JÚLIA CRISTINA CARDOSO CARRARO

CARACTERIZAÇÃO QUÍMICA E NUTRICIONAL DA LINHAÇA MARROM E DOURADA SOBRE PARÂMETROS FISIOLÓGICOS DE RATOS WISTAR

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Nutrição para obtenção do título de *Magister Scientiae.*

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APROVADA: 16 de fevereiro de 2012.

João Bosco Gonçalves de Barros

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Sérgio Luis Pinto da Matta

Maria do Carmo Gouveia Pelúzio
(Presidente da Banca)

"São as dúvidas que nos fazem crescer,
porque nos obrigam a olhar sem medo
para as muitas respostas de uma mesma pergunta."
(Paulo Coelho)

"Um pouco de ciência nos afasta de Deus.
Muito, nos aproxima."
(Louis Pasteur)

Dedico essa dissertação
aos meu pais, Júlio e Conceição, pelo apoio,
torcida, orações e confiança!

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E a todos que contribuíram de alguma forma para a realização desse trabalho,

O meu “muitíssimo” obrigada!

SUMÁRIO

ABREVIATURAS E SIGLAS

AF – Ácido fítico

ALA – Ácido α-linolênico

ARR – Atividade de retirada de radical

BHT – Butil-hidroxitolueno

CLAE – Cromatografia líquida de alta eficiência

DCNT – Doenças crônicas não transmissíveis

DPPH – 2,2-difenil-1-picril hidrazil

LD – Linhaça dourada

LM – Linhaça marrom

MDA – Malondialdeído

SDG – Diglicosídeo secoisolariciresinol

SECO – Secoisolariciresinol

TBARS – Testes das substâncias reativas ao ácido tiobarbitúrico

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RESUMO

CARRARO, Júlia Cristina Cardoso Carraro, M.Sc., Universidade Federal de Viçosa, fevereiro de 2012. **Caracterização química e nutricional da linhaça marrom e dourada sobre parâmetros fisiológicos de ratos Wistar.** Orientadora: Sônia Machado Rocha Ribeiro; Coorientadores: Laércio dos Anjos Benjamim e Hércia Stampini Duarte Martino.

A semente de linhaça tem se destacado em pesquisas científicas como alimento funcional, devido à sua rica composição em compostos bioativos, mostrando-se eficaz na redução de risco para dislipidemias, diabetes, cânceres, entre outras doenças. No entanto, existem duas variedades comumente comercializadas da semente, marrom e dourada, que podem ter composições químicas e, consequentemente, efeitos fisiológicos diferentes. Deste modo, este trabalho teve como objetivo investigar diferenças na composição química e efeitos fisiológicos sobre redução da colesterolemia e nível de peroxidação de lipídios entre as duas variedades da semente. Foram avaliados: a composição química, perfil de lipídios, teor de vitamina E, ácido fítico, compostos fenólicos totais, secoisolariciresinol e capacidade antioxidante de farinhas provenientes das duas sementes; os níveis de lipídios séricos e peroxidação de lipídios no soro e homogenatos de tecidos de animais alimentados com dieta padrão (AIN 93) ou padrão adicionada de 12% de farinhas de linhaça marrom ou dourada. A linhaça marrom apresentou maior conteúdo em proteínas, fibras alimentares, compostos fenólicos, entre eles, o secoisolariciresinol, vitamina E e, consequentemente, maior capacidade antioxidante. No entanto, as diferenças na composição química das farinhas não repercutiram em diferentes efeitos fisiológicos, sendo que ambas reduziram igualmente a razão colesterol total/ HDL e os níveis de peroxidação de lipídios no soro, testículo e pulmão.

ABSTRACT

CARRARO, Júlia Cristina Cardoso Carraro, M.Sc., Universidade Federal de Viçosa, February, 2012. **Chemical and nutritional characterization of brown and golden flaxseed on physiological parameters of wistar rats**. Adviser: Sônia Machado Rocha Ribeiro; Co-advisers: Laércio dos Anjos Benjamim and Hércia Stampini Duarte Martino.

Flaxseed has excelled in scientific research like a functional food, due to its rich composition of bioactive compounds, being effective in reducing risk for dyslipidemia, diabetes, cancers and other diseases. However, there are two varieties of seeds commonly sold, brown and gold, which may have different chemical compositions and, thus, different physiological effects. Thus, this study aimed to investigate differences in chemical composition and physiologic effects on reducing cholesterol level and lipid peroxidation of Wistar rats, between the two varieties of seed. Were evaluated: chemical composition, lipid profile, content of vitamin E, phytic acid, total phenolics, secoisolariciresinol and antioxidant capacity of the two seed flour, serum lipid levels and lipid peroxidation in serum and homogeneous tissues of animals fed with a standard diet (AIN 93) or standard added with 12% of brown or golden flaxseed flour. The brown flaxseed had a higher protein content, dietary fiber, phenolic compounds, among them, secoisolariciresinol, vitamin E and, consequently, higher antioxidant capacity. However, differences in chemical composition of flour not reflected in different physiological effects. Both the flours reduced equally the ratio of total cholesterol / HDL and the levels of lipid peroxidation in serum, testis and lung.

INTRODUÇÃO GERAL

Considerações gerais e justificativa do estudo

A elevação da expectativa de vida média da população, paralelamente ao aumento da incidência de doenças crônicas não transmissíveis (DCNT) tem levantado uma questão mundial sobre a necessidade de se adotar uma alimentação saudável, buscando-se os efeitos positivos dos alimentos na redução de risco das mesmas.

Nessa perspectiva, a linhaça, semente oleaginosa proveniente da planta do linho *(Linum usitatissimum L.)*, contém vários compostos bioativos que podem promover a saúde, diminuindo o risco de DCNT (BLOEDON & SZAPARY, 2004; BASSETT *et al.*, 2009).

Seu conteúdo em proteínas é semelhante ao da soja (OOMAH, 2001) e é considerada a mais rica fonte de ácido α-linolênico (ALA) no reino vegetal, uma vez que contém cerca de 40% de lipídios, com 50% destes sendo constituídos de ALA (PELLIZZON *et al.*, 2007). A linhaça ainda é rica em fibras (OOMAH, 2001) e compostos fenólicos, especialmente lignanas (HO *et al.*, 2007; STRANDÅS *et al.*, 2008).

Em função de sua composição química, a semente tem recebido, nas últimas décadas, grande destaque na mídia e em pesquisas científicas, uma vez que são atribuídos aos seus compostos bioativos efeitos antiinflamatório (FAINTUCH *et al.*, 2007), antioxidante (TORRES-SÁNCHEZ *et al.*, 2009), ação estrogênica e anti-estrogênica (RAFTER, 2002), redução de risco de doenças como câncer, especialmente os hormônio-dependentes (RAFTER, 2002; GEORGE *et al.*, 2007), dislipidemia (PATADE *et al.*, 2008), e diabetes do tipo 2 (PAN *et al.*, 2007).

A população em geral tem recebido muitas informações sobre os benefícios do consumo de linhaça por meio da mídia, principalmente por causa da promessa de se obter propiedades de estimulação do funcionamento intestinal e redução da colesterolemia.

No entanto, duas variedades da semente são normalmente comercializadas, a dourada, importada do Canadá, e a marrom, produzida em regiões frias do Brasil.

Existem alegações veiculadas pela mídia a respeito da superioridade da semente dourada em relação à marrom, mas pouco se sabe sobre as diferenças entre as duas variedades da semente. Sendo assim, justifica-se a investigação da composição química de ambas as variedades de linhaça e se possíveis diferenças na composição podem refletir em efeitos fisiológicos diversos.

HIPÓTESES

As variedades de linhaça marrom e dourada podem diferir na composição química, o que resulta em efeitos funcionais de intensidade variada sobre parâmetros séricos de risco de DCNT.

OBJETIVOS:

Geral

Avaliar os efeitos fisiológicos da linhaça em ratos Wistar machos com ênfase nas propriedades hipocoleresterolêmica e antioxidante.

Específicos

1. Avaliar a composição química de sementes de linhaça marrom e dourada;
2. Avaliar a capacidade antioxidante de sementes de linhaça marrom e dourada;
3. Comparar o efeito da ingestão de linhaças marrom e dourada sobre a concentração sérica de lipídios e de níveis de malondialdeído no soro e em homogenatos de tecidos de ratos;

Utilizando-se ensaio biológico, a proposta geral do presente trabalho foi esclarecer diferenças nas propriedades funcionais das duas variedades de sementes de linhaça disponíveis no mercado brasileiro para o consumo

humano. Foi investigado o efeito sobre o perfil de lipídios séricos e status oxidativo, visando seus efeitos positivos.

Esta dissertação está organizada nos seguintes itens:

i) Embasamento teórico sobre os efeitos fisiológicos da linhaça na forma do artigo: “Flaxseed and human health: reviewing benefits and adverse effects” publicado na revista Food Reviews International;
ii) Metodologia geral (descrição detalhada sobre material e métodos);
iii) Resultados apresentados na forma do artigo: “Chemical composition and physiological properties of whole brown and golden flaxseed flours in Wistar rats” submetido à revista Plant Foods for Human Nutrition.

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Flaxseed and Human Health: Reviewing Benefits and Adverse Effects

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Flaxseed and Human Health: Reviewing Benefits and Adverse Effects

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Consumption of flaxseed has been increased due to its possible functional properties in health. However, its chronic consumption may offer risks considering the effects of lignans in men and in pregnant women, as well as the existence of other phytochemicals and toxic factors with adverse health effects in the seed. The present review focuses on the growing body of evidence on the potential health benefits of flaxseed in humans, with supporting evidence from human and animal studies. It also raises questions that provide input for future research on the effects of flaxseed ingestion in terms of nutrient bioavailability and human fertility.

Keywords disease prevention, flaxseed, health, phytochemicals

Introduction

The increase in life expectancy of the population in parallel with increase in the incidence of noncommunicable chronic diseases (NCCDs) has brought up the question for the necessity of adapting a healthy diet and understanding the positive effects of foods for reducing the risk of these diseases.

NCCDs such as cardiovascular diseases (CVDs), certain cancers, type 2 diabetes, and obesity are reaching epidemic proportions worldwide.[1] This scenario justifies obtaining an understanding of the functional properties of bioactive compounds in foods, with the objective of protecting against such diseases.

Flaxseed, of Mesopotamic origin, has been cultivated since 5000 BC, being used until the 1990s principally for the fabrication of cloths and papers. Flaxseed oil and its subproducts are used in animal feeds. In the last two decades, the use of flaxseed in human diets has increased throughout the world. Flaxseed has potential to be considered a functional food, as it already is in Canada, in a way which is relevant to either the state of well-being and health or the reduced risk of diseases.[2,3] However, to stimulate the safe use of flaxseed as a functional food, it is necessary to understand more on the magnitude of possible adverse effects of phytochemicals on the bioavailability of nutrients and human fertility. Reviews on flaxseed and its health benefits have been recently published, relating its benefits to isolated chronic diseases, but an overview on the state of the art of the health

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benefits of flaxseed as a whole encompassing various chronic noncommunicable diseases, and how its components affect obesity, CVD, diabetes, cancer, and other pathogenic conditions is still missing. The objective of the present paper was to review the studies on functional properties of flaxseed and possible adverse effects, considering studies on both human and animals.

Methods

The electronic databases MEDLINE, SCOPUS, SCIENCE DIRECT, SCIELO, and LILACS were utilized to identify studies published over the past 5 years addressing functional effects of flaxseed and its bioactive compounds, mainly fiber, n-3 fatty acid and lignan, using text terms with appropriate truncations and relevant indexing terms. Information on flaxseed composition was obtained from all publications available in other databases, regardless of publication year. The search terms were as follows:

(1) Block A: flaxseed/linseed, flaxseed fiber, linolenic acid, flaxseed lignin, cadmium, cianogenic compounds, phosphate myoinositol, trypsin inhibitors, bioactive peptides.
(2) Block B: cardiovascular diseases, serum lipid profile, risk cancer, prevention cancer, obesity, body weight, metabolic syndrome, diabetes mellitus, glucose metabolism impaired, insulin resistance, immune response, infectious, therapeutic agent, fertility, reproduction, hormonal effect.
(3) Block C: human, men, women, animal, review

This review is organized into the following sections: Chemical Composition of Nutrients and Phytochemicals in Flaxseed, Flaxseed and the Reduction of Risk for Noncommunicable Chronic Diseases, Other Beneficial Physiological Effects of Flaxseed, Flaxseed and Hormonal Modulation, Adverse Effects of Flaxseed Components, and Conclusions.

Chemical Composition of Nutrients and Phytochemicals in Flaxseed

Flaxseed is a food with high energetic density (4.5 kcal/g) and protein content (20%), being equivalent to that described for soybean.[2] Although the protein is not considered a complete protein due to the presence of limiting amino acids,[4] it contains peptides with bioactivities related to the decrease in risk factors of CVD.[5] Flaxseed contains a greater quantity of dietary fiber (18%) than many legumes (5–10% in the cooked food), being composed of soluble (25%) and insoluble fibers (75%).[6] Both fiber fractions have shown physiologically beneficial actions. Soluble dietary fiber exerts physiologic effects on the stomach and small intestine modulating postprandial glycemic responses and the main effect of insoluble fiber involves enhancement of insulin sensitivity.[7]

Of all lipids in flaxseed (approximately 30%), 53% are α-linolenic acids (ALAs), 17% linoleic acids (LAs), 19% oleic acids, 3% stearic acids, and 5% palmitic acids, which provides an excellent n-6: n-3 fatty acid ratio of approximately 0.3:1.[8] Therefore, the seed may be an alternative for supplying this fatty acid to populations concentrated in regions of the world where there is not large access to marine foods, which are the best sources of n-3 fatty acids. However, the conversion of ALA into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is limited in the biological media (∼0.3 to 8.0% to EPA and less than 1.0% to DHA).[9] Still, this aspect is of great importance considering that alterations in eating habits related to industrialization caused an increase in consumption of foods with elevated levels of n-6 fatty acids and a reduction in ingestion

of n-3 fatty acids. The n-6/n-3 ratio currently ranges from 10:1 to 20:1, instead of a traditional range of 1–2:1,[10] which is associated to the increase in noncommunicable chronic diseases.

In relation to composition of minerals, the contents of calcium, magnesium and phosphorus are highlighted,[11] being that a 30-g portion of the seed constitutes 7% to 30% of the recommended dietary allowances (RDAs) for these minerals. Flaxseed is also rich in vitamin E, predominantly the isomer γ-tocopherol.[12] Although α-tocopherol is preferentially absorbed in relation to the γ isomer,[13] it has been considered the most effective against oxidation of low-density lipoprotein (LDL).[14]

Other bioactive compounds of flaxseed are from the class of phenolic compounds, including lignans, *p*-coumaric acid, and ferulic acid.[15] Flaxseed is considered a good source of known lignans, where the lignan content (301.1 mg/100 g) is principally composed of secoisolariciresinol diglucoside (SDG) (294.2 μg/100 g), matairesinol (0.55 mg/100 g), lariciresinol (3.04 mg/100 g), and pinoresinol (3.32 mg/100 g).[16] After ingestion, SDG is converted to enterolignans (enterodiol and enterolactone) by the intestinal microflora,[17] and these metabolites (phytoestrogens) are absorbed and can provide health benefits due to their weak estrogenic or antiestrogenic effects, as well as antioxidant effects and inducing of phase 2 proteins.[18] The bioavailability of lignans from flaxseed has been shown in studies that verified that urinary excretion of its metabolites is proportional to the quantity ingested and this was not altered with processing and heating of the flaxseed.[19] Considering that information on lignan levels in the foods is scarce, the quantity of lignans ingested by humans is still uncertain. Studies have verified that ingestion of lignans by 115 Canadian women varied from 45 to 92,083 μg/day, with average of 403 μg/day and the serum concentration of enterolactone was maximally 374.2 nmol/L.[20]

Table 1 summarizes the chemical composition of nutrients and phytochemicals in flaxseed.

Flaxseed contains bioactive peptides, such as cyclolinopeptide A, which have strong immunosuppressive and antimalarial activities, inhibiting the human malarial parasite *Plasmodium falciparum* in culture.[21] Also, a peptide mixture with high levels of branched-chain amino acids (BCAAs), and low levels of aromatic amino acids (AAAs), has shown antioxidant properties by scavenging 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and antihypertensive properties by inhibiting the angiotensin I–converting enzyme.[5]

The presence of n-3 fatty acids, soluble fibers, vitamin E, lignans, and other phenolic and peptide compounds found in flaxseed offers potential for it to exert diverse actions thought to benefit health, e.g., anti-inflammation, vessel relaxation, antioxidant, hypocholesterolemic, anticarcinogenic, and attenuation of the postprandial insulin response. Fig. 1 shows the action mechanisms of bioactive compounds found in flaxseed that promote health and reduction of risk factors for noncommunicable chronic diseases.

Flaxseed and Reduction of Risk for Noncommunicable Chronic Diseases

Obesity

The growing interest in consumption of flaxseed is in part due to the recognized importance of increasing the ingestion of dietary fiber and polyunsaturated fatty acids. Dietary fibers have a direct relation to health, diminishing caloric density of the diet, and studies on whole flaxseed effects demonstrated reduction of the fat absorption by fecal excretion in animal and human.[22,23]

Table 1
Chemical composition of nutrient and phytochemicals in flaxseed

Nutrients/bioactive compounds	Quantity/100 g of seed	Nutrients/bioactive compounds	Quantity/100 g of seed
Energy	450 kcal	Biotin	6 mg
Protein	20.0 g	Carotenes**	Not detected
Carbohydrates*	29.0 g	α-Tocopherol**	7 mg
Total fats	41.0 g	δ-Tocopherol**	10 mg
Linolenic acid	23.0 g	γ-Tocopherol**	552 mg
Dietary fiber	28.0 g	Calcium	236 mg
Lignans	10–2600 mg	Copper	1 mg
Ascorbic acid	0.50 mg	Magnesium	431 mg
Tiamin	0.53 mg	Manganese	3 mg
Riboflavin	0.23 mg	Phosphorus	622 mg
Niacin	3.21 mg	Potassium	831 mg
Pyridoxin	0.61 mg	Sodium	27 mg
Pantothenic acid	0.57 mg	Zinc	4 mg
Folic acid	112 mg		

Table adapted from reference 12.
*Values include dietary fiber.
**Values in mg/kg of flaxseed lipids.

Studies have shown the benefits of elevated ingestion of dietary fibers for the prevention of weight gain and accumulation of abdominal fat in both men and women.[24] Ingestion of 30 g of flaxseed per day for 12 weeks reduced central obesity in 283 individuals with metabolic syndrome.[25] Patients with morbid obesity who ingested toasted flaxseed flour (30 g/day) during 2 weeks presented no significant alterations in body fat; however, the authors suggested that the short duration of the study may explain the absence of effects on body weight. The authors did, however, observe a significant reduction in serum levels of inflammatory markers (C-reactive protein and serum amyloid A), which were initially high in these individuals.[26]

Studies on animals showed possible benefits of n-3 fatty acid from flaxseed, decreasing the retention of fat in the liver of genetically obese animals. It has been suggested that the seeds may be used as a therapeutic strategy in the treatment of hepatic steatosis associated with obesity.[27] More recent studies in animals have demonstrated the bioactivity of ALA from flaxseed in the reduction of hepatic steatosis, indicating that a dietary deprivation of n-3 fatty acid for 3–7 months was sufficient to provoke alterations of the fatty acid content in the liver of 6-week-old normal rats with steatosis, mimicking visceral obesity and insulin resistance similar to those observed in metabolic syndrome. Dietary supplementation with 5% (*w*/*w*) flaxseed oil returned the hepatic fatty acids profile to control levels[28] and the plasma glucose concentration and insulin resistance index decreased in rats.[29]

Other beneficial effects of flaxseed on the metabolism of adipose tissue may be attributed to the bioactivity of the lignans. Generally, phytoestrogens tend to accumulate in the adipose tissue for decades, potentially causing long-lasting endocrine effects.[30] Isolated lignan of flaxseed, administered to C57BL/6 mice for 4 weeks at a level of 0.5% to 1% in a high-fat diet, induced the expression of adiponectin mRNA in the

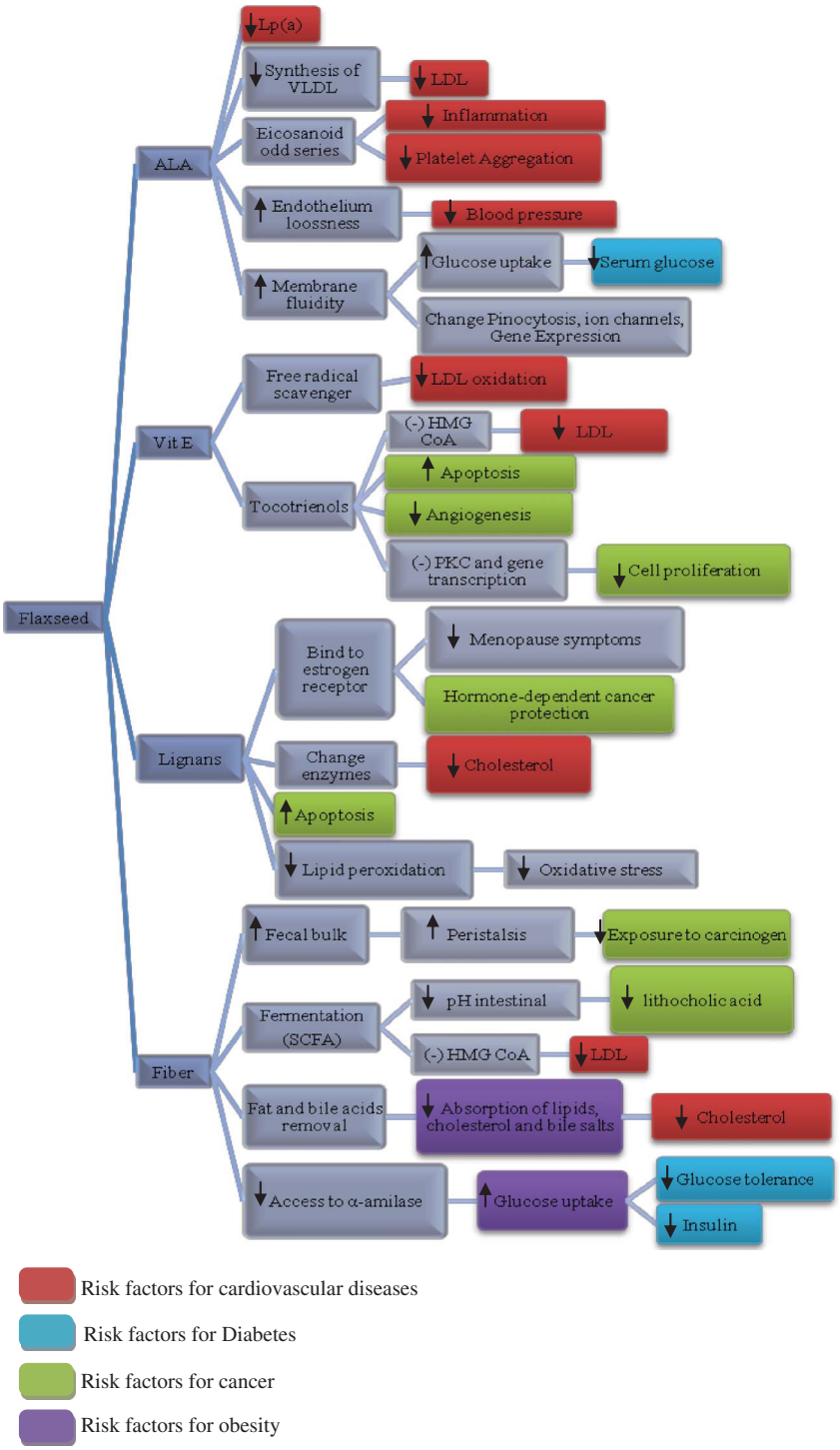

Figure 1. Action mechanisms of flaxseed bioactive compounds on risk reduction of noncommunicable chronic diseases. Lp(a) = Lipoprotein a; HMG CoA = 3-hydroxy-3-methylglutaryl–coenzyme A reductase; Vit E = vitamin E; PKC = protein kinase C; SCFA = short-chain fatty acids. ↑ = increase; ↓ = decrease. (color figure available online.)

white adipose tissue.[31] This also resulted in reduced visceral and liver fat accumulation, hyperinsulinemia, and hyperleptinemia.[31] A study with humans showed similar effects. Postmenopausal overweight women with a high dietary lignan intake had a significant lower body mass index and total body fat mass, as well as a better glucose disposal rate compared with women in the low lignan intake.[20] Tominaga et al. (2009), however, demonstrated that there are differences between the isomers of secoisolariciresinol (SECO) on adipogenesis. The (−)-SECO isomer accelerated the production of adiponectin in 3T3-L1 adipocytes, contrarily, (+)- and meso-SECO isomers decreased production. Accumulation of triglycerides in the adipocytes was annulled by the three isomers, however, were more strongly reduced by the (−)-SECO isomer, showing that only this isomer has an effective estrogenic activity on adipogenesis.[32]

Diet supplementation with flaxseed has been shown to prevent weight gain in individuals with well-controlled type 2 diabetes.[33] A study of flaxseed supplementation during lactation in rats, however, showed negative effects, producing early insulin sensitivity and hyperleptinemia in offspring, as well as indicating hormonal imprinting that could result in selective insulin resistance with increased risk for later development of diabetes mellitus.[34]

In conclusion, although it is shown that consumption of flaxseed does not necessarily promote weight loss, the contained bioactive compounds may positively modulate mechanisms that result in the decrease of comorbidities associated with obesity. However, such effects are controversial during lactation.

Dyslipidemia and CVD

Flaxseed is a rich source of three bioactive compounds with demonstrated cardioprotective effects: ALA, dietary fiber, and phytoestrogen lignans.

Pellizzon et al. (2007) investigated the hypolipidemic mechanism of flaxseed in animals and verified that there was no alteration in gene expression of enzymes of the cholesterol metabolism, suggesting that the hypolipidemic effect was attributed to dietary fibers.[8] Beyond the hypocholesterolemic effect, more recently other cardioprotection mechanisms have been investigated that may be modulated by the actions of bioactive compounds of flaxseed: lignans, other phenolic compounds, and n-3 fatty acids, including antioxidant, hormonal,[35] and anti-inflammatory effects.[36]

Studies related to ingestion of flaxseed and its bioactive compounds with the decreased risk of CVD are presented in Table 2.

The serum lipid profile is the cardiovascular risk factor most intensely investigated in studies on functional properties of flaxseed and its bioactive components. In hypo- and normocholesterolemic individuals, ingestion of flaxseed (30–40 g/day) for periods greater than 30 days improved biochemical parameters, such as cholesterolemia, lipoprotein a (Lp(a)) and the homeostatic model assessment (HOMA) index.[37] Studies with flaxseed and its bioactive components have been performed with postmenopausal women, showing positive effects, including hypocholesterolemic and antidiabetic effects of supplementation.[37–39] The great interest in this type of study is justified because women after menopause present an increase in risk of CVD due to the decreased estrogen level, favoring a more atherogenic serum lipid profile.[40] Recently it was suggested that 300 mg of SDG/day during 8 weeks appeared to be necessary to observe positive effects on the risk factors for CVD in humans.[43] This value is equivalent to approximately 100 g of flaxseed. It is well reported that ALA can modify gene transcription levels of some key molecules such as nuclear factor-kappa B (NF-κB) and sterol binding receptor protein-1c (SBRP-1c),

Table 2
Effects of flaxseed and its bioactive compounds on risk factors for cardiovascular disease

Reference	Characteristics of the experiment	*n*	Sex	Dosage	Duration	Observed effects
Humans						
Bloedon et al. (2008)[37]	Postmenopausal women with LDL between 130 and 200 mg/dL	62	Female	40 g/day of bread products containing flaxseed	5 weeks 10 weeks	↓LDL, HDL ↓HDL, Lp(a), HOMA-IR
Hallund et al. (2008)[119]	Double-blind crossover study—postmenopausal women	22	Female	Low-fat muffins and 500 mg of SDG	6 weeks	↓CRP
Patade et al. (2008)[39]	Control-case—hypercholesterolemic postmenopausal women	55	Female	30 g flaxseed/day	3 months	↓TC and LDL
Dodin et al. (2008)[120]	Control-case of healthy menopausal women	199	Female	40 g/day	12 months	↑ALA, DPA, and total n-3 fatty acids in the plasma and had a limited affect on the metabolism of the apolipoproteins. However ↑Lp(a).
Paschos et al. (2007)[121]	Prospective, two-group, parallel-arm design with dyslipidemic men	59	Male	8 g flaxseed oil/day	12 week	↓ALA systolic and diastolic blood preasures compared with linoleic acid (safflower oil)
Hallund et al. (2006)[42]	Randomized, double-blind, placebo-controlled, crossover with healthy postmenopausal woman	22	Female	Low-fat muffins and 500 mg of SDG	6 weeks	No effect on the parameters analyzed (plasmatic lipid profile and antioxidant capacity)

(*Continued*)

Table 2
(Continued)

Reference	Characteristics of the experiment	*n*	Sex	Dosage	Duration	Observed effects
Mandaşescu et al. (2005)[41]	Hyperlipidemic patients that received hypo-lipidic diet, hypo-lipid diet + statins, or hypo-lipidic diet + flaxseed	40	—	20 g ground flaxseed/day	60 days	↓17.2% TC; 3.9% LDL; 36.3% TG and 33.5% TC/HDL, no difference between the flaxseed and statins group
Animals						
Makni et al. (2008)[122]	Rats received a hypercholesterolemic diet (1% cholesterol) supplemented with flaxseeds and pumpkin seeds	30	Male	33% of the diet was composed of flaxseed flour and pumpkin seeds to forma n-6 and n-3 fatty acids proportion of: 5/1	30 days	↓TC and LDL and ↑HDL
Dupasquier et al. (2007)[123]	Rats submitted to different high cholesterol diets with or without addition of flaxseed)	105	Female	1%, 5%, or 10% in the regular and atherogenic diets	24 weeks	The expression of markers, including proliferating cell nuclear antigen (PCNA), IL-6, and VCAM-1, increased in aortic tissue by hypercholesterolemic diets, was normalized when flaxseed was included in the diet.
Pellizon et al. (2007)[8]	hApoBtg mice (transgenic which superexpressed the apolipoprotein B-100, elevating levels of LDL) and *knockout* $LDL^{-/-}$/$apobec^{-/-}$ mice received a western diet, containing 0.1% cholesterol and 30% fat, continuing with this diet or diet with flaxseed	14 25 11	Male (hApoBtg) Female (hApoBtg) Male ($LDL^{-/-}$/ $apobec^{-/-}$)	20% flaxseed in the diet	21 days	↓Plasmatic and hepatic cholesterol, without altering hepatic lipogenic genes, attributing the hypolipidemic effect of flaxseed to the lower absorption of cholesterol and/or reabsorption of biliary acids.

Cintra et al. (2006)[22]	Rats submitted to a hyperlipidemic diet (1% cholesterol, 10% soy oil and 5% pork fat)	60	Male	10% flaxseed	28 days	↓TC, HDL, liver weight, and hepatic cholesterol; ↑fecal excretion of lipids
Vijaimohan (2006)[124]	Rats submitted to a hyperlipidemic diet	24	Male	1 g flaxseed oil/kg of weight	60 days	↓Weight gain, liver weight, TC, TG, phospholipids, AGL, LDL, HDL, VLDL, LDL/HDL, TC/HDL
Prasad et al. (1998)[125]	Rabbits submitted to a hypercholesterolemic diet (addition of 1% of cholesterol to the commercial diet)	24	—	7.5 g flaxseed with lower n-3 fatty acid levels per kg of the diet	8 weeks	↓69% of the atherosclerotic plaque, 31% TC, 34% TC/HDL, 32% LDL, TG; suggesting that the effect is in function of the antioxidants
Prasad (1997)[126]	Rabbits submitted to a hypercholesterolemic diet (addition of 1% of cholesterol to the commercial diet)	30	—	7.5 g flaxseed/kg diet	8 weeks	↓42% of the atherosclerotic plaque

LDL = low-density lipoprotein; HDL = high-density protein; Lp(a) = lipoprotein a; HOMA-IR = homeostatic model assessment; CRP = C-reactive protein; TC = total cholesterol; TG = triglycerides; ALA = α-linolenic acid; DPA = docosapentaenoic acid; VLDL = very-low-density protein; IL-6 = interleukin-6; VCAM-1 = vascular cell adhesion protein 1. ↑ = increase; ↓ = decrease.

which regulate expression of adhesion molecules or receptors involved in triglyceride synthesis.[44] Proteome analysis of peripheral blood mononuclear cells from seven healthy men, before and after a 1 week intervention with 0.4 g of flaxseed/kg body weight per day showed enhanced enterolactone plasma levels and modulation of the steady-state levels of 16 proteins related to protective effects in atherosclerosis.[45]

The effects of flaxseed on risk factors for CVD in studies performed on animals are similar to those conducted in humans. Rats, mice, and rabbits presented positive responses for biochemical and histological parameters, indicating the hypocholesterolemic activity of flaxseed, generally related to the greater fecal content of lipids.[22,46] However, there remains controversy in relation to action of the high-density lipoprotein (HDL) fraction, since general studies do not present the LDL/HDH ratio, which is a more determining factor for CVD risk than the isolated concentration of the two lipoproteins.

Supplementation with SDG may also be associated with prevention of CVD by means of increasing angiogenesis. Penumathsa et al. (2007) showed this effect in three models: i) in vitro model: human coronary arteriolar endothelial cells treated with SDG showed a increase in tubular morphogenesis compared with the control, increasing expression of angiogenic factors as vascular endothelial growth factor (VEGF), kinase insert domain-containing receptor (KDR), Fit-1, angiopoietin-1, Tie-1, and phosphorylated endothelial nitric oxide synthase (p-eNOS); ii) ex vivo model: rats treated orally with 20 mg SDG/kg of body weight/day, during 2 weeks, showed a increased level of aortic flow and functional recovery after 2 hours of reperfusion following 30 minutes of ischemia, also decreasing cardiomyocyte apoptosis; iii) in vivo model: in myocardial infarction model, SDG increased capillary density and myocardial function.[47]

Effects of flaxseed on low-grade chronic inflammation are still conflicting. Some studies show that inflammatory markers, including the C-reactive protein (CRP), were reduced with ingestion of flaxseed and its bioactive compounds.[48] The study performed by Duda et al. (2009), however, attributed anti-inflammatory effects to only n-3 fatty acids from fish oil, and not from vegetable sources such as flaxseed oil.[49] Phospholipase A2 associated to the analyzed lipoprotein in another study also did not suffer modification with supplementation of fish oil (∼2 g EPA/DHA + 9 g olive oil/day) or flaxseed oil (∼11 g/day) during 8 weeks.[50]

There are findings that flaxseed oil does not affect serum lipids in studies with animals, with a weak effect on reduction of triglyceridemia, presenting variable effects on inflammatory markers, but lignans generally reduce total and LDL cholesterol and increase HDL cholesterol.[51] In adults of both genders, no effect on cardiovascular health parameters (serum lipid profiles and oxidation of LDL cholesterol) was observed after 3 months of flaxseed oil ingestion (1 mg per day).[52]

However, scientific evidence suggests that flaxseed, with its set of bioactive compounds that act synergistically, has a summed beneficial effect with a positive impact on lipidemia, which is not always observed with one of its isolated components. The cholesterol-lowering effects seem more apparent in hypercholesterolemic postmenopausal women.[53] The hypocholesterolemic effect of flaxseed consumption may, however, be a useful strategy in primary health service based on prevention and control of dyslipidemia, therefore focusing on the promotion of health and prevention of cardiovascular disease risk.

Diabetes and Blood Glucose Control

The beneficial effects of flaxseed ingestion on blood glucose control have also been demonstrated (Table 3).

Table 3
Effects of flaxseed and its bioactive compounds on blood glucose control and type 2 diabetes mellitus

Reference	Characteristics of the experiment	*n*	Sex	Dosage	Duration	Observed effects
Humans						
Pan et al. (2009)[53]	Double-blind crossover study—diabetic type 2 individuals of the same community	70	26 men and 44 postmenopausal women	360 mg of lignans per day	12 weeks each period with 8 weeks of wash-out	↓CRP, but not IL-6 and RBP4
Barre et al. (2008)[55]	Double-blind control case—diabetic individuals	32	18 men and 14 women	60mg ALA/ kg weight/day	3 months	There was no reduction in the levels of HbA1c and insulin.
Pan et al. (2007)[38]	Double-blind crossover study—diabetic patients with moderate hypercholesterolemia	68	25 men and 43 women	360 mg of flaxseed	12 week period with 8 weeks of wash-out	↓HbA1c, without changes in fasting glycemia, insulin, resistance to insulin, and serum lipid profile.
Animals						
Kelley et al. (2009)[127]	C57BL/6N rats (animals susceptible to obesity, diabetic tumors and induced atherosclerosis) received supplementation of *trans*-10,*cis*-12-CLA = conjugated linoleic acid or CLA and flaxseed oil	30	Females	CLA—0.5%CLA + flaxseed oil—0.5%:0.5%	8 weeks	↓Glycemia by 20% and liver weight by 37%. *This isomer has been related to the resistance to insulin and nonalcoholic hepatic steatosis in animals.
Prasad (2001)[128]	Zucker diabetic fat rats (ZDF), DM2 development models	36	Females	40 mg/kg/day of isolated flaxseed lignans	10 weeks	Only 20% of rats treated with lignans developed DM2, ↑serum MDA. The diabetes development mechanism was related to the increase in oxidative stress.

HbA1c = glycated hemoglobin; CRP = C-reactive protein; IL-6 = interleukin-6; RBP4 = retinol biding protein 4; DM2 = diabetes mellitus 2; MDA = malondialdehyde. ↑ = increase; ↓ = decrease.

Dietary fibers, lignans, and n-3 fatty acids, present in flaxseed and other integral cereals, such as beans and nuts have a protective effect against diabetes risk.[18] The effects of flaxseed to aid in the treatment of diabetes are inconsistent. Human intervention studies show modest effects with maximal decreases on the order of 10% in blood serum glucose or improved isolated effects, such as on glycated hemoglobin (HbA1c).[38,54] Supplementation of the human diet with lignans (360 mg/day) reduced HbA1c and CRP, decreasing low-grade chronic inflammation without altering parameters related to insulin.[38,53] Ingestion of 10 g/day of flaxseed oil had no effect on fasting blood serum glucose, insulin, or HbA1c levels.[55]

Studies with lignans have demonstrated greater effectiveness in reducing blood serum glucose than flaxseed oil, both in humans as well as animal models. The positive results may be found in the parameters of glycemic control (blood glucose and HbA1c) and inflammatory markers, which are suggested to be involved in the development of diabetes, such as CRP, interleukin-6 (IL-6), and retinal-binding protein 4 (RBP4).[56,57] Utilization of flaxseed for glycemic control may also be associated to the decrease in risk of obesity and dyslipidemia, since these are risk factors for the development of diabetes and resistance to insulin.[20,25,28,29]

Flaxseed gum reduced blood glucose and cholesterol in an interventional study, in which 60 type 2 diabetic patients were studied.[54] A controlled trial including 34 adults with well-controlled type 2 diabetes who consumed milled flaxseed (32 g/day) or flaxseed oil (13 g/day) daily for 12 weeks demonstrated, however, no glycemic control.[33]

Although the effects of flaxseed on metabolic control in diabetes mellitus are controversial, in studies on animals it has been demonstrated that dietary supplementation can be beneficial against oxidative stress and increasing defense systems,[58] as well as improving diabetic nephropathy by reducing histopathological changes[59] and decreasing the incidence of diabetic macrovascular complications through improvement of the lipid profile.[60]

In general, it was verified that ingestion of flaxseed by diabetic patients or animals presented limited results that need to be further investigated. Some compounds present in flaxseed, utilized in their isolated form, were efficient in improving glycemic control[38,53]; however, the isolated flaxseed oil did not present beneficial effects in humans.[55]

Cancer

Interest in research on the association between flaxseed ingestion and risk of cancer emerged when epidemiologic evidences suggested a beneficial relationship. Populations that ingested greater quantities of flaxseed tended to present lower rates of hormonal-dependent cancers.[61] It is believed that the association between ingestion of flaxseed and cancer is explained by the bioactivity of lignans.[62]

The effects of flaxseed and its bioactive compounds in relation to prevention or treatment of some cancer types are presented in Table 4.

Experimental evidence in animals has shown anticarcinogenic effects of flaxseed or pure lignans in many types of cancer.[18] Flaxseed oil inhibited the growth and development of tumors in the breast of laboratory animals.[12] In fact, it has been demonstrated that lignans can modulate development of breast cancer in MCF-7 and MDA-MB-231 cell lines.[63] Furthermore, expression of the estrogen receptor is modulated by lignan extracts from flaxseed in a concentration-dependent manner in MCF-7.[63] Decrease of risk biomarkers for breast cancer in premenopausal women after administration of the plant lignan SDG has been observed[64] and in ovariectomized mice receiving 10% flaxseed in their

Table 4
Effects of flaxseed and its bioactive compounds on cancer

Reference	Characteristics of the experiment	*n*	Sex	Dosage	Duration	Observed effects
Humans						
Demark-Wahnefried et al. (2008)[67]	Men with prostate cancer	161	Male	30 g of flaxseed/day or reduction of dietary fat (<20% of the total energy)	21 days prior to prostatectomy	Supplementation of flaxseeds but no reduction of dietary fat ↓cell proliferation in the tumors
Thompson et al. (2005)[129]	Double-blind control case—women with recently diagnosed breast cancer	32	Female	muffins containing 25 g flaxseed flour or placebo	5½ weeks	↓Proliferation of cancer cells; ↑cellular apoptosis and lignan excretion
Demark-Wahnefried et al. (2001)[130]	Men with prostate cancer who are to be submitted to intervention	25	Male	30 g of flaxseed and a low-fat diet	4 weeks	↓Proliferation of cancer cells, total testosterone and free androgen; ↑apoptosis
Animals						
Chen et al. (2009)[131]	Ovarectomized athymic mice with breast cancer cells (MCF-7) established	48	Female	Flaxseed (100 g/kg diet); SDG (1 g/kg diet) or flaxseed hull (18 g/ kg diet)	8 weeks	FS, SDG, and FH ↓tumor size; inhibited cell proliferation, but only FS and SDG induced higher apoptosis and decreased growth factor.

(*Continued*)

Table 4
(Continued)

Reference	Characteristics of the experiment	*n*	Sex	Dosage	Duration	Observed effects
Chen et al. (2007)[132]	BALB/C nu/nu mice (athymic), ovarectomized with established MCF-7 tumors	8–10 in eachgroup	Female	Flaxseed in the diet of 5% and 10% or tamoxifen (drug used in the treatment of breast cancer by competition with type β estrogen receptors)	8 weeks	The groups that received 5% and 10% flaxseed in the diet inhibited tumor growth in 26% and 38%, respectively, where the treatment with 10% showed results similar to that of tamoxifen. This reduction was associated to the increase in dosage-dependent cellular apoptosis.
Williams et al. (2007)[133]	Fisher 344 rats	48	Male	7% and 14% flaxseed oil and 10 and 20% flaxseed flour	13 weeks	>80% of the aberrant crypt foci in the colon, precursor lesions of colon cancer, and glutatione-*S*-transferase activity
Jungeström et al. (2007)[134]	Ovarectomized athymic mice with breast câncer cells (MCF-7) established	—	Female	10% flaxseed on diet		Flaxseed and its lignans, enterodial and enterolactone, counteracted E2-induced growth and angiogenesis in solid tumor.

FS = flaxseed; SDG = secoisolariciresinol diglucoside; FH = flaxseed hull; E2 = estradiol. ↑ = increase; ↓ = decrease.

diet for 2 or 25 weeks.[65] Despite acting as an inhibitor to the development of cancer, recent evidence has shown that lignan and flaxseed oil reduced the growth of tamoxifen-treated tumors by mechanisms involving signaling pathways,[66] suggesting their potential use to aid in chemotherapy of some cancer types.

The action of estrogens in the male reproductive system may confirm a protection factor for prostate cancer in humans.[67] Therefore, flaxseed has been cited as a useful food in the strategy of dietary intervention to reduce risk and improve the prognostic of prostate cancer.[68] Lignans presented antimitotic, antioxidant, and antiangiogenic effects, as well as actuating in the reduction of testosterone by means of inhibiting enzymes, which resulted in a decrease in tumor growth in studies with humans, animals, and cell cultures.[69,70]

In summary, despite experimental evidence of the bioactivity of lignans in the progression of cancerous lesions, epidemiological results are controversial because the determinants of plasma enterolactone are very different between populations.[71] Other factors may be involved in the protective mechanism, including the source of the lignans and synergic effects with other bioactive components contained in flaxseed.

The treatment of human colon cancer with SDG or its metabolites, either isolated or combined, resulted in a decrease in the number of cancerous cells. Inhibition of cell growth by lignans and their metabolites appear to be mediated by apoptotic and cytostatic mechanisms.[72] Rosa et al. (2010) demonstrated that the addition of flaxseed oil to the standard diet of Wistar rats diminished the adherence of lymphocytes in the intestinal mucus when compared to the addition of other oils.[73] Because it contains elevated levels of dietary fibers, flaxseed may also offer protection against cancer, principally colon cancer.[74]

n-3 fatty acids in general, as well as EPA and DHA, may also be related to the reduced development of colon tumors induced in rats, where n-6 fatty acids exerted the opposite effect.[75] Enhanced tumor-reducing effects were observed with flaxseed oil-trastuzumab interaction in breast cancer,[76] suggesting the anticarcinogenic effect of ALA by means of inhibiting enzyme activity adhered to the membrane of the cells.

Various mechanisms are proposed for the action of flaxseed on reducing the risk of cancer. In general, the strongest evidences are related to the action of its bioactive compounds, diminishing proliferation and progression of tumors. From the current knowledge available, there are strong suggestions that the bioactivity of flaxseed for protection against cancer, especially breast cancer, is attributed to the lignans.[77]

Other Beneficial Physiological Effects of Flaxseed

Various other physiological effects of flaxseed have been described, as well as their relationship with reduced risks of NCCDs (Table 5).

ALA exerts positive effects on the modulation of the immune response, inhibiting excessive inflammatory responses and being beneficial in allergic conditions.[78]

Lignans may also exert antibacterial, cytotoxic, and antiviral activities.[79,80] Rajesha et al. (2010) reported that SDG extracts of two Indian flaxseed varieties presented antibacterial activity against diverse pathogens in vivo.[81] Intense immune responses contributed significantly to the pneumonia pathogen, where this regulation is fundamental for such patients. Saini et al. (2010) investigated the effect of supplementing flaxseed oil for 4 and 9 weeks to mice infected by *Streptococcus pneumoniae.* Long-term supplementation resulted in reduction of the histopathological length of the lungs, as well as markers of oxidative stress (malondialdehyde [MDA], myeloperoxidase, and nitric oxide), with reduction in the levels of proinflammatory cytokines (tumor necrosis factor α [TNF-α], IL-1) and

Table 5
Other beneficial physiological effects of flaxseed and its bioactive compounds

Reference	Characteristics of the experiment	*n*	Sex	Dosage	Duration	Observed effects
Humans						
Cornish et al. (2009)[135]	Randomized double-bind placebo controlled study during exercise training on metabolic syndrome gand osteoporosis risk in older adults.	100	Males and females	543 mg lignan	6 months	Males taking flaxseed lignin complex reduce metabolic syndrome score, but this was not seen in females. Flaxseed had no effect on bone mineral density or content.
Dodin et al. (2005)[136]	Case control study with postmenopausal women	199	Female	40 g flaxseed	12 months	No positive correlation was encountered between the bone mass loss and flaxseed consumption.
Animals						
Dugani (2009)[84]	Rats were fed with flaxseed mucilage and oil, pretreated with ethanol.	30 (oil)20 (mucilage)	Males	2.5, 5, and 10 mL/kg of flaxseed oil by oral application; 10 and 20 mL of mucilage/kg of weight 1 mL ethanol 75%	—	↓The number and length of gastric ulcers induced by ethanol. The greatest effect was encountered with oil (5 mL/kg).

Zhan et al. (2009)[137]	Barrows feeding linseed diet on inflammation-related genes and growth performance	24	Males	10% dietary linseed	0, 30, 60, and 90 days	mRNA expression of PPARγ in muscle and spleen increased with duration of linseed diet feeding. TNF-α and IL-6 expression in muscle, adipose tissue, spleen and serum concentration of TNF-α decrease.
Rajesha et al. (2006)[81]	Albino rats were fed with flaxseed, followed by CCl_4, to diminish the catalase catalase, superoxide dismutase (SOD), and peroxidase antioxidante enzymes.	24	Male and females	5% and 10% flaxseed in the diet (0.75 and 1.5 g/kg weight)	14 days	CCl_4, increased the activity of enzyme and lipid peroxidation in the liver by 1.2×. 5% flaxseed restored activity of the catalase, SOD, and peroxidase enzymes by 35.6%, 47.76%, and 53% respectively. 10% of flaxseed restored enzyme activities at 39.7%, 181.42%, and 123.7%, respectively.
Ward et al. (2001)[138]	Rats received SDG from flaxseed, when born, by means of lactation and after weaning in the growth (adolescent) and maintenance diets (adult).	20 pregnant rats and the litter	Male and females	5% and 10% of SDG in the 2.5, 5, and 10mL of Flaxseed oil/kg of weight by ors/application or 10 and 20 mL of mucilage/kg of weight, 30 min before 1.0 mL of 75% (v/v) ethanol.	21 (weaning), 50 (adolescent), and 132 (adult) days	Rats which received SDG continuously in two concentrations showed greater ferum strength until adolescence, but without alteration in bone mineral content. As adults there was no significant difference in regards to strength.

SDG = secoisolariciresinol diglucoside; CCl_4 = carbon tetrachloride; SOD = superoxide dismutase; mRNA = messenger ribonucleic acid; PPARγ = peroxisome proliferator-activated receptor-γ; TNF-α = tumor necrosis factor-α; IL-6 = interleukin-6. ↑ = increase; ↓ = decrease.

increase in anti-inflammatories (IL-10). Supplementation for 4 weeks did not show to be effective.[78]

It was therefore verified that the effect of the bioactive compounds of flaxseed may be beneficial both in low-grade inflammation to reduction levels of CRP and serum amyloid A, as well as inflammatory markers in obese individuals[82] and clinical inflammatory processes.[83]

The antioxidant effect of flaxseed was also described in studies on animals,[79] as well as effects against diseases of the gastrointestinal tract. Therefore, the mucilage obtained from flaxseed may exert antiulcer effects.[84] Positive effects were also encountered in patients with irritable bowel syndrome who received 17 g of flaxseed per day during 3 months, demonstrating a reduction in constipation and in other abdominal symptoms.[85]

In summary, the ingestion of 30 g of flaxseed per day, which is a quantity utilized in some studies with humans, provides a considerable quantity of the bioactive compounds studied, being efficient to supply the dosages used in the majority of the studies.

Flaxseed and Hormonal Modulation

An additional benefit of lignans is hormonal modulation, causing a decrease in hot flashes which are characteristic of menopause.[86] This is a result of the weak estrogenic activity of lignans.[87] However, in a study performed with 38 women who were 1 to 10 years post menopause, daily consumption of breads containing 25 g of flaxseed for 12 weeks alleviated menopausal symptoms, but was not more effective than the placebo group.[88] Therefore, the usefulness of flaxseed in the treatment of menopausal symptoms remains in question.

Lignans may reduce the level of free circulating testosterone and when bonded together are excreted in the bile, potentially reducing the risk of polycystic ovary syndrome in susceptible women, since this syndrome is associated to high levels of androgens.[89,90] This therapeutic use has yet to be tested.

Competition of lignans with estrogen for receptor sites causes dual effects. Considering that lignan possesses a weak hormonal action, during phases of life when there is a large production of estrogen, the chronic ingestion of flaxseed may exert an antiestrogenic action because it competes with estrogen for the same receptors. By means of this mechanism, flaxseed may protect women with risk of cancer by decreasing hormonal signalization involved in the beginning of tumor development. Sturgeon et al. (2008) reported that flaxseed may modestly reduce levels of estrone, principally circulating estrogen during the postmenopause period in overweight and obese women.[91] But the question arises as to whether this weak estrogenic action in women presenting no risk of cancer has some adverse effect.

O'Neil et al.[92] showed that estrogenic activity or antiestrogenic activity of flaxseed, in the form of flour, is dependent on the tissue, exposure to estradiol, and duration of flaxseed utilization. The accumulation of lignans in tissues is also different between the genders, showing that there are many variables involved in the action of such compounds on the organism.[93]. Therefore, more studies on this subject are required to determine the beneficial effects, or even potential risk, of flaxseed.

The masculine reproductive system also is influenced by phytoestrogens present in the diet, being responsive to estrogens as a fetus until reaching adulthood as long as estrogen receptors are expressed and exposure to such hormones may result in abnormal development of the reproductive system.[94] In rat embryos, estrogen receptors (ER-β and ER-α) were encountered in precursor cells of oocytes and spermatogonia, and in somatic cells,

respectively.[95] Neonatal exposure of rats to estrogens resulted in the reduction of the spermatic concentration, plasmatic testosterone,[96] the number and function of Sertoli cells,[97] the distension of the rat testis and the epithelial height of the efferent duct,[98] and increased apoptosis of germinative cells due to expression of gonadal testosterone, thus decreasing sperm production.[99]

Female Wistar rats were fed with diets containing low concentrations of phytoestrogens in the period prior to conception until weaning of the pups. After weaning, the male pups continued receiving this diet until reaching adulthood, at which time they began to receive a diet with high concentrations of phytoestrogens during 24 hours. The group that received high doses of phytoestrogens as adults presented higher sperm counts, decreased number of rounded and elongated spermatids, increased in seminiferous tubules in the lumen, apoptosis of spermatocytes, and rounded spermatids. However, there was no decrease in the levels of testicular testosterone and plasmatic gonadotropin. The results suggested a negative effect of phytoestrogens on the independent spermatogenesis of the hypothalamus- pituitary-testicular axis.[100] Ruhlen et al. (2008), however, observed an increase in the endogenous estradiol of pups whose mothers received low doses of phytoestrogens in the diet, when compared to those whose mothers received high doses. This caused adverse effects on the reproductive system, including the reduction in size of the testicles, epididymis, and seminal vesicle, and increase in size of the prostate. Females of the offspring presented precocious puberty and greater uterine responsiveness to estrogens.[101]

Lipids present in flaxseed may also exert influences on the spermatogenic process. Lipids are the principal components of sperm and changes in its composition may cause modifications in physiological events related to sperm production.[102] Decrease in the n-6:n-3 fatty acid ratio promoted by the ingestion of flaxseed results in a rearrangement in the spermatic composition, positively affecting fluidity, which is increased, as well as the integrity and viability of the spermatozoide membrane associated to its fusion with the oocytes[103] and greater velocity,[102] showing the positive effect of n-3 fatty acid.

A positive effect may also be attributed to the ingestion of antioxidants, including vitamin E and the phenolic compounds present in flaxseed, associated to greater sperm count and greater motor force of spermatozoa in men.[104]

Therefore, based on the results shown in studies with animals, it is concluded that the utilization of flaxseed in the diet must be performed with caution during gestation, since the exact effects that flaxseed phytoestrogens may cause on the male reproductive system are not known. Studies on humans are also necessary to increase understanding of the positive and negative effects of flaxseed on males related to the reproductive system and fertility.

Adverse Effects of Flaxseed Components

The presence of phytoestrogens with adverse health effects and toxic compounds in flaxseed cannot be neglected. Flaxseed contains the inhibitors: trypsin,[105] *myo*-inositol phosphate,[106] cadmium,[107] and cyanogenic glycosides.[108]

Wanasundara et al. (1999), reported that flaxseed possesses small quantities (13.3 mg/ g crude protein) of protease inhibitors, which are reduced to trace quantities after germination.[105] It is known that trypsin inhibitors present in the diet have been known for decades to diminish growth in animals, since they decrease the digestion and consequent absorption of proteins by the inhibition of proteases.[109] Protease inhibitors are thermolabile, given the increase in digestibility of flaxseed proteins in animals after thermal treatment.[110]

Generally, seeds containing elevated concentrations of phytic acid in the inositol penta (IP-5) and hexaphosphate (IP-6), which present a property of binding with proteins and divalent minerals, have decreased bioavailability.[107,111]

Flaxseed contains roughly 0.526 μg of cadmium per kg of seed,[110] where this content is the result of artificially fertilized soil.[112] Cadmium is potentially toxic to the human organism, since it is a heavy metal that when accumulated in the kidney may cause renal dysfunction, as well as pulmonary emphysema,[108] aminoaciduria, glycosuria, phosphaturia, and even compromise mineral reabsorption, making organisms susceptible to osteomalacia.[113] Accumulation of cadmium also causes damage to the renal tubes, resulting in proteinuria.[112] It is known that cadmium, together with mercury, is one of the most toxic metals[112]; it has been established that ingestion of cadmium should not surpass 1 μg/kg weight per day.[113] Considering the content of cadmium in flaxseed as previously cited, consumption of flaxseed in the habitual diet does not offer risk of intoxication to consumers, since excessively large quantities of the seed would be necessary to provide the content considered as toxic to the organism.

Flaxseed contains 264–354 mg of cyanogenic compounds per 100 g of seed, being 10–11.8 mg of linamarin/100 g, 136–162 mg of linustatin/100 g, and 105–183 mg of neolinustatin/100 g of flaxseed.[114] These compounds are toxic to the human organism and it is estimated that ingestion of 100 mg may be lethal to adult individuals.[115] The chronic effects of cyanogenic compound ingestion are manifested in the nervous system and are observed in populations that ingest high quantities of cyanate in foods.[116] Other symptoms related to intoxication by cyanogenic glycosides are headaches and tachycardia.[113] However, these compounds present instability when subjected to thermal and mechanical processes,[114,115] including cooking in microwaves, autoclaving, and boiling.[116]

Average tolerance of ingestion of cyanogenic compounds without adverse effects, as established by the World Health Organization (WHO) (2003), is 0.11 mg/kg weigh in the form of cyanogen chloride and thus, an individual weighing 60 kg may consume up to 0.66 mg.[117] Considering the concentration of cyanogenic compounds in flaxseed as reported in literature,[115] the daily ingestion of flaxseed indicated (30 g) may contain on average 106 mg of cyanogenic compounds, which is above the tolerable level.

Based on the previous considerations, the consumption of flaxseed is recommended in the form of flour, after thermal treatment, because not only are the concentrations of compounds with adverse effects eliminated or reduced, trituration of the seed increases bioavailability of the bioactive compounds.[118]

Conclusion

The modulation of risk factors for NCCDs by flaxseed is amply described in the literature, principally regarding its effect on risk factors for CVD and hormone-dependent cancers. Such effects are controversial when analyzed in function of isolated bioactive compounds, but are shown to be very favorable when the consumption of flaxseed as a whole is considered, generally with doses varying from 20 to 40 g/day. There is still controversy regarding its action on blood glucose.

The beneficial effects, however, show differences in function of the form of product presentation: integral seed, defatted flour, or oil. The oil of flaxseed does not contain dietary fibers or lignans, whereas defatted flour is not a source of n-3 fatty acids. Therefore, the health benefits related to flaxseed depend on the type of product and form of consumption of the seed.

These positive effects allow for suggesting that flaxseed is a food that may be utilized in strategies for taking care of health. In chronic use, the presence of phytoestrogens with adverse health effects must not be neglected; however, the risk of toxicity by cyanogenic compounds may be reduced or eliminated with mechanical and thermal processing. There are still doubts, however, on security related to chronic consumption by some population groups: pregnant women and men in their reproductive ages, in function of the possible adverse effects due to hormonal action of the lignans.

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METODOLOGIA GERAL

O presente trabalho foi desenvolvido nos Laboratórios de Desenvolvimento de Novos Produtos e Análise Sensorial, Nutrição Experimental, Análise de Alimentos e Bioquímica Nutricional do Departamento de Nutrição e Saúde, e Laboratório de Biologia de Peixes, do Departamento de Veterinária.

Elaboração da farinha de linhaça

As sementes de linhaça (*Linum usitatissimum L.*) das variedades marrom e dourada foram adquiridas no comércio local da cidade de Viçosa, MG - Brasil (20°45´14´´, latitude S; 42°52´54´´, longitude W).

Para a obtenção das farinhas, as sementes foram submetidas ao tratamento térmico a 150 ˚C, em estufa de circulação de ar, por 15 minutos. As sementes foram então trituradas em liquidificador em velocidade média, e passadas por peneira de 20 *mesh*, separando-se o farelo, que foi moído novamente. As farinhas foram acondicionadas em embalagens de polipropileno e armazenadas em temperatura ambiente até o momento das análises ou elaboração das dietas. As condições de processamento utilizadas foram pré-estabelecidas anteriormente e asseguram a preservação do ALA e a estabilidade oxidativa da farinha (MORAIS *et al.*, 2011).

Análise da composição química das farinhas de linhaça marrom e dourada

Composição centesimal

O teor de umidade foi determinado em estufa de circulação e renovação de ar (Marconi®, modelo MA 035) a 105 ^{o}C até peso constante (AOAC, 1997).

O teor de proteínas foi analisado segundo o método semimicro de Kjeldahl, para a quantificação de nitrogênio total (AOAC, 1997),

sendo utilizado o fator de conversão de nitrogênio em proteínas de 6,25.

Os lipídios totais foram determinados pelo método de extração intermitente, utilizando-se extrator de Soxhlet (AOAC, 1997). O conteúdo de cinzas foi determinado por meio de calcinação das amostras em mufla a 550 °C (AOAC, 1997).

O teor de fibra alimentar total da farinha de linhaça foi determinado pelo método enzimático-gravimétrico (AOAC, 1997), utilizando-se kit enzimático (Sigma®). Esse método está fundamentado na porção não hidrolisada do alimento que resiste à digestão enzimática seqüencial com α-amilase, protease e amiloglicosidase. As farinhas utilizadas para a determinação de fibras totais foram processadas em moinho microanalítico.

O teor de carboidratos foi obtido por meio da diferença entre 100 e a soma do conteúdo de proteínas, gorduras, fibra alimentar, umidade e cinzas. Este procedimento está previsto na Resolução da Diretoria Colegiada (RDC) nº 360, de 23 de dezembro de 2003 (BRASIL, 2003).

O conteúdo calórico da farinha de linhaça foi calculado de acordo com a composição do alimento em proteínas, lipídios e carboidratos, sendo utilizados os fatores de conversão 4, 9 e 4 Kcal/g destes macronutrientes, respectivamente.

Quantificação de ácidos graxos da amostra

O perfil de ácidos graxos das amostras foi determinado por cromatografia gasosa.

A fração de lipídios das amostras das farinhas (0,1 g) foi obtida de acordo com a metodologia descrita por Folch, Lees e Stanley (1956). Após extração, os lipídios foram submetidos à saponificação e esterificação, segundo metodologia de Hartman e Lago (1973). Alíquotas das amostras (1,0 µL) contendo os ésteres metílicos foram injetadas (duplicata) em cromatógrafo a gás equipado com auto-injetor (Shimadzu, model AOC-17) e um integrador (Shimadzu, model C-R7A). A coluna capilar Carbowax (30 mm x 0,25 mm de diâmetro

interno) foi mantida a 200 ^{o}C por 10 minutos, com elevações de 6 ^{o}C até 240 ^{o}C, durante 16,6 minutos. As condições cromatográficas foram: temperaturas do injetor e detector de 240 ^{o}C e 260 ^{o}C, respectivamente, modo *split* e razão 1:10. O gás de arraste foi o nitrogênio a um fluxo de 0,5 mL/minutos, pressão de 100 Kpa. Uma curva analítica de ALA, variando de 0 a 1.000 ppm, com r^2=0,996, foi utilizada para a quantificação do ácido graxo ômega-3.

Teor de ácido Fítico

O teor de ácido fítico (AF) foi determinado seguindo o procedimento descrito por Latta e Eskin (1980), com modificação da resina para Dowex-AGX-4, conforme Ellis e Morris (1986).

Extração

O AF das amostras foi extraído a partir de 10 g de farinha em solução de HCl 0,8 M, na proporção de 1:10 (p/v) e agitação constante, por 2 horas, em 100 x g. Em seguida, o material foi centrifugado a 980 x g, por 10 minutos para a obtenção do sobrenadante contendo o AF. Uma alíquota (1,0 mL) do sobrenadante foi colocada em um balão volumétrico e o volume completado para 25 mL com água deionizada. Após homogeneização, uma alíquota (2,0 mL) foi utilizada para eluição na coluna Dowex-AGX-4 previamente preparada.

Para a coluna cromatográfica, 0,5 g da resina Dowex-AGX-4 foi dissolvida em água deionizada (5,0 mL) em béquer e transferida para uma coluna de vidro (20 x 1,0 cm de diâmetro interno) contendo 1 cm de lã de vidro no fundo. Após a drenagem da água da coluna, a mesma foi eluída com solução de NaCl 0,7 M (10 mL) e depois com água deionizada.

Uma alíquota (2,0 mL) do extrato contendo o AF foi colocado na coluna previamente preparada, seguindo-se eluição com solução de NaCl 0,1 M (10 ml), descartando-se o eluato. A seguir, a coluna foi eluída com solução de NaCl 0,7 M (10,0 mL) e o eluato contendo o AF foi coletado em um béquer.

Ensaio

Alíquotas (3,0 mL) do eluato contendo o AF foram então transferidas para tubos de ensaio, adicionando-se, a seguir, 1,0 mL do reativo de Wade (ácido sulfosalicílico e cloreto férrico). A absorvância foi lida a 500 nm, utilizando-se espectrofotômetro Shimadzu UV-Visível 1601 (Kyoto, Japão).

A curva analítica foi construída utilizando solução de fitato dodecassódico (Sigma®) em água deionizada, em concentrações variando de 22,5 a 135 µg/mL, sendo as condições idênticas às descritas para as amostras. A equação da reta, obtida a partir do gráfico de regressão linear, foi utilizada para calcular e quantificar o teor de AF das amostras. Os resultados foram expressos em grama de AF por 100 g de farinha.

Estimativa de fenólicos totais

O teor de compostos fenólicos nas amostras foi estimado utilizando-se o reagente de Folin-Ciocalteu, que oxida os compostos fenólicos em ambiente alcalino, e reduz o fosfomolibidato presente no reagente, formando um composto de coloração azul que pode ser lido em uma faixa de 720 a 765 nm, conforme descrito por Singleton et al. (1999).

Extração

A extração foi feita em triplicata, por adição de 10 mL de solução metanólica a 60% (v/v) a 0,1 g da amostra, sendo agitada por 30 minutos a 10,6 x g em temperatura ambiente e o volume completado para 15 mL com água destilada.

Ensaio

A uma alíquota de 0,5 mL do extrato foi adicionado 0,5 mL de solução aquosa de carbonato de sódio 7,5% e 0,5 mL de solução aquosa de Folin-Ciocalteau a 20%.

A mistura foi agitada em vórtex e incubada em temperatura ambiente por 30 minutos. A absorbância foi lida a 765 nm, utilizando-se espectrofotômetro Shimadzu UV-Visível 1601 (Kyoto, Japão).

Uma curva analítica padrão de ácido gálico em concentrações variando de 0,01 a 0,1 g/L foi utilizada para calcular a concentração de compostos fenólicos, a qual foi expressa em equivalentes de ácido gálico (EAG) por 100 g de farinha.

Teor de secoisolariciresinol (SECO)

O teor do isômero SECO foi determinado conforme utilizado por Charlet *et al.* (2002).

A farinha de linhaça (600 mg) foi desengordurada através de tratamento com hexano (5 mL) por 1 hora, desprezando-se o sobrenadante.

Extração

O extrato contendo lignanas foi obtido a partir da farinha de linhaça desengordurada com 12 mL de solução aquosa de metanol (70%) por 3 horas a 60 °C em agitação constante, e filtrada em papel de filtro.

O extrato foi então hidrolisado com 18 mL de HCl (concentração final de 2 M) a 100 °C sob agitação por 2,5 horas, e o hidrolisado extraído duas vezes com 10 mL de acetato de etila/hexano (1:1 v/v). A fração orgânica foi evaporada e o extrato seco ressuspendido em 1 mL de metanol.

Ensaio

A quantificação foi realizada em HPLC (SHIMADZU SPD-10A VP acoplado ao UV-Visivel) com coluna C18 de fase reversa e detecção a 204 nm. A fase móvel foi constituída por solução aquosa de acetonitrila (60% v/v) suplementada com 0,1% de ácido fosfórico e 0,1% de trietilamina, com razão de fluxo de 1,0 $mL.min^{-1}$ (tempo de retenção de aproximadamente 3 minutos). Foi construída uma curva padrão de SECO de 0 a 100 ppm.

Teor de vitamina E

O teor de vitamina E foi determinado a partir da quantificação dos isômeros de vitamina E por cromatografia líquida de alta eficiência (CLAE).

Extração

O processo de obtenção dos extratos e de quantificação por CLAE foram baseados em Lee (1999).

Amostras de 4 g de farinha de linhaça foram acrescidas de 4 mL de água ultrapura aquecida a 80 ºC. Em seguida, 10 mL de isopropanol, 1 mL de hexano contendo 0,05% de butil-hidroxi-tolueno (BHT) e 5 g de sulfato de sódio anidro foram adicionados ao tubo de extração. Gradativamente, foram adicionados 25 mL da mistura solvente de extração (hexano:acetato de etila, 85:15 v/v). As amostras foram, então, trituradas em microtriturador em velocidade média durante 1 minuto. Uma vez trituradas, as amostras foram filtradas a vácuo em funil de büncher utilizando-se papel de filtro e mantendo o resíduo no tubo de extração. Esta etapa de extração foi repetida adicionando-se 5 mL de isopropanol e 30 mL da solução solvente, com posterior homogeneização e filtração à vácuo.

O extrato foi concentrado em evaporador rotativo a 70 °C por 2 minutos e transferido para balão volumétrico, completando o volume para 25 mL com mistura solvente.

Uma alíquota de 5 mL foi seca em nitrogênio gás e recuperada em 2 mL de hexano para leitura em CLAE.

Análise dos isômeros de vitamina E

A análise dos isômeros de vitamina E foi realizada em cromatógrafo Shimadzu SCL 10AT VP, equipado com bomba de alta pressão (LC-10 AT VP), injetor automático com loop de 50 µL (SIL-10AF), coluna cromatográfica LiChrosorb (Si60 Phenomenex 250 x 4 mm, 5 µm), detector de fluorescência (RF-10ª XL, 290 nm de

excitação e 330 nm de emissão), software “Multi System” (Class VP 6.1). A fase móvel foi constituída de hexano:isopropanol (99,6:0,4) ajustados para pH 2,5 com ácido acético glacial. A taxa de fluxo foi de 1,0 mL/minuto e o tempo de corrida foi de 30 minutos.

As alíquotas recuperadas em hexano foram filtradas utilizando unidades filtrantes com porosidade de 0,45 µm.

Foram injetados dois volumes diferentes para cada amostra, a fim de se obter a detecção de todos os compostos em quantidades apropriadas para a identificação e quantificação. Assim, foram utilizados 5 µL para γ-tocoferol e 50 µL para os demais isômeros de menor concentração (α-tocoferol e δ-tocoferol).

Capacidade antioxidante das farinhas

Foram obtidos extratos hidroalcoólicos seguindo procedimentos idênticos aos descritos na determinação de compostos fenólicos (BLOOR, 2001).

A determinação do potencial antioxidante das diferentes farinhas foi realizada por meio do teste de atividade de retirada do radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH), conforme descrito por Blois (1958), e pelo teste do poder redutor (OYAIZU, 1986).

Atividade de retirada de radical DPPH

No teste do DPPH, a atividade de retirada de radical (ARR) das amostras foi mensurada em uma alíquota do extrato (100 µL) em concentração de 0,01 g/mL (amostra/solução extratora). Foi adicionado 1,5 mL de solução metanólica de DPPH 0,1 mM, sendo a mistura agitada por um minuto e deixada em repouso por 30 minutos em temperatura ambiente e no escuro. Na sequência, absorbância foi lida em espectrofotômetro Shimadzu UV-Visível 1601 (Kyoto, Japão) a 517 nm e a ARR calculada pela seguinte fórmula:

$$ARR\ (\%): [1- (A_{amostra} – A_{branco\ da\ amostra}/ A_{controle})] \times 100$$

Onde A: absorbância a 517 nm.

Poder redutor de íons férricos

O teste do poder redutor foi determinado adicionando-se uma alíquota de 1 mL do extrato a 1 mL de tampão fosfato 0,2 M pH 6,6 e a 1,5 mL de solução aquosa de ferrocianeto de potássio a 1%. Após 30 minutos de incubação a 50 °C, foram adicionados 1,5 mL de ácido tricloroacético a 10% e a mistura agitada em vórtex. Em seguida, 2 mL da camada superior foram retirados e misturados a 2 mL de água destilada e 0,5 mL de cloreto férrico 0,1%. A absorbância foi lida em 700 nm, utilizando-se espectrofotômetro Shimadzu UV-Visível 1601 (Kyoto, Japão).

Ensaio Biológico

Desenho Experimental

Ratos *Wistar* machos com 8 semanas de idade foram divididos em 3 grupos (n=10 por grupo): 1) dieta controle AIN-93M; 2) dieta AIN-93M adicionada de farinha de linhaça marrom tratada termicamente com calor seco, de forma a fornecer 50% das necessidades diárias de fibra do animal (12% da dieta), corrigida para os demais nutrientes; e 3) dieta AIN-93M adicionada de farinha de linhaça dourada marrom tratada termicamente com calor seco, de forma a fornecer 50% das necessidades diárias de fibra do animal (12% da dieta), corrigida para os demais nutrientes. Os animais receberam dieta e água *ad libitum* durante 30 dias, após os quais foram eutanasiados para coleta de plasma, testículo, fígado, pulmão e rins. Foram analisados o consumo alimentar, ganho de peso, perfil de lipídios séricos e peroxidação de lipídios em todos os tecidos coletados, sendo realizadas comparações entre linhaça dourada e linhaça marrom.

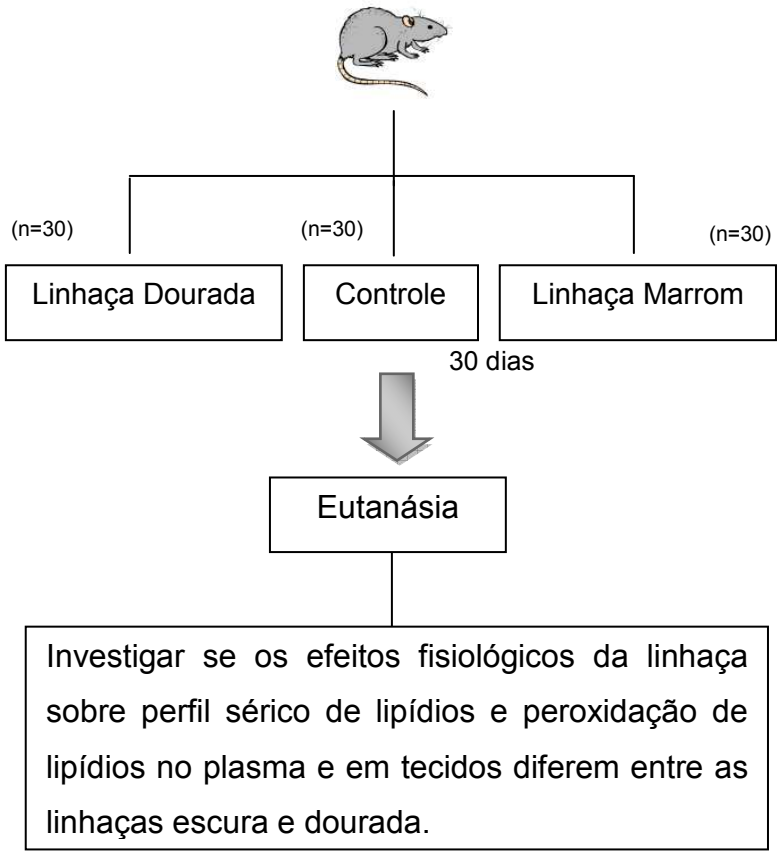

Figura 1: Desenho Experimental

Animais e dietas experimentais

Foram utilizados 30 *Ratus norvegicus albinus* linhagem *Wistar* machos, provenientes do Biotério do Centro de Ciências Biológicas e da Saúde da Universidade Federal de Viçosa. Os animais foram mantidos em gaiolas individuais, em ambiente de temperatura (22±2˚C) e fotoperíodo (12/12 horas) controlados, recebendo diariamente dieta e água destilada *ad libitum*. Foram distribuídos em 3 grupos com dez animais cada, de acordo com o desenho experimental (Figura 1), recebendo dietas experimentais conforme Tabela 1. As quantidades de ingredientes foram ajustadas para se obter dietas isocalóricas para todos os grupos.

Tabela 1: Composição das dietas experimentais (g/100g de mistura).

Ingredientes	AIN-93M	AIN-93M + LM	AIN-93M + LD
Caseína (82% proteína)*	14,00	11,50	11,70
Sacarose	10,00	10,00	10,00
Amido de Milho	46,50	42,12	42,57
Amido Dextrinizado	15,50	15,50	15,50
Óleo de Soja	4,00	3,50	0,00
Celulose Microfina*	5,00	2,50	2,50
Mistura Mineral*	3,50	3,50	3,50
Mistura Vitamínica*	1,00	1,00	1,00
L-cistina*	0,18	0,18	0,18
Bitartarato de Colina*	0,25	0,25	0,25
Farinha de linhaça	0,00	12,00	12,80

AIN-93M= controle manutenção (animais adultos); LM= linhaça marrom; LD= linhaça dourada. *Produtos adquiridos da Rhoster®.

As dietas foram elaboradas conforme Reeves (1997), com freqüência semanal e mantidas em freezer convencional (-20ºC).

Coleta de amostras biológicas ex-vivo

Ao final do experimento, os animais foram anestesiados com isoflurano e eutanasiados por exsanguinação. Para cada animal foram coletados 10 mL de sangue, sendo centrifugado a 2.400 x g, por 15 minutos para obtenção do soro, e armazenado a -20 ºC (para análises bioquímicas séricas) e -80 ºC (para análise de peroxidação). Foram excisados os seguintes órgãos: fígado, pulmão, rim e testículo. O pulmão, rim e o testículo direito de cada animal, bem como o fígado, foram liofilizados para análises de peroxidação de lipídios.

Parâmetros avaliados

Consumo alimentar e variação de peso corporal

O consumo alimentar foi avaliado por meio do registro semanal de ingestão de dieta pelos animais (diferença entre a oferta e as sobras deixadas nas gaiolas).

A variação do peso corporal foi avaliada por meio de pesagem semanal dos animais durante o experimento.

Perfil de lipídios séricos

Os níveis de colesterol total, triglicerídeos séricos e HDL foram determinados por meio do método enzimático colorimétrico, utilizando-se *kits* comerciais (Human do Brasil®), de acordo com recomendações do fabricante. Após as reações, as leituras de absorbância foram feitas em espectrofotômetro (Shimadzu®, modelo UV – 1601) a 500 nm. As concentrações foram expressas em mg/dL.

Peroxidação de lipídios

Os níveis de malondialdeído (MDA) foram estimados no soro e em homogenatos de fígado, pulmão, rim e testículo, por meio do Teste de Substâncias Reativas ao Ácido Tiobarbitúrico – TBARS, de acordo com metodologia descrita por Buege e Aust (1978).

Para obtenção dos homogenatos, os tecidos liofilizados foram ressuspensos em tampão fosfato 0,1M, pH 7,4, na proporção 1:10 (m/v), sendo utilizadas amostras de 0,5g de fígado; 0,25g de rim; 0,3g de pulmão e 0,2 g de testículo. Foram adicionados 20 µM de hidroxibutiltolueno (BHT) à solução, sendo homogeneizada em vórtex e, posteriormente, centrifugada a 2940 x g por 10 minutos. Alíquotas de 0,5 mL do soro e dos homogenatos dos tecidos foram adicionados em tubos de ensaio contendo a solução de TBARS (ácido tricloroacético a 15% e ácido tiobarbitúrico a 0,375%, dissolvidos em HCL 0,25 N). A mistura permaneceu em banho-maria a 90 °C por 15 minutos. Após a incubação, esta foi resfriada e centrifugada a 1000 x g. O sobrenadante foi utilizado para leitura de absorbância a 535 nm em espectrofotômetro (Shimadzu®, modelo UV – 1601). Os resultados foram expressos em nanomol de equivalentes de MDA por miligrama de proteína nas amostras de homogenatos ou nmol/mL de plasma, utilizando-se o coeficiente de extinção molar de $1,56 \times 10^5\ M^{-1}.cm^{-1}$ (BUEGE & AUST, 1978).

Concentração de proteínas totais nos homogenatos

Para a determinação de proteínas totais nos homogenatos de tecidos foi empregado o método de biureto, utilizando-se *kit* comercial (Proteínas Totais - Bioclin®). Para a realização da análise foram seguidas as recomendações do fabricante. A leitura de absorbância foi realizada a 545 nm, utilizando-se espectrofotômetro (Shimadzu®, modelo UV – 1601). A concentração de proteína foi expressa em g/dL.

Análises estatísticas

Os dados foram expressos como média e desvio padrão, sendo utilizados os testes de One Way ANOVA ou Kruskal-Wallis, de acordo com a distribuição dos dados, seguidos de teste de Tukey ou Dunn, respectivamente. Teste de Correlação de Pearson foi utilizado para determinar a existência de correlação entre os dados de compostos fenólicos totais e capacidade antioxidante das farinhas de linhaça.

Para estas análises, utilizou-se o programa Sigma STAT versão 2.03, sendo o nível de significância adotado de 5%.

Aspectos éticos

O presente estudo foi aprovado pelo Comitê de Ética em Experimentação Animal da Universidade Federal de Minas Gerais (CETEA/UFMG) sob o protocolo de número 213/2009.

RESULTADOS

Chemical composition and physiological properties of whole brown and golden flaxseed flours in male Wistar rats

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CHEMICAL COMPOSITION AND PHYSIOLOGICAL PROPERTIES OF WHOLE BROWN AND GOLDEN FLAXSEED FLOURS IN MALE WISTAR RATS

ABSTRACT

Two varieties of flaxseed are marketed for human consumption in Brazil: brown and golden seeds; and there is little information about differences in functional properties between both. This study compared hypocholesterolemic and antioxidant effects of brown and golden flaxseed flours in rats, and characterized the chemical composition of macronutrients and bioactive compounds of both varieties. Animals were fed with a basal AIN-93M diet added with brown flaxseed flour or golden flaxseed flour, at a level sufficient to provide 50% of the recommended dietary fiber. The data were analyzed by ANOVA followed by Tukey (α = 5%). Brown flaxseed showed greater protein and fiber content, antioxidant capacity, and phenolic compounds and vitamin E contents. Both flaxseeds showed a reduction only in TC/HDL ratio. Although the brown flaxseed flour has showed a better chemical composition to lower risk of chronic non-communicable diseases in relation to the golden flaxseed flour, this was not reflected in the physiological effects on male *Wistar* rats.

KEYWORDS: Linum usitatissimum; brown flaxseed; golden flaxseed; bioactive compounds; cholesterol.

ABREVIATIONS

AC: Antioxidant capacity
ALA: α-linolenic acid
BD: Basal diet
BF: Brown flaxseed
BHT: butylated hydroxytoluene
DPPH: 2,2-diphenyl-1-picryl hidrazyl
GAE: Galic acid equivalent

GC: gas chromatography
GF: Golden flaxseed
HDL: High density lipoprotein
HPLC: High performance liquid chromatography
IP-2: inositol biphosphate
IP-3: inisitol triphosphate
IP-5: inositol pentaphosphate
IP-6: inositol hexaphosphate
LDL: Low density lipoprotein
MDA: Malondialdehyde
P/S: Polyunsaturated/saturated fatty acids
PBS: Phosphate buffered saline
RP: Reducing power
RSA: radical scavenging assay
SDG: Secoisolariciresinol Diglucoside
TC: Total cholesterol
TG: Triglyceride
TP: Total phenolics
VLDL: Very low density lipoprotein

1. INTRODUCTION

Flaxseed (*Linum usitatissimum L.*) is an oilseed with high nutritional value being considered the richest source of α-linolenic acid (ALA) among plants [1]. Flaxseed also has a high content of phenolic compounds, especially in lignans, a molecule structurally similar to estrogen [2].

Flaxseed has received great attention in health researches due to the functional properties of its bioactive compounds, which can act to reduce risk of chronic diseases. Bioactive effects of flaxseed include anti-inflammatory [3], antioxidant [4], estrogen and antiestrogen actions [5] and in reducing the risk of diseases such as cancers, especially breast and prostate cancer [5], dyslipidemia [6], and type 2 diabetes mellitus [7].

As an assistant in the therapy of reducing the risk of cardiovascular disease, one of the main effects to be achieved in the short term with the

intake of flaxseed, is the reduction of cholesterol [8]. Flaxseed contains several bioactive compounds that have been reported to reduce cholesterol: ω-3 [9], fiber [10], antioxidants [11] and lignans [12]. The effect obtained by intake of flaxseed depends on its form, since flaxseed oil contains no fiber and lignans and defatted flaxseed is not a source of ω-3 [12].

In Brazil, a gradual increase of the interest on flaxseed physiological effects has been observed in the last years. There are two varieties of flaxseed available for human consumption in the Brazilian market: brown and golden flaxseed. No comparative studies were found in literature on the physiological properties between the two seed varieties. Thus, the objective of this study was to compare the effects of consuming brown flaxseed (BF) and golden flaxseed (GF) on weight, food intake, serum lipid profile and markers of oxidative stress in rats, as well to characterize the chemical composition of both seed varieties.

2. MATERIAL AND METHODS

2.1. Flaxseed samples

Flaxseed samples were acquired from local market and subjected to heat treatment in a circulated air oven (150 °C for 15 min) to eliminate anti-nutritional compounds. Seeds were crushed in a blender at medium speed for 5 minutes, and sieved to obtain a particle size of 20 *mesh*.

2.2. Biological assay

2.2.1. Diets

The basal diet (BD) consisted of a semi-purified AIN-93M diet [13]. Brown (BF) and golden flaxseed (GF) diets were prepared by supplementing the AIN-93M formula with whole brown or golden flaxseed flours, respectively, as a source of fiber at the level of 50% the recommendation as proposed for rodents [13], and adjusted for total calories, macronutrients and fiber.

2.2.2. Animals and experimental design

Male wistar rats were purchased at 8 weeks of age (Center of Experimental Animals of the University Federal of Viçosa) and maintained in individual cages at controlled temperature (21 ± 2°C) with a daily 12-h light-dark cycle and received both diet and water ad libitum.

Thirty rats were randomly divided into three groups of 10 rats per group: control, BF, and GF groups. Four weeks after beginning the experiment, the animals were euthanized under anesthesia. Blood was collected from each animal for lipid peroxidation assay and serum lipid profile analysis. Testis on the right side and liver were immediately removed and frozen at -80ºC until lyophilization for peroxidation assay in homogenates. Blood serum was separated by centrifugation at 1,600 x g for 10 minute at 4°C for analysis of total cholesterol (TC), triglycerides (TG) and HDL.

The experimental protocol of the study was approved by the Ethic Committee in Animal Research of the Federal University of Minas Gerais, Brazil (protocol number 213/2009).

2.2.3. Evaluated parameters

Diet intake and weight gain

Food intake and body weight gain were monitored throughout the study and the results were expressed as means over a period of one week.

Lipid peroxidation in serum and tissue homogenates

The lyophilized tissues were homogenized using stirring after adding 0.1 M phosphate buffered saline (PBS) (pH 7.4) containing 20 µMol of butylated hydroxytoluene (BHT) to tissue samples at a ratio of 10:1. Tissue volumes used were 0.5 g of liver; 0.25 g of kidney; 0.3 g of lung and 0.2 g of testis. The material was homogenized again in a vortex and then centrifuged at 1,000 x g for 10 minutes to obtain the supernatant.

Lipid peroxidation was analyzed using the assay of thiobarbituric acid reactive substances – TBARS according by Buege and Aust [14].

Serum lipid profile

Blood serum was obtained by centrifugation of blood at 1,000 x g for 10 minutes and total cholesterol (TC), high-lipoprotein cholesterol (HDL-C) and triglycerides (TG) was analyzed by enzymatic “kits” (Bioclin). The TC/HDL-c ratio was calculated, and absorbance was measured at 700 nm in a spectrophotometer Shimadzu UV-Visible 1601 (Kyoto, Japão).

2.3. Proximate chemical composition

The chemical composition analysis of the flours was performed according to the standard methods described by the AOAC [15] and the carbohydrate content of both flours was calculated as the difference between the total sample (100%) and moisture, proteins, lipids, ash and fiber.

2.4 Fatty acid content

Lipids were extracted as recommended by Folch [16] and saponified and esterified as proposed by Hartmann et al. [17]. Identification of the fatty acid methyl esters was performed by gas chromatography using the GC-17A Shimadzu/Class model, with a fused silica column SP-2560 (biscianopropil polysiloxane), 30 m x 0.25 mm diameter and a flame ionization detector. The temperature program presented an initial temperature of 200°C held for 10 minutes, and a posterior heating of 6°C per minute up to 240°C where this temperatures was maintained for 16.6 minutes. Temperature of the vaporizer was 240°C and the temperature of the detector was 260°C. The carrier gas used was nitrogen at 0.5 mL/minute, and pressure of 100 Kpa. The split of the sample in the injector was 1/10 and 1 µL of the solution was injected.

2.5. Vitamin E content

The content of vitamin E was quantified from the concentration of vitamin E isomers determined by high performance liquid chromatography (HPLC). The process of acquisition and quantification was based on Lee [18].

Analysis of the vitamin E isomers was performed using the Shimadzu SCL 10AT VP chromatograph, equipped with a high-pressure pump (LC-10 AT VP), automatic injector with 50 mL loop (SIL-10AF), LiChrosorb chromatography column (Si60 Phenomenex 250 x 4 mm, 5 mm),

fluorescence detector (RF-10ª XL, 290 nm excitation and 330 nm emission) and "Multi System" software (Class VP 6.1). The mobile phase consisted of hexane: isopropanol (99.6:0.4) adjusted to pH 2.5 with glacial acetic acid. The flow rate was 1.0 mL/min and the run time was 30 minutes.

The aliquots recovered in hexane were filtered using filter units with 0.45
μm and two different volumes were injected for each sample in order to detect all compounds in quantities appropriate for identification and quantification, being 5 mL for γ-tocopherol and 50 mL for the other isomers of lower concentrations (α- tocopherol and δ-tocopherol).

2.6. Content of phytic acid

The phytic acid content was determined according to the procedures described by Latta & Eskin [19], with modification of the resin for Dowex-AGX-4, according to Ellis & Morris [20]. For chromatography column, 0.5 g of the Dowex-AGX-4 resin was dissolved in deionized water (5.0 mL) in a beaker and transferred to a glass column (20 x 1.0 cm internal diameter) containing 1 cm glass wool at the bottom (added with aid of a tweezers).

2.7. Total phenolic content

Extracts containing the phenolic compounds were obtained as recommended by Bloor et al. [21].

The total phenolic content (TP) was determined using the Folin-Ciocalteu assay [22]. The results were expressed in milligrams of gallic acid equivalents (GAE) per 100g of whole flour, utilizing a calibration curve for gallic acid in the concentration range of 0.005-0.080 mg/L.

2.8. Antioxidant capacity (AC)

2.8.1. DPPH radical scavenging activity (RSA):

The ability of the extracts to scavenge DPPH free radicals was determined by the method described by Blois [23]. The inhibition value was calculated according to the following equation:

Scavenging activity (%) = [(Abs$_{control}$ - Abs$_{sample}$)/Abs$_{control}$] x 100

**Abs: absorbance*

2.8.2. Reducing power (RP):

The RP of the extracts was measured as described by Oyaizu [24].

2.9. Statistical analysis

All assays were performed in triplicate. Statistical analyses were conducted using the SPSS Science SigmaStat software, version 2.03. Differences between sample means for chemical composition were analyzed by t test and physiological effects by one-way ANOVA followed by Tukey, or Kruskall-Walis followed by Dunn's test, according to the normality of the data, at $p < 0.05$.

3. RESULTS

Food intake and weight gain of the animals did not differ among groups (data not shown). There was no difference in lipid peroxidation in homogenates of liver and kidney between the groups, but in the testis, lung and serum, the groups receiving BF and GF showed statistically equal reduction of malondialdehyde (MDA) equivalents concentrations among themselves but lower than BD ($P < 0.005$) (Table 1).

Table 1: Lipid peroxidation in serum and tissue homogenates of rats fed with BD and supplemented with BF GF flours

BF and GF don't decreased TC levels, however, the TC/HDL ratio was reduced by the both, showing a slight improvement in relation to serum lipids levels (Figure 1). There were no differences between groups for concentrations of HDL, VLDL and triglycerides (data not shown).

Figure 1: Total cholesterol and TC/HDL ratio in serum of animals fed the BD (control) and supplemented with brown (BF) or golden flaxseed (GF).

The chemical composition of brown and golden flaxseed flours are presented in Table 2.

Table 2: Chemical composition (g/100g) and antioxidant capacity of the brown and golden flaxseed flours.

In general, the macronutrient composition was similar among the two seeds. The greatest differences were observed in the carbohydrate content, which was 137% higher in GF in relation to BF and the protein content, which was 17.5% greater in the BF. Dietary fiber also was statistically higher in BF.

The levels of stearic and palmitic acids were higher in BF flour, however without altering the polyunsaturated/saturated acids ratio (P/S) of the flours, which were 8.102 and 8.285, respectively.

The vitamin E content was approximately 50% higher in the BF, and the major isomer was γ-tocopherol in both flaxseeds. Phytic acid content obtained from the flours of two seed varieties was similar. However, the phenolic content was 20% greater in BF, and this resulted in its greater antioxidant capacity in both assays (Table 2).

4. DISCUSSION

The physiological effects of brown and golden flaxseed were investigated, since there are health claims related to consumption of this oilseed such as increasing the dietary intake of ω-3, and improving the serum lipid profile in hypercholesterolemic individuals [6]. Whereas it is common in plants there are differences in chemical composition between varieties of the same botanic family [25]; herein, these differences were investigated. Brown flaxseed is cultivated in southern Brazil, and golden one is imported because the local climatic conditions are not suitable for its cultivation.

The chemical composition of the flour was analyzed to verify the association between the physiological effect and the content of bioactive compounds.

Studies on the antioxidant capacity of foods have attracted great interest in recent years, because it has been demonstrated that the selection of foods with greater antioxidant potential may affect positively biochemical parameters, including factor of risks of non-communicable chronic diseases [26].

There were no differences in food consumption among animal groups which allow us to assume that differences in the parameters evaluated in this study can be attributed to the intake level of bioactive compounds, and not the quantity of diet consumed.

Composition parameters that showed greater differences in content between two flaxseed varieties were dietary fibers, vitamin E, total phenolics and antioxidant capacity, which were higher for the BF. However BF had no a better hypocholesterolemic effect than GF, which suggests that such differences in chemical composition were no biologically significant in relation to the positive effects promoted by the ingestion of flaxseed.

Effects of flaxseed intake on the reduction of TC/HDL in this study can be

attributed to the action of α-linolenic acid [27], fibers [28] or the lignans found in flaxseed [29]. The hypocholesterolemic mechanisms of ALA are still controversial, although they have been observed in studies with animals [9]. The isolated action of soluble fibers present in flaxseed may diminish absorption of cholesterol and bile acids [30]. Regarding lignans, some studies suggest that SDG are involved in reducing serum cholesterol through the modulation of the enzymes 7-α-hydroxylase and acyl CoA cholesterol transferase, which catalyze reactions in the metabolic pathway of cholesterol [31].

Some differences in protein, fat and carbohydrate can be expected in foods of plant origin, independent of variety, since nutrient content in raw food may be influenced by other factors including climate, agricultural and maturation [32]. Nevertheless, the similarity in ALA levels for BF and GF contradicts the common believe that the content of this fatty acid is higher in the golden flaxseed; thus, not ensuring greater cardioprotective effects by one variety in relation to the other one.

This study showed a direct association between TP and AC, in agreement with studies of other foods [33-34]. However, despite the BF presenting greater antioxidant content and higher antioxidant capacity in the in vitro tests, the effects of both flours on lipid peroxidation in serum and tissue homogenates were similar. This demonstrates that significant differences in food chemical composition not always result in biological effects statistically different, maybe due to different levels of antioxidant enzymes in animals. Ingestion of flaxseed decreased lipid peroxidation in some tissues and did not alter this parameter in other tissues which, indirectly, show us that there are differences in the bioaccessibility and bioefficacy of bioactive compounds of flaxseed among the different rat tissues.

Phytic acid, phenolic compounds and vitamin E could affect the antioxidant capacity of the samples.

The presence of phytic acid in the seed needs to be discussed under two approaches. Phytic acid can be an anti-nutritional factor and a bioactive molecule, depending on its chemical form. The forms IP-2 (inositol bisphosphate) and IP-3 (inositol triphosphate) are considered beneficial because they can prevent the formation of kidney stones and can act as antioxidants in biological environments, while inositols IP-5 (inositol pentaphosphate) and IP-6 (inositol hexaphosphate) act as antinutritional factors [35]. In the present study the forms of inositol were not analyzed, but in previous analyses in our laboratories with brown flaxseeds, it was found that phytic acid is in forms IP-5 and IP-6 [36], and therefore does not influence the antioxidant potential of the seed, but the bioavailability of nutrients, mainly at the level of mineral absorption.

Seeds in general are rich in phenolic compounds [37] and their inclusion in the diet is a useful strategy to increase the supply of this class of compounds. As seen in both seeds, differences in levels of phenolic compounds between varieties of other plants have been reported, as are secondary compounds involved in defense mechanisms [38]. The difference in content of phenolic compounds between the two flours may be the factor responsible for the greater antioxidant potential of the brown seed, as shown

in studies with other foods, which this is a direct association between TP and AC [33-34]. The antioxidant activity of lignans may occur due to inactivation of fatty acid free radicals and reactive oxygen species, as well as indirectly in the endogenous antioxidant systems [39].

Although the vitamin E content was also greater in brown variety and exerts antioxidant activity [40], this was probably not the factor that influenced the antioxidant capacity, since the analyzed extracts were aqueous. The main isomer in both varieties was the γ-tocopherol, what is the most effective among the isomers in preventing oxidation of LDL [41], although there are affirmations that between eight different molecules belonging to the vitamin E family, the α-tocopherol isomer showed the highest ferric reducing antioxidant power [42].

5. CONCLUSION

The results suggest that BF has a greater antioxidant potential and content of dietary fibers, phenolic compounds and vitamin E, however, it may not be associated with a more intense hypocholesterolemic and antioxidant response in *Wistar* rats, since both varieties showed the same effect on serum lipid profile and lipid peroxidation in tissues.

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TABLES AND FIGURE CAPTIONS

Table 1: Lipid peroxidation in serum and tissue homogenates of rats fed the basal diet (control) and supplemented with brown (BF) or golden flaxseed (GF) fours

	MDA Equivalents				
	Liver*	Kidney*	Testis*	Lung*	Serum**
Control	0.70^{a}±0.45	0.76^{a}±0.33	0.23^{a}±0.07	1.80^{a}±0.54	2.02^{a}±0.88
BF	0.74^{a}±0.44	0.88^{a}±0.45	0.17^{b}±0.03	1.13^{b} ±0.28	0.76^{b} ±0.32
GF	0.98^{a}±0.60	0.63^{a}±0.12	0.17^{b} ±0.04	1.24^{b} ±1.07	0.83^{b} ±0.45

Means with different letters in lines present statistically significant differences (0.005). * data expressed in nmol/g protein. ** data expressed in nmol/mL of serum.

Table 2: Chemical composition (g/100g) and antioxidant capacity of the brown and golden flaxseed flours

Chemical composition	***BF***	***GF***
Moisture	7.29[a]±0,04	7.47[a]±0,05
Carbohydrates[#]	4.33	10.57
Proteins	24.18[a]±0,85	20.59[b]±1,19
Ash	3.28[a]±0,01	2.99[a]±0,0
Lipids	41.34[a]±0,3	39.95[a]±0,17
Dietary fibers	19,61[a]±0,26	18,45[b]±0,42
Calories[#]	486.1	484.15
Fatty acid content		
Octadecenoic Acid	3.23[a]±0.2	3.52[a]±0.53
Octadecanoic Acid	1.52[a]±0.04	1.39[b]±0.05
Hexadecanoic Acid	1.64[a]±0.05	1.40[b] ±0.09
Octadecadienoic Acid	3.77[a]±0.31	3.9[a]±0.61
Octadecatrienoic Acid (ALA)	7.5[a]±0.8	7.18[a]±1.11
Content of other biactive compounds		
α-Tocopherol*	96.07[a]±8.8	112.23±5.25
γ-Tocopherol*	8340.65[a]±268.77	5541.78[b]±491.02
δ-Tocopherol*	179.80[a]±8.86	105.65 [b] ±37.64
Total Vit E *	8616.52[a]±254.56	5746.73 [b] ±89.84
Phytic Acid **	202,78[a]±85,22	168,45[a]±40,72
Total Phenolic Compounds***	537.23 ± 34.06[a]	445.29 ± 16.7[b]
Secoisolariciresinol	614.78 ± 23.49[a]	449.49 ± 22.68[b]
Antioxidant Assays		
RSA%	29.71 ± 3.09[a]	20.85 ± 1.1[b]
RP (Abs)	0.28 ± 0.01[a]	0.22± 0.02[b]

[#] Carbohydrates and calories were not statistical analysis since they were calculated by subtracting the total (100g) by moisture, protein, fat, ash and fiber; and by multiplying the carbohydrate and protein content by 4 kcal and lipids by 9 kcal , respectively.
* µg/100g
** mg/100g
*** mg of gallic acid equivalent (GAE)/100g
RSA = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity
RP = Reducing Power in absorbance
Means with different letters in lines present statistical difference at the level of 5% (t-test).

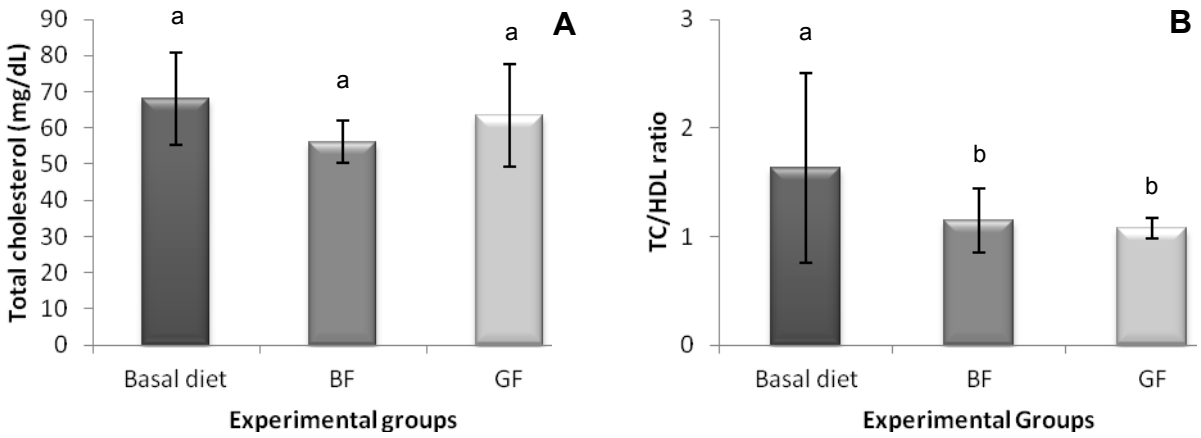

Fig 1: Total cholesterol (A) and TC/HDL (B) ratio in serum of animals fed the basal diet (control) and supplemented with brown (BF) or golden flaxseed (GF)

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