor, e menor área sob a curva nos primeiros 60 minutos pós-prandiais. Em nosso estudo, os valores de glicose sanguínea dos animais no período pós-prandial apresentaram-se mais estáveis e com menores valores para as áreas sob a curva (índice glicêmico-IG). Isto demonstra que mesmo ratos normoglicêmicos alimentados com dietas contendo feijões com elevados teores de fibra solúvel podem apresentar uma melhor regulação do metabolismo da glicose sanguínea, pois, devido ao baixo IG, estas dietas possivelmente produzem menores picos de insulina.

OU et al.(2001) estudaram *in vitro* os mecanismos pelos quais a fibra dietética reduz a glicemia pós-prandial. Concluíram que 3 são os mecanismos possíveis: as fibras dietéticas aumentam a viscosidade do suco intestinal, reduzindo a difusão da glicose; ligam-se à glicose reduzindo sua concentração e sua disponibilidade na luz do intestino; retardam a ação da alfa-amilase devido ao encapsulamento do amido e da enzima, além de atuarem de forma inibitória diretamente sobre a enzima. JENKINS et al. (1978) estudaram os efeitos de diferentes tipos de fibra durante um teste de tolerância à glicose em 6 voluntários sadios. Observaram que fibras dietéticas com viscosidade elevada são mais adequadas para uso terapêutico em pacientes diabéticos por reduzirem os níveis glicêmicos pós-prandiais, sendo esta redução diretamente proporcional à viscosidade da fibra. Assim, a ingestão de feijões com altos teores de fibra solúvel teria um efeito regulador da glicemia pós-prandial nestas situações clínicas.

PAWLAK, KUSHNER, & LUDWIG (2004) estudaram os efeitos em ratos de dietas com diferentes índices glicêmicos (IG) devido ao tipo de carboidrato (HC) utilizado (HC de alto IG; HC de baixo IG). Observaram que a dieta com alto IG causou maior acúmulo de gordura, menor massa magra, maiores áreas sob a curva da glicemia e da insulina, e maiores níveis de triglicerídeos plasmáticos. Concluíram que, como fator independente, o IG pode causar obesidade e aumentar os riscos de diabete e doenças do coração em ratos. BRENNAN (2005) também relatou conclusões semelhantes em seu estudo de revisão: dietas contendo alimentos de elevado IG estão relacionadas com aumento de peso, obesidade e diabete, provavelmente devido à alteração na expressão da enzima ligada à síntese de lipídeos, modificação da resposta hormonal e estimulação da gliconeogênese. Em nosso estudo, através da análise do ganho de peso dos animais e do peso do tecido adiposo epididimal, observamos uma menor retenção de gordura corporal nos animais alimentados com dietas contendo feijão em relação aos controles. Este dado nos leva a crer que, a despeito da não medição dos valores de insulina pós-prandial, possivelmente estes eram inferiores nos ratos alimentados com dietas contendo feijão que nos controles. Assim, devido à estreita relação entre IG, pico de insulina e deposição de gordura, pacientes com obesidade ou em controle de peso poderiam ser beneficiados da ingestão regular de feijões em sua dieta. Isto porque feijões, além de possuírem teores elevados de fibra solúvel, também apresentam amido digestível considerado de baixo IG que, segundo BRENNAN (2005), seria o fator primordial no impacto glicêmico determinado por um alimento.

Sendo assim, o consumo de feijões com teores elevados de fibra solúvel dentro de um plano alimentar poderia auxiliar no controle e prevenção de doenças metabólicas como o diabete melito, as deslipidemias, a obesidade e as doenças cardiovasculares. Para isto, a busca por cultivares que apresentem este perfil e que mantenham estes teores ao longo do tempo deveria ser estimulada.

5 CONCLUSÕES

Quanto às características físico-químicas das sementes de feijão concluímos que:

1. A composição química das sementes variou significativamente de uma cultivar para outra sendo possível categorizá-las em quatro diferentes grupos de acordo com os macronutrientes (PB, FT, FI, FS, AD e AR) bem como pelos micronutrientes (Fe, Zn, Mn, Cu, Ca, Mg e P); as cultivares Guateian 6662 e Rio Tibagi apresentaram os melhores perfis de nutrientes, com altos teores de PB, FS, AD, Fe e Zn.;

2. Exceto pelos níveis de massa seca, umidade e fibra dietética total, as características físico-químicas das cultivares estudadas mantiveram-se estáveis ao longo das duas safras;

3. A estocagem de feijões cozidos e armazenados sob refrigeração ou sob congelamento não alterou os níveis de FT, FI ou FS, mas reduziu os teores de AD e aumentou os teores de AR, principalmente em sementes cujos teores de AR eram menores antes do cozimento.

Quanto aos efeitos das dietas experimentais em ratos normolipidêmicos e normoglicêmicos concluímos que os animais alimentados com dietas contendo cultivares de feijão apresentaram redução dos níveis séricos de colesterol total, menor índice glicêmico, valores mais estáveis de glicemia pós-prandial e menor retenção de gordura corporal, sendo que estes efeitos foram mais marcantes no grupo alimentado com a dieta Iraí (FS/FT: 0,26).

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7 ANEXOS

ANEXO 1 - Roteiro para autores

Guia para redação e edição de manuscrito científico a ser submetido à revista LWT- FOOD SCIENCE AND TECHNOLOGY

Guide for Authors

LWT - Food Science and Technology is an international journal that publishes innovative papers in the fields of food chemistry, biochemistry, microbiology, technology and nutrition. The work described should be innovative either in the approach or in the methods used. The significance of the results either for the science community or for the food industry must also be specified. Contributions that do not fulfil these requirements will not be considered for review and publication. Submission of a paper will be held to imply that it presents original research, that it has not been published previously, and that it is not under consideration for publication elsewhere.

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Reviews. Concise reviews of a scientific or applied field should include all major findings and bring together reports from a number of sources.

Research Papers. Reports of complete, scientifically sound, original research which contributes new knowledge to its field. The paper must be organised as described below.

Research Notes. Brief reports of scientifically sound, original research of limited scope of new findings. Research Notes have the formal organisation of a full paper. Such notes will receive priority of publication.
LWT - Food Science and Technology is an international journal that publishes innovative papers in the fields of food chemistry, biochemistry, microbiology, technology and nutrition. The work described should be innovative either in the approach or in the methods used. The significance of the results either for the science community or for the food industry must also be specified. Contributions that do not fulfil these requirements will not be considered for review and publication. Submission of a paper will be held to imply that it presents original research, that it has not been published previously, and that it is not under consideration for publication elsewhere.

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Language Manuscripts should be written in English. Authors whose mother tongue is not English are strongly advised to have their manuscripts checked by someone familiar with English scientific writing. The Editors reserve the right to make any necessary linguistic alterations without consulting the authors.

Preparation of Manuscripts Authors should aim at producing 6 printed A4 pages (research papers) or 3 printed pages (research notes), which corresponds to approximately 18 and 9 pages of double-spaced type, respectively. All papers should follow the new style for LWT. Please see Volume 36 issue 1 onward. Manuscripts should be double-spaced throughout, with a left-hand margin of not less than 4 cm. On the first page, immediately below the title, give authors' names, affiliations and business addresses. Number the lines of each page. If your manuscript cites submitted but unpublished papers, send one copy of each of these papers with your manuscript.

The SI system (Systeme International d'Unites, often referred to as 'International Units') must be used for reporting units of measurement. Do not use %, ppm, M, N, etc. as units for concentrations. If analytical data are reported, replicate analyses must have been carried out and the number of replications must be stated.

Standard error or other evidence of reliability of data must be given.

Editorial Style *Title*. The title of the papers should be short but informative.

Abstract. Each article should include an abstract, not exceeding 200 words.
Introduction. In the Introduction, briefly review important prior publications and state the

reasons for the investigation being reported.

Methods. Results. Discussion. Following the Introduction, authors are free to structure papers as appropriate. However, for the sake of clarity and uniformity, the above or similar section headings are recommended. If necessary, each section may be divided into further subsections, but do not use more than two levels for subtitles.
The Materials and Methods section must provide enough detail that a competent worker can repeat the experiments. However, detailed descriptions of well-known methods should be avoided in the experimental section. References to the relevant literature are sufficient. The Discussion should not be a compilation of current literature, but a consideration of the significance and consequences of the authors' present findings.

References: All publications cited in the text should be presented in a list of references following the text of the manuscript. In the text refer to the author's name (without initials) and year of publication (e.g. "Steventon, Donald and Gladden (1994) studied the effects..." or "...similar to values reported by others (Anderson, Douglas, Morrison & Weiping, 1990)..."). For 2-6 authors all authors are to be listed at first citation. At subsequent citations use first author et al.. When there are more than 6 authors, first author et al. should be used throughout the text. The list of references should be arranged alphabetically by authors' names and should be as full as possible, listing all authors, the full title of articles and journals, publisher and year. The manuscript should be carefully checked to ensure that the spelling of authors' names and dates are exactly the same in the text as in the reference list. References should be given in the following form:

Watt, D. K., Brasch, D. J., Larsen, D. S., & Melton, L. D. (1999). Isolation, characterisation, and NMR study of xyloglucan from enzymatically depectinised and non-depectinised apple pomace. *Carbohydrate Polymers*, 39(2), 165-180.

Closs, C. B., Roberts, I. D., Conde-Petit, B., & Eschler, F. (1997). Phase separation and rheology of aqueous amylopectin/ galactomannan systems. In E. J. Windhab, & B. Wolf. Proceedings of the 1st international symposium on food rheology and structure (pp. 233-237). Hannover: Vincentz Verlag.

Stephen, A. M. (1995). Food polysaccharides and their applications. New York: Marcel Dekker. Wurzburg, O. B. (1986). Cross-linked starches. In O. B. Wurzburg, Modified starches: properties and uses (pp. 41). Boca Raton, FL: CRC Press.

Tables
Tables should be numbered and headed by a short but informative title. The experimental conditions, as far as they are necessary for understanding, should be given. Use no vertical and few horizontal lines. Probabilities may be indicated by $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Tables should be submitted on separate sheets.

Figures and Illustrations
Figures must be provided on separate sheets. Do not repeat material already included in tables. Figures should be comprehensible without reference to the text. All drawings and graphs should not exceed 20 x 20.5 cm in size. All illustrations should be consecutively numbered. Keys to graphs etc., should not appear on the figure, but only in the figure legend. Legends should consist of a short title followed by a brief description of experimental

conditions and, where necessary, a key. Where it is necessary to use photographs, these should again be clearly labelled and numbered. Authors are strongly advised to consult Elsevier's artwork instructions before uploading any graphics files for electronic submission. These are available at http://authors.elsevier.com/artwork.

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Key Words
Four to five pertinent key words should be provided. If possible the Food Science and Technology Abstracts (FSTA) Thesaurus should be used (IFIS Publ., Shinfield, Reading RG2 9BB, UK http://www.foodScienceCentral.com).

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ANEXO 2 - Roteiro para autores

Guia para redação e edição de manuscrito científico a ser submetido à revista British Journal of Nutrition

The *British Journal of Nutrition* is an international peer-reviewed journal which publishes original papers, review articles (including those critically re-examining published information and the conclusions drawn from it), technical notes and short communications in all branches of nutritional science. Short communications will be expedited through the review process. The underlying aim of all work should be, as far as possible, to develop nutritional concepts**. Prospective authors should note that they (or their institutions) now retain the copyright of their material published in the *British Journal of Nutrition*.** As a contributor you are asked to follow the guidelines set out below. Prospective authors may also contact the Editorial Office directly on + 44 (0)207 371 6225 (telephone), +44 (0)207 602 1756 (fax), or edoffice@nutsoc.org.uk (email).

Papers submitted for publication should be written in English and be as concise as possible. **The *BJN* now operates an on-line submission and reviewing system (eJournalPress). Authors should submit to the following address: http://bjn.msubmit.net/**. Receipt of papers will be acknowledged immediately.

Papers should be accompanied by a statement signed by each author (or by the submitting author on behalf of the others) to the effect that the conditions laid down in the Directions to Contributors are accepted. The statement should affirm that the submission represents original work that has not been published previously and which is not currently being considered by another journal, and that if accepted for the *British Journal of Nutrition* it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Editor-in-Chief. It should also confirm that each author has seen and approved the contents of the submitted manuscript. **The submission letter should be accompanied by a completed copy of the 'Licence to Publish' (in lieu of copyright transfer) as reproduced in the January 2004 issue (vol. 91, no 1) of the *British Journal of Nutrition* or on the Nutrition Society's web pages** (http://www.nutritionsociety.org); the Society no longer requires copyright of the material published in the journal, only a 'Licence to Publish', the authors or their institution retaining the copyright.

When substantial revisions are required to manuscripts, authors are given the opportunity to do this once only, the need for any further changes should at most reflect only minor issues. Should there be a change in the authorship then a new Licence to Publish should be submitted to reflect that change.

Authors' names should be given without titles or degrees and one forename may be given in full. The name and address of the institution where the work was performed should be given. Any necessary descriptive material about the author, e.g. Beit Memorial Fellow, should appear at the end of the paper in the acknowledgments section.

Manuscripts should bear the name and address, together with telephone and fax numbers and email address, of the person to whom correspondence is to be sent and should also give a shortened version of the paper's title, not exceeding forty-five letters and spaces in length, suitable for a running title in the published pages of the work.

If a paper requiring revision is not resubmitted within 3 months, it may, on resubmission, be deemed a new paper and the date of receipt altered accordingly.

Short Communications. Papers submitted as Short Communications should consist of an Abstract (250 words maximum), and no more than 3000 words of text (including references).

Each Short Communication can include up to two Tables or one Table and one Figure, but these will be at the expense of text (one half-page Table or Figure is equivalent to about 500 words in two columns or 250 words in one column).

Nutrition Discussion Forum. Letters are invited which discuss, criticize or develop themes put forward in papers published in the *Journal*, or which deal with matters relevant to it. They should not, however, be used as a means of publishing new work.

Acceptance will be at the discretion of the Editorial Board, and editorial changes may be required. Wherever possible, letters from responding authors will be included in the same issue.

Form of Papers Submitted for Publication. The onus of preparing a paper in a form suitable for sending to press lies with the author. Authors are advised to consult a current issue in order to make themselves familiar with the practice of the *British Journal of Nutrition* as to typographical and other conventions, layout of tables and so on. Papers will not be accepted as part of a numbered series; instead there should be a short common title separated by a colon from a subtitle more specific to the paper. Sufficient information should be given to permit repetition of the published work by any competent reader of the *Journal*. Authors are invited to nominate up to four potential referees who may then be asked by the Editorial Board to help review the work.

Papers should be in double-spaced typescript on paper with wide margins (2 cm or more). At the ends of lines, words should not be hyphenated unless hyphens are to be printed. A space of 50mm should be left at the top of the first sheet. Line-numbered paper is encouraged.

Spelling should generally be that of the *Concise Oxford Dictionary* (1995), 9th ed. Oxford: Clarendon Press. Paper should normally be divided into the following parts:

(a) *Abstract*: each paper must open with an abstract of not more than 250 words. The abstract should be a single paragraph of continuous text outlining the aims of the work, the experimental approach taken, the principal results, and the conclusions and their relevance to nutritional science.

(b) *Introduction*: it is not necessary to introduce a paper with a full account of the relevant literature, but the introduction paragraph should indicate briefly the nature of the question asked and the reasons for asking it.

(c) *Experimental methods adopted*: methods should appear after the introduction.

(d) *Results*: these should be given as concisely as possible, using figures or tables as appropriate.

(e) *Discussion*: while it is generally desirable that the presentation of the results and the discussion of their significance should be presented separately, there may be occasions when combining these sections may be beneficial. Authors may also find that additional or alternative sections such as 'conclusions' may be useful.

(f) *Acknowledgments*: these should be given in a single paragraph after the discussion and be as brief as possible.

(g) *References*: these should be given in the text thus: Sebrell & Harris (1967) showed that ..., or ... has been shown (Wallace & West, 1982); where a paper to be cited has more than two authors, citations should appear thus: (Peto *et al*. 1981). Where more than one paper has appeared in one year for which the first name in a group of three or more authors is the same, the reference should be given as follows: Adams *et al*. (1962*a*,*b*,*c*) ..., or ... (Adams *et al*. 1962*a*,*b*,*c*). In the text, references grouped together should be given in chronological order thus: ... (Wallace & West, 1982; Lau, 1988). At the end of the paper, on a page(s) separate from the text, references should be listed in alphabetical order according to the name of the first author of the publication quoted and should include the author's initials and the title of the paper. When an article has more than ten authors only the names of the first three authors should be given followed by *et al*. Names and initials of authors of unpublished work should be given in the text and not included in the References. Titles of journals should appear in their abbreviated form using the NICB LinkOut page http://**www.ncbi.nlm.nih.gov/entrez/journals/loftext_noprov.html**). References to books and monographs should include the Publisher's name, the town of publication and the number of the edition to which reference is made. Thus:

Ablett JG & McCance RA (1971) Energy expenditure of children with kwashiorkor. *Lancet* **ii**, 517–519.

Adams RL, Andrews FN, Gardiner EE, Fontaine WE & Carrick CW (1962*a*) The effects of environmental temperature on the growth and nutritional requirements of the chick. *Poultry Sci* **41**, 588–594.

Adams RL, Andrews FN, Rogler JC & Carrick CW (1962*b*) The protein requirement of 4-week-old chicks as affected by temperature. *J Nutr* **77**, 121–126.

Adams RL, Andrews FN, Rogler JC & Carrick CW (1962*c*) The sulfur amino acid requirement of the chick from 4 to 8 weeks as affected by temperature. *Poultry Sci* **41**, 1801–1806.

Agricultural Research Council (1981) *The Nutrient Requirements of Pigs*. Slough: Commonwealth Agricultural Bureaux.

Clément K, Vaisse C, Lahlou N, *et al*. (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**, 398–401.

Edmundson W (1980) Adaptation to undernutrition: how much food does man need? *Soc Sci Med* **14** D, 19–126.

European Communities (1971) *Determination of Crude Oils and Fats, Process A.* Part 18, *Animal Feeding-stuffs*, pp. 15–19. London: H. M. Stationery Office.

Hegsted DM (1963) Variation in requirements of nutrients – amino acids. *Fed Proc* **22**, 1424–1430.

Heneghan JB (1979) Enterocyte kinetics, mucosal surface area and mucus in gnotobiotes. In *Clinical and Experimental Gnotobiotics. Proceedings of the VIth International Symposium on Gnotobiology*, pp. 19–27 [TM Fliedner, H Heit, D Niethammer and H Pflieger, editors]. Stuttgart: Gustav Fischer Verlag.

Hill DC (1977) Physiological and biochemical responses of rats given potassium cyanide or linamarin. In *Cassava as an Animal Feed. Proceedings of a Workshop held at University of Guelph, 1977. International Development Research Centre Monograph* 095e, pp. 33–42 [B Nestel and M Graham, editors]. Ottawa, Ont., Canada: International Development Research Centre.

Lau EMC (1988) Osteoporosis in elderly Chinese (letter). *Br Med J* **296**, 1263.

Louis-Sylvestre J (1987) Adaptation de l'ingestion alimentaire aux dépenses energétiques (Adaptation of food intake to energy expenditure). *Reprod Nutr Dév* **27**, 171–188.

Martens H & Rayssiguier Y (1980) Magnesium metabolism and hypomagnesaemia. In *Digestive Physiology and Metabolism in Ruminants*, pp. 447–466 [Y Ruckebusch and P Thivend, editors]. Lancaster: MTP Press Ltd.

Ministry of Agriculture, Fisheries and Food (1977) *Energy Allowances and Feeding Systems for Ruminants. Technical Bulletin* no. 33. London: H.M. Stationery Office.

Peto R, Doll R, Buckly JD & Sporn MB (1981) Can dietary beta-carotene materially reduce human cancer rates? *Nature* **290**, 201–208.

Sebrell WH Jr & Harris RS (1967) *The Vitamins*, 2nd ed., vol. 1. London: Academic Press.

Technicon Instruments Co. Ltd (1967) *Technicon Methodology Sheet* N-36. Basingstoke: Technicon Instrument Co. Ltd.

Van Dokkum W, Wesstra A & Schippers F (1982) Physiological effects of fibre-rich types of bread. 1. The effect of dietary fibre from bread on the mineral balance of young men. *Br J Nutr* **47**, 451–460.

Wallace RJ & West AA (1982) Adenosine 5' triphosphate and adenylate energy charge in sheep digesta. *J Agric Sci (Cambridge)* **98**, 523–528.

Wilson J (1965) Leber's disease. PhD Thesis, University of London.

World Health Organization (1965) *Physiology of Lactation. Technical Report Series* no. 305. Geneva: WHO.

References to material available on websites should include the full Internet address, and the date of the version cited. Thus:

Department of Health (1997) Committee on Toxicity of Chemicals in Food Consumer Products and the Environment. Statement on vitamin B_6 (pyridoxine) toxicity. http://www.open.gov.uk/doh/hef/B_6.htm

Mathematical Modelling of Nutritional Processes. Papers in which mathematical modelling of nutritional processes forms the principal element will be considered for publication provided: (i) they are based on sound biological and mathematical principles, (ii) they advances nutritional concepts or identifies new avenues likely to lead to such advances, (iii) assumptions used in their construction are fully described and supported by appropriate argument, (iv) they are described in such a way that the nutritional purpose is clearly apparent, (v) the contribution of the model to the design of future experimentation is clearly defined.

Units. Results should be presented in metric units according to the International System of Units (see *Quantities, Units, and Symbols* (1971) London: The Royal Society, and *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences* (1972) London: The Royal Society – as reproduced in *Proceedings of the Nutrition Society* (1972) **31**, 239–247).

Energy measurements should be expressed in joules.

For substances of known molecular weight, e.g. glucose, urea, Ca, Na, Fe, K, P, values should be expressed as mol/l: for substances of indeterminate molecular weights, e.g. phospholipids, proteins, and for trace elements, e.g. Cu, Zn, then g/l should be used.

Time. The 24 h clock should be used, e.g. 15.00 hours.

Statistical Treatment of Results.

Data from individual replicates should not be given for large experiments, but may be given for small studies. The methods of statistical analysis used should be described, and references to statistical analysis packages included in the text, thus: Statistical Analysis Systems statistical software package version 6.11 (SAS Institute, Cary, NC, USA). Information such as analysis of variance tables should be given in the paper only if they are relevant to the discussion. A statement of the number of replicates, their average value and some appropriate measure of variability is usually sufficient.

Comparisons between means can be made by using either confidence intervals or significance tests. The most appropriate of such measures is usually the standard error of a difference between means (SED), or the standard errors of the means (SE or SEM) when these vary between means. The standard deviation (SD) is more useful only when there is specific interest in the variability of individual values. The degrees of freedom associated with SED, SEM or SD should also be stated. The number of decimal places quoted should be sufficient but not excessive. Note that pH is an exponential number, as are the log(10) values often quoted for microbial numbers. Statistics should be carried out on the scalar rather than the expontential values.

If comparisons between means are made using confidence intervals (CI), these may be presented as, e.g. ‘difference between means 0·73 g (95 % CI 0·314, 1·36)’. If significance tests are used, a statement that the difference between the means for two groups of values is (or is not) statistically significant should include the level of significance attained, preferably as an explicit *P* value (e.g. P =0·016 or P =0·32) rather than as a range (e.g. P<0·05 or P>0·05}. It should be stated whether the significance levels quoted are one-sided or two-sided. Where a multiple comparison procedure is used, a description or explicit reference should be given. Where appropriate, a superscript notation may be used in tables to denote levels of significance; similar superscripts should denote lack of a significant difference.

Where the method of analysis is unusual, or if the experimental design is at all complex, further details (e.g. experimental plan, raw data, confirmation of assumptions, analysis of variance tables, etc.) should be included.

Figures. In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, 🕘, 🕘, _, σ, 🕘, ν, ×, . Curves and symbols should not extend beyond the experimental points. Scale-marks on the axes should be on the inner side of each axis and should extend beyond the last experimental point. Ensure that lines and symbols used in graphs and shading used in histograms are large enough to be easily identified when the figure is reduced to fit the printed page.

Figures and diagrams should be provided with numbers and lettering, preferably using computer-based graphical programmes. The names of the authors, title of the paper and the figure number should be given in pencil on the back of each figure. Legends for all figures should be typed on a separate sheet and numbered. Each figure, with its legend, should be comprehensible without reference to the text. The approximate position of each should be indicated in the margin of the text.

Plates. The *Journal* will now also consider the inclusion of colour plates. The size of photomicrographs may have to be altered in printing; in order to avoid mistakes, the magnification should be shown by scale on the photograph itself. The scale with the appropriate unit together with any lettering should be drawn by the author, preferably using appropriate software.

Tables. Tables should carry headings describing their content and should be comprehensible without reference to the text. The dimensions of the values, e.g. mg/kg, should be given at the top of each column. Tables should be typed on separate sheets at the end of the text. Tables should not be subdivided by ruled lines. Abbreviations in tables must be defined in footnotes. Signs for footnotes should be used in the sequence: *†‡§|¶, then ** etc. (omit * or †, or both, from the sequence if they are used to indicate levels of significance). The approximate position should be indicated in the margin of the text.

Key Words. Authors are asked to supply three or four key words or phrases (each containing up to three words) on the title page of the typescript. Please see a recent issue of the *British Journal of Nutrition Cumulative Index* for examples of approved key words.

Chemical Formulas. These should be written as far as possible on a single horizontal line. With inorganic substances, formulas may be used from first mention. With salts, it must be stated whether or not the anhydrous material is used, e.g. anhydrous $CuSO_4$, or which of the different crystalline forms is meant, e.g. $CuSO_4.5H_2O$, $CuSO_4.H_2O$.

Descriptions of Solutions, Compositions and Concentrations. Solutions of common acids, bases and salts should be defined in terms of molarity (M), e.g. 0·1 M-NaH_2PO_4.

Compositions expressed as mass per unit mass (w/w) should have values expressed as ng, µg, mg or g per kg; similarly for concentrations expressed as mass per unit volume (w/v), the denominator being the litre. Concentrations or compositions should not be expressed on a percentage basis. The common measurements used in nutritional studies, e.g. digestibility, biological value and net protein utilization, should be expressed as decimals rather than as percentages, so that amounts of available nutrients can be obtained from analytical results by direct multiplication. See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences*. London: The Royal Society, 1972 (para. 8).

Nomenclature of Vitamins. Most of the names for vitamins and related compounds that are accepted by the Editors are those recommended by the IUNS Committee on Nomenclature. See *Nutrition Abstracts and Reviews* A (1978) **48**, 831–835.

*Acceptable name Other names**

Vitamin A

Retinol Vitamin A_1

Retinaldehyde, retinal Retinene

Retinoic acid (all-*trans* or 13-*cis*) Vitamin A_1 acid

3-Dehydroretinol Vitamin A_2

Vitamin D

Ergocalciferol, ercalciol Vitamin D_2 calciferol

Cholecalciferol, calciol Vitamin D_3

Vitamin E

α-, β- and γ-tocopherols plus
tocotrienols

Vitamin K

Phylloquinone Vitamin K_1

Menaquinone-n (MK-n) † Vitamin K_2

Menadione Vitamin $K_{3,}$
menaquinone,
menaphthone

Vitamin B_1

Thiamin Aneurin(e), thiamine

Vitamin B_2

Riboflavin Vitamin G, riboflavine,
Lactoflavin

Niacin

Nicotinamide Vitamin PP

Nicotinic acid

Folic Acid

Pteroyl(mono)glutamic acid Folacin, vitamin B_c or M

Vitamin B_6

Pyridoxine Pyridoxol

Pyridoxal

Pyridoxamine

Vitamin B_{12}

Cyanocobalamin

Hydroxocobalamin Vitamin B12*a* or Vitamin B12*b*
Aquocobalamin
Methylcobalamin
Adenosylcobalamin
Inositol
Myoinositol Meso-inositol
Choline
Pantothenic acid
Biotin Vitamin H
Vitamin C
Ascorbic acid
Dehydroascorbic acid

*Including some names which are still in use elsewhere, but are not used by the *British Journal of Nutrition*.

†Details of the nomenclature for these and other naturally occurring quinones should follow the Tentative Rules of the IUPAC-IUB Commission on Biochemical Nomenclature (see *European Journal of Biochemistry* (1975) **53**, 15–18).

Generic descriptors. The terms **vitamin A**, **vitamin C** and **vitamin D** may still be used where appropriate, for example in phrases such as ‘vitamin A deficiency’, ‘vitamin D activity’.

Vitamin E. The term **vitamin E** should be used as the descriptor for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of α-tocopherol. The term **tocopherols** should be used as the generic descriptor for all methyl tocols. Thus, the term **tocopherol** is not synonymous with the term **vitamin E**.

Vitamin K. The term **vitamin K** should be used as the generic descriptor for 2-methyl-1,4-naphthoquinone (menaphthone) and all derivatives exhibiting qualitatively the biological activity of phylloquinone (phytylmenaquinone).

Niacin. The term **niacin** should be used as the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.

Vitamin B_6. The term **vitamin B_6** should be used as the generic descriptor for all 2-methylpyridine derivatives exhibiting qualitatively the biological activity of pyridoxine.

Folate. Due to the wide range of carbon-substituted, unsubstituted, oxidized, reduced and mono- or polyglutamyl side-chain derivatives of pteroylmonoglutamic acid which exist in nature, it is not possible to provide a complete list. Authors are encouraged to use either the generic name or the correct scientific name(s) of the derivative(s), as appropriate for each circumstance.

Vitamin B_{12}. The term **vitamin B_{12}** should be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. The term **corrinoids** should be used as the generic descriptor for all compounds containing the corrin nucleus and thus chemically related to cyanocobalamin. The term **corrinoid** is not synonymous with the term **vitamin B_{12}**.

Vitamin C. The terms **ascorbic acid** and **dehydroascorbic acid** will normally be taken as referring to the naturally occurring L-forms. If the subject matter includes other optical isomers, authors are encouraged to include the L- or D-prefixes, as appropriate. The same is true for all those vitamins which can exist in both natural and alternative isomeric forms.

Amounts of vitamins and summation. Weight units are acceptable for the amounts of vitamins in foods and diets. For concentrations in biological tissues, SI units should be used;

however, the authors may, if they wish, also include other units, such as weights or international units, in parentheses.

See Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences (1972) paras. 8 and 14–20. London: The Royal Society.

Nomenclature of Fatty Acids and Lipids. In the description of results obtained for the analysis of fatty acids by conventional gas–liquid chromatography, the shorthand designation proposed by Farquhar JW, Insull W, Rosen P, Stoffel W & Ahrens EH (*Nutrition Reviews* (1959), **17**, Suppl.) for individual fatty acids should be used in the text, tables and figures. Thus 18 : 1 should be used to represent a fatty acid with eighteen carbon atoms and one double bond; if the position and configuration of the double bond is unknown. The shorthand designation should also be used in the abstract. If the positions and configurations of the double bonds are known, and these are important to the discussion, then a fatty acid such as linoleic acid may be referred to as *cis*-9,*cis*-12-18 : 2 (positions of double bonds related to the carboxyl carbon atom 1). However, to illustrate metabolic relationship between different unsaturated fatty acid families, it is sometimes more helpful to number the double bonds in relation to the terminal methyl carbon atom, *n*. The preferred nomenclature is then: 18 : 3*n*-3 and 18 : 3*n*-6 for α-linolenic and γ-linolenic acids respectively; 18 : 2*n*-6 and 20 : 4*n*-6 for linoleic and arachidonic acids respectively and 18 : 1*n*-9 for oleic acid. Positional isomers such as α- and γ-linolenic acid should always be clearly distinguished. It is assumed that the double bonds are methylene-interrupted and are of the *cis*-configuration (see Holman RT in *Progress in the Chemistry of Fats and Other Lipids* (1966) vol. 9, part 1, p. 3. Oxford: Pergamon Press. Groups of fatty acids that have a common chain length but vary in their double bond content or double bond position should be referred to, for example, as C_{20} fatty acids or C_{20} polyunsaturated fatty acids. The modern nomenclature for glycerol esters should be used, i.e. triacylglycerol, diacylglycerol, monoacylglycerol *not* triglyceride, diglyceride, monoglyceride. The form of fatty acids used in diets should be clearly stated, i.e. whether ethyl esters, natural or refined fats or oils. The composition of the fatty acids in the dietary fat and tissue fats should be stated clearly, expressed as mol/100 mol or g/100 g total fatty acids.

Nomenclature of Micro-organisms. The correct name of the organism, conforming with international rules of nomenclature, should be used: if desired, synonyms may be added in parentheses when the name is first mentioned. Names of bacteria should conform with the current Bacteriological Code and the opinions issued by the International Committee on Systematic Bacteriology. Names of algae and fungi must conform with the current International Code of Botanical Nomenclature. Names of protozoa should conform with the current International Code of Zoological Nomenclature.

Nomenclature of Plants. For plant species where a common name is used that may not be universally intelligible, the Latin name in italics should follow the first mention of the common name. The cultivar should be given where appropriate.

Other Nomenclature, Symbols and Abbreviations. Authors should follow current numbers of the *British Journal of Nutrition* in this connection. The IUPAC rules on chemical nomenclature should be followed, and the Recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (see *Biochemical Journal* (1978) **169**, 11–14). The symbols and abbreviations, other than units, are essentially those listed in *British Standard* 5775 (1979–1982), *Specifications for Quantities, Units and Symbols*, parts 0–13. Day should be abbreviated to d, for example 7 d, except for ‘each day’, ‘7th day’ and ‘day 1’.

Elements and simple chemicals (e.g. Fe and CO_2) can be referred to by their chemical symbol or formula from the first mention in the text; titles can be taken as an exception. Well-known abbreviations for chemical substances may be used without explanation, thus: RNA for ribonucleic acid and DNA for deoxyribonucleic acid. Other substances that are mentioned frequently may also be abbreviated, the abbreviation being placed in parentheses at the first mention, thus: free fatty acids (FFA), and an alphabetical list of abbreviations used should be included on a separate sheet of paper. Terms such as 'bioavailability' or 'available' may be used providing that the use of the term is adequately defined.

Spectrophotometric terms and symbols are those proposed in *IUPAC Manual of Symbols and Terminology for Physicochemical Quantities and Units* (1979) London: Butterworths. The attention of authors is particularly drawn to the following symbols: m (milli, 10^{-3}), μ (micro, 10^{-6}), n (nano, 10^{-9}) and p (pico, 10^{-12}). Note also that ml (millilitre) should be used instead of cc, μm (micrometre) instead of μ (micron) and μg (microgram) instead of γ.

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Anexo 3

Valores para peso de 1000 grãos e de macronutrientes das cultivares de feijão das safras 1 (2001/2002) e 2 (2002/2003)*

Cultivar	P 1000 grãos		Proteína Bruta		Amido Disponível		Amido Resistente		Fibra Total		Fibra Solúvel		Extrato Etéreo	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
TPS BONITO	234,00	233,00	22,81	22,73	33,08	33,78	4,70	4,41	24,74	25,11	4,55	7,59	1,42	1,01
TPS BIONOBRE	223,60	215,60	25,68	27,16	28,73	29,12	2,91	3,14	19,36	21,73	4,03	5,73	1,01	0,80
GUAPO BRILHANTE	211,20	230,40	24,16	23,14	35,61	39,41	3,00	3,56	20,60	20,25	4,61	3,86	1,48	1,90
TPS NOBRE	211,00	224,00	26,22	25,37	29,89	34,41	3,22	4,12	22,55	23,02	5,18	4,79	1,93	1,97
IRAÍ	320,80	339,40	21,24	22,50	29,72	31,97	3,18	3,20	22,95	26,06	3,61	6,45	1,35	0,87
FTS SOBERANO	157,60	205,00	29,06	24,62	33,40	38,90	2,99	3,29	19,44	22,58	3,44	3,85	1,56	1,95
GUATEIAN 6662	185,40	189,40	27,45	27,96	33,25	34,02	2,91	2,98	21,05	21,20	9,18	6,43	1,41	1,42
CARIOCA	260,40	259,60	25,44	24,93	35,06	32,12	3,20	3,13	21,38	20,32	7,22	3,68	1,23	1,25
RIO TIBAGI	180,60	151,80	28,95	27,52	27,10	28,06	3,59	3,47	19,25	22,29	4,99	7,51	1,74	1,57
MINUANO	215,60	214,00	23,82	24,52	32,98	28,90	3,55	3,80	22,92	28,01	3,14	8,53	1,53	1,31
PÉROLA	264,80	282,40	26,97	26,09	23,46	29,28	4,56	4,20	20,32	20,77	3,31	1,26	1,44	1,22
FTS MAGNÍFICO	235,80	261,80	26,75	25,84	28,36	29,35	3,98	4,58	21,16	20,12	3,71	2,32	4,77	3,88
MACANUDO	212,80	223,40	23,42	24,73	29,04	25,99	2,35	2,57	20,23	18,41	4,22	2,35	0,85	1,36
DIAMANTE NEGRO	217,80	207,80	27,79	26,27	20,02	23,66	2,54	2,47	19,91	21,28	3,22	5,86	1,62	1,51
VALENTE	203,40	191,60	26,66	24,68	21,56	25,46	2,62	2,46	18,09	20,90	3,81	6,02	1,67	2,26
IAPAR 31	222,40	252,20	24,66	23,70	29,07	28,50	4,18	3,61	18,40	21,08	3,38	5,26	1,33	1,15
Média	222,33	230,09	25,69	25,11	29,39	30,81	3,34	3,44	20,77	22,07	4,47	5,09	1,65	1,59
CV (%)	16,96	18,78	8,62	6,53	15,71	14,55	20,97	19,29	8,70	11,15	36,29	40,70	53,00	46,36

*Valores (média de 3 repetições) expressos em g/100 g de MS.

Anexo 4

Valores de micronutrientes das cultivares de feijão das safras 1 (2001/2002) e 2 (2002/2003)*

Cultivar	Fe		Zn		Mn		Cu		Ca		Mg		P	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
TPS BONITO	9,56	9,37	3,53	3,55	1,50	1,54	1,47	1,36	117,39	117,52	23,98	21,82	274,80	337,42
TPS BIONOBRE	8,83	10,57	3,40	3,38	1,65	1,66	1,56	1,51	361,34	426,37	24,47	22,75	294,47	349,70
GUAPO BRILHANTE	8,39	9,42	3,28	3,51	1,47	1,47	1,38	1,51	343,32	333,80	22,77	23,26	338,47	355,31
TPS NOBRE	10,11	n.d.	4,02	3,46	1,92	1,71	1,43	1,57	394,29	427,24	23,96	24,84	350,75	329,52
IRAÍ	7,50	8,85	4,02	2,72	1,51	1,30	1,17	1,18	325,84	349,92	17,60	20,80	365,96	374,26
FTS SOBERANO	9,30	10,52	3,74	3,69	1,80	1,63	1,48	1,60	351,71	346,09	24,93	24,80	375,18	360,32
GUATEIAN 6662	9,96	9,88	3,21	3,34	1,83	1,58	1,68	1,89	429,75	468,63	26,73	26,03	392,87	348,00
CARIOCA	8,89	9,97	2,81	3,33	1,37	1,58	1,30	1,28	406,34	422,54	24,62	24,36	376,14	379,01
RIO TIBAGI	9,12	10,32	3,59	3,69	1,45	1,56	1,59	1,86	411,55	426,29	24,78	22,50	304,98	291,84
MINUANO	8,68	9,77	3,29	3,20	1,42	1,40	1,45	1,54	328,41	404,99	23,45	24,85	329,17	328,94
PÉROLA	9,04	10,17	3,29	3,90	1,53	1,56	1,52	1,54	406,63	402,98	25,90	24,65	346,11	349,86
FTS MAGNÍFICO	9,25	9,72	3,47	3,36	1,84	1,83	1,50	1,42	380,64	395,97	24,87	24,68	354,17	325,62
MACANUDO	6,36	9,49	2,91	3,39	1,12	1,43	1,22	1,35	348,01	163,12	22,82	22,26	339,10	333,62
DIAMANTE NEGRO	8,76	9,58	3,76	3,52	1,51	1,53	1,46	1,55	138,39	171,02	23,52	23,44	343,10	369,13
VALENTE	9,62	9,46	3,72	3,62	1,63	1,61	1,41	1,49	163,16	174,53	24,14	22,22	353,91	335,77
IAPAR 31	9,55	8,44	3,19	3,09	1,51	1,33	0,84	0,90	156,33	200,15	22,92	22,76	369,33	369,25
Média	8,93	9,70	3,45	3,42	1,57	1,55	1,40	1,47	316,44	326,95	23,84	23,50	344,28	346,10
CV (%)	10,44	6,01	10,14	7,99	13,11	8,88	14,15	16,17	34,03	36,27	8,32	6,08	9,13	6,47

*Valores (média de 3 repetições) expressos em g/100 g de MS.
n.d. = não determinado.