MARINA LEOPOLDINA LAMOUNIER CAMPIDELLI

CARACTERIZAÇÃO QUÍMICA E AVALIAÇÃO BIOLÓGICA DE AMÊNDOA E PASTA ALIMENTÍCIA DE BARU (*Dipteryx alata* VOG.) EM RATOS SUBMETIDOS À DIETA HIPERLIPÍDICA

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação, em Ciência dos Alimentos, para obtenção do título de Doutor.

Prof. Dr. João de Deus de Souza Carneiro
Orientador

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CHEMICAL CHARACTERIZATION AND BIOLOGICAL EVALUATION OF ALMOND AND BARU FOOD (*Dipteryx alata* VOG.) IN RATS SUBMITTED TO THE HYPERLIPID DIET

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, para a obtenção do título de Doutor.

APROVADA em 23 de junho de 2020.

Prof. Dr. Luciano José Pereira	UFLA
Dr. Rafael Ricon de Oliveira	UFLA
Profa. Dra. Maressa Caldeira Morzelle	UFMT

Prof. Dr. João de Deus de Souza Carneiro
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LAVRAS – MG
2020

“À você minha filha Antonella, meu bem mais precioso,

que, ao nascer, me fez renascer”.

DEDICO ❤

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RESUMO GERAL

A amêndoa de baru (*Dipteryx alata* Vog.) é uma oleaginosa nativa do Cerrado brasileiro que apresenta elevados teores de compostos químicos e nutricionais possibilitando a redução do desenvolvimento de doenças crônicas degenerativas não transmissíveis. O presente estudo teve como objetivos (i) determinar os parâmetros de qualidade e a composição físico-química, química, mineral e bioativa da amêndoa de baru submetida à diferentes processos de secagens; (ii) desenvolver uma pasta alimentícia adicionada de diferentes teores de amêndoa de baru e avaliar a composição centesimal, mineral, sensorial e microbiológica, bem como os parâmetros químicos e bioativos; e (iii) investigar o efeito do consumo de dietas hiperlipídicas adicionadas de amêndoa de baru e de pasta alimentícia de amêndoa de baru na atividade metabólica, antioxidativa e anti-inflamatória de ratos Wistar. Dentre os principais resultados, foi demonstrado que os processos de secagens à 65 °C e à 105 °C por 30 minutos aumentaram os níveis de acidez total, ácido gálico, rutina, catequina, ácido trans-cinâmico, vanilina, ácido *m*-cumárico, tocoferóis, ácidos graxos monoinsaturados e atividade antioxidante (DPPH•). No entanto, reduziram os teores de ácido cafeico, ácido clorogênico, antocianinas, ácido *p*-cumárico, ácido ferúlico, ácido *o*-cumárico, quercetina e ácidos graxos poliinsaturados. Além disso, ambos processos não alteraram a presença de compostos fenólicos, taninos, antioxidantes (medido pelo sistema beta caroteno/ácido linoleico) e esteróis totais, além da composição físico-química (exceto umidade), mineral e parâmetros de qualidade (exceto acidez total).Verificou-se, que a adição de 35% de amêndoa de baru na pasta alimentícia foi capaz de aumentar os teores de umidade, lipídeos, cálcio, valor energético total, vitamina C, antioxidantes (DPPH•), ácido gálico, cálcio, magnésio, enxofre, manganês e ácido oleico presentes. Em relação a avaliação sensorial não foi detectada diferença estatística entre as formulações para sabor, impressão global (recebendo o escore médio hedônico de gostei moderamente) e intenção de compra (com escore médio hedônico de provavelmente compraria). Referente ao experimento *in vivo*, foi evidenciado que o consumo das dietas hiperlipídicas adicionadas de amêndoa de baru e pasta alimentícia de amêndoa de baru foram responsáveis pela modulação metabólica, pois à níveis séricos, reduziram colesterol total, lipoproteína de baixa densidade e Índices de Castelli, além de aumentarem lipoproteína de alta densidade e não alteraram as enzimas hepáticas (exceto alanina aminotransferase). As dietas também desempenharam efeito antioxidante pois foram observados no fígado aumento nos teores do Poder Redutor do Ferro (FRAP), na atividade enzimática da glutationa redutase e na expressão gênica de *Manganese Superoxide Dismutase*, além da redução dos níveis circulantes de Malonaldeído. No entanto, não promoveram efeito anti-inflamatório, visto que não houve modulação dos genes *Interleucina-6* e *Ciclooxigenase-2*. Como conclusão geral, observou-se que a adição de amêndoa de baru ou pasta alimentícia elaborada com amêndoa de baru nas dietas hiperlipídicas, foram capazes de desencadear uma modulação metabólica e antioxidante em ratos Wistar. Dessa forma, a inclusão da amêndoa de baru e novos produtos elaborados a partir dela na dieta pode ser uma alternativa eficaz na manutenção do estado de saúde e na possível minimização de doenças crônicas degenerativas não transmissíveis.

Palavras-chave: Novos produtos. Compostos bioativos. Estresse oxidativo. Expressão gênica.

GENERAL ABSTRACT

Baru almond (*Dipteryx alata* Vog.) is an oilseed native to the Brazilian Cerrado that has high levels of chemical and nutritional compounds, enabling the reduction of the development of non-transmissible chronic degenerative diseases. The present study had as objectives (i) to determine the quality parameters and the physicochemical, chemical, mineral and bioactive composition of baru almond submitted to different drying processes; (ii) develop a food paste with different baru almond contents and evaluate the chemical, mineral, sensory and microbiological composition, as well as the chemical and bioactive parameters; and (iii) to investigate the effect of consumption of high fat diets with baru almond and baru almond paste on the metabolic, antioxidative and inflammatory of Wistar rats. Among the main results, it was demonstrated that the drying processes at 65 °C and 105 °C for 30 minutes increased the levels of total acidity, gallic acid, rutin, catechin, trans-cinnamic acid, vanillin, *m*-cumharic acid, tocopherols, monounsaturated fatty acids and antioxidant activity (DPPH•). However, they reduced the levels of caffeic acid, chlorogenic acid, anthocyanins, *p*-cumaric acid, ferulic acid, *o*-cumaric acid, quercetin and polyunsaturated fatty acids. In addition, both processes did not alter the presence of phenolic compounds, tannins, antioxidants (as measured by the beta carotene/linoleic acid system) and total sterols, in addition to the physical-chemical composition (except moisture), mineral and quality parameters (except total acidity). It was found that the addition of 35% baru almond in the food paste was able to increase the contents of moisture, lipids, calcium, total energy value, vitamin C, antioxidants (DPPH•), gallic acid, calcium, magnesium, sulfur, manganese and oleic acid present. Regarding the sensory evaluation, no statistical difference was detected between the formulations for flavor, global impression (receiving the average hedonic score of liked moderately) and purchase intention (with average hedonic score of probably buying). Regarding the *in vivo* experiment, it was evidenced that the consumption of hyperlipidic diets added with baru almond and baru almond food paste were responsible for the metabolic modulation, since at serum levels, they reduced total cholesterol, low density lipoprotein and Castelli Indexes, in addition to increasing high-density lipoprotein and did not alter liver enzymes (except alanine aminotransferase). Diets also had an antioxidant effect, as increased levels of Iron Reducing Power (FRAP), enzymatic activity of glutathione reductase and gene expression of *Manganese Superoxide Dismutase* were observed in the liver, as well as a reduction in circulating levels of Malonaldehyde. However, they did not promote an anti-inflammatory effect, since there was no modulation of the *Interleukin-6* and *Cyclooxygenase-2* genes. As a general conclusion, it was observed that the addition of baru almond or food paste made with baru almond in hyperlipidic diets, were able to trigger a metabolic and antioxidant modulation in Wistar rats. Thus, the inclusion of baru almond and new products made from it in the diet can be an effective alternative in maintaining health status and in the possible minimization of chronic non-transmissible degenerative diseases.

Keywords: New products. Bioactive compounds. Oxidative stress. Gene expression.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Os hábitos alimentares da sociedade ocidental moderna configuram-se como um problema de saúde pública e, em decorrência do desbalanceamento nutricional, é crescente a incidência de obesidade. Esta, por sua vez, está associada a alterações imunometabólicas oxidativas, e neurológicas e, tais como dislipidemias, diabetes *mellitus*, doenças neurodegenerativas, dentre outras. Essas enfermidades prevalecem, na população mundial, causando o óbito de 41 milhões de pessoas a cada ano, o equivalente a 71% de todas as mortes no mundo (WHO, 2018).

O alto consumo de gordura saturada também contribui para esse cenário, visto que a sua ingestão em excesso é capaz de promover um desequilíbrio entre a produção de radicais livres e a efetividade do sistema antioxidante endógeno (SAHA *et al*., 2017). Como consequência, há um desequilíbrio homeostático, implicando na oxidação de biomoléculas com perdas de suas funções biológicas. Dentre os principais resultados desse processo, ressalta-se a apoptose celular, responsável pelo desenvolvimento de doenças crônicas degenerativas não transmissíveis (DCDNT) (CUNHA *et al*., 2017a; KATERJI; FILIPPOVA; DUERKSEN-HUGHES, 2019; SOARES *et al*., 2015b; VIAL *et al*., 2011).

Medidas alternativas para o controle dessas alterações biológicas, por meio do uso de substâncias biologicamente ativas, têm gerado resultados promissores e estudos comprovam a relação entre o consumo diário de frutos do cerrado (mais especificamente as sementes oleaginosas) e a redução da incidência de DCDNT. Isso se deve, em grande parte, à capacidade dos componentes fitoquímicos presentes nesses frutos de modularem os biomarcadores envolvidos na patogênese dessas enfermidades (FRAGUAS *et al*., 2014; LEMOS *et al*., 2016).

Entre os frutos do cerrado, destaca-se a amêndoa de baru (*Dipteryx alata* Vog.), que apesar das informações disponíveis serem limitadas, principalmente às funções *in vivo*, contém compostos químicos e bioativos permitindo com que essa oleaginosa se destaque frente às demais (FRAGUAS *et al*., 2014; LEMOS *et al*., 2016). Contém compostos químicos e bioativos, tais como, ácidos graxos monoinsaturados (oleico), minerais (boro, zinco, cobre, manganês e magnésio), moléculas antioxidantes, polifenóis (catequina, rutina e ácidos gálicos, caféicos, clorogênicos, *o*-cumáricos e trans-cinâmicos) e vitaminas (C e E – tocoferois alfa e gama) (CAMPIDELLI *et al*., 2019, 2020; FARIA *et al*., 2015; LEMOS,

2012; SANO; RIBEIRO; BRITO, 2014). Além disso, possui altos níveis de lipídeos, proteínas, aminoácidos, eficiente digestibilidade, alto teor de minerais (tais como cálcio, ferro, magnésio, potássio e zinco) e fibras alimentares (em média 15%) (CAMPIDELLI *et al*., 2019; FRAGUAS *et al*., 2014). Em sinergismo, essas substâncias proporcionam alto potencial na inibição da oxidação, podendo desempenhar efeitos positivos na prevenção de doenças ligadas ao estresse oxidativo (CORY *et al*., 2018; PRASAD *et al*., 2011; ROS, 2015).

Apesar dos inúmeros benefícios da amêndoa de baru *in natura*, é elevado o número de consumidores que optam por produtos prontos para o consumo. Diante disso, a inclusão dessa oleaginosa em subprodutos na dieta pode atender à atual demanda dos consumidores que desejam produtos práticos e com eficácia na saúde. Logo, explorar o potencial funcional dessa amêndoa, por meio de novas formas de consumo, torna-se uma alternativa viável. No entanto, são escassos os trabalhos que comprovam a ação dos compostos bioativos da amêndoa de baru e de seus produtos no organismo vivo, sendo a realização de estudos biológicos uma alternativa viável e eficaz para uma melhor compreensão da sua ação endógena.

Apesar dos benefícios à saúde que essa amêndoa proporciona, uma prática recorrente entre os produtores dessa oleaginosa, é a utilização de altas temperaturas visando à inativação dos constituíntes antinutricionais, a melhora dos atributos sensoriais e o aumento de sua vida útil. Como consequência desse processo, pode haver alteração e destruição de seus compostos bioativos, dificultando a efetividade da sua atividade funcional. Além disso, há uma carência de trabalhos científicos que avaliem o impacto que as diferentes temperaturas provocam em suas propriedades químicas. Logo, faz-se necessária a compreensão do processo de secagem mais eficiente na manutenção das propriedades funcionais da amêndoa de baru.

Dessa forma, analisando a possibilidade de um uso eficiente da amêndoa baru, por meio de sua incorporação em novos produtos, aliado ao desejo de identificar e comprovar novas propriedades funcionais e biológicas eficazes na saúde, neste trabalho, objetivou-se:

(i) Estudar a composição físico-química, química, mineral e bioativa da amêndoa de baru submetida à diferentes processos de secagem (Artigo 1 e 2 da Tese: Publicados nas revistas *Journal of Culinary Science & Technology* e *Graxas y Aceites*, respecivamente);

(ii) Desenvolver uma pasta alimentícia adicionada de diferentes teores de amêndoa de baru e avaliar a composição centesimal, mineral, sensorial e microbiológica, bem como os parâmetros químicos e bioativos (Artigo 3 e 4 da Tese: Publicados nas revistas *Research, Society and Development* e *Graxas y Aceites*, respectivamente);

(iii) Investigar o efeito do consumo de dietas hiperlipídicas adicionadas de amêndoa de baru e pasta alimentícia na atividade metabólica, antioxidativa e anti-inflamatória de ratos Wistar (Artigo 5 da Tese: Submetido na revista *Journal of Nutritional Biochemistry*).

2 REFERENCIAL TEÓRICO

2.1 Radicais livres e o estresse oxidativo

Radical livre pode ser considerado uma molécula ou um átomo que possui número ímpar de elétrons em sua última camada eletrônica, tendo em vista que, na estrutura normal, esses elétrons se encontram em pares. A presença de elétrons não pareados aumenta a reatividade química desses compostos, conferindo-lhes instabilidade. Isso ocorre, em decorrência de sua tendência para capturar (oxidar) ou ceder (reduzir) elétrons das células à sua volta. Embora todos os compostos reativos participem das reações de oxirredução, não são todos que possuem elétrons não-pareados, na última camada, dessa forma, os radicais livres (RLs) devem ser chamados de espécies reativas de oxigênio (EROs) e espécies reativas de nitrogênio (ERNs) (MIROŃCZUK-CHODAKOWSKA; WITKOWSKA; ZUJKO, 2018; VIZZOTTO, 2017; WEIDINGER; KOZLOV, 2015).

A formação dos RLs é um processo fisiológico e contínuo, ocorrendo no citoplasma, nas membranas celulares e nas mitocôndrias (que são as principais geradoras dos RLs). Esses radicais podem desempenhar benefícios ao organismo, exercendo funções metabólicas relevantes, no entanto, quando estão em quantidades elevadas, também são responsáveis pela geração de substâncias deletérias, tornando-se prejudiciais às células (BARBOSA *et al*., 2010; NASCIMENTO, 2016; VIZZOTTO, 2017).

Dentre os principais responsáveis pela formação dos RLs, estão os fatores endógenos e exógenos, sendo a produção de energia (respiração celular), fagocitose, regulação do crescimento, defesa celular, imunidade, síntese de substâncias biológicas e transporte de glicose nas células, exemplos das principais fontes endógenas. Alguns fatores externos como poluição, radiação solar, estresse, maus hábitos alimentares, consumo de antibióticos, tabaco e álcool, são descritos como os principais fatores exógenos responsáveis pela formação dos RLs (MIROŃCZUK-CHODAKOWSKA; WITKOWSKA; ZUJKO, 2018; VIZZOTTO, 2017).

Independente da fonte de obtenção desses agentes químicos, é necessário que exista um equilíbrio entre a produção (pelos agentes oxidantes) e a eliminação (pelo sistema de defesa antioxidante). Quando há produção em concentrações maiores que o organismo consegue metabolizar e eliminar, é instalada uma situação denominada estresse oxidativo. Tal processo conduz à oxidação de biomoléculas com consequente perda de suas funções biológicas e/ou desequilíbrio homeostático, cuja manifestação é o dano oxidativo potencial

contra células e tecidos (BARBOSA *et al*., 2008). Dentre as principais consequências do estresse oxidativo no organismo, cita-se:

a) **Proliferação celular** - algumas células podem responder ao estresse oxidativo por meio do aumento da taxa de divisão celular;
b) **Adaptação das células** - aumento das defesas celulares, como catalase, superóxido dismutase e glutationa, deixando a célula totalmente, parcialmente ou superprotegida (a célula estará mais resistente frente a futuros insultos oxidativos mais intensos). Além disto, os alvos de dano oxidativo podem ser redirecionados, ou ainda, a produção basal de ERO pode ser reduzida;
c) **Dano celular** – os RLs podem desencadear dano a um ou mais tipos de biomoléculas, como lipídios, proteínas, DNA, carboidratos, etc. Em casos de dano menor, a célula pode sobreviver com algum dano oxidativo persistente e irreparável, ou ainda promover o seu reparo;
d) **Senescência** - sobrevivência da célula, mas com o sistema de divisão celular comprometido;
e) **Morte celular** - após o dano na estrutura da célula pode haver o desencadeamento do processo de morte celular. Danos oxidativos ao DNA, mitocôndria, ou em outros alvos celulares, podem causar morte celular por apoptose ou por necrose.

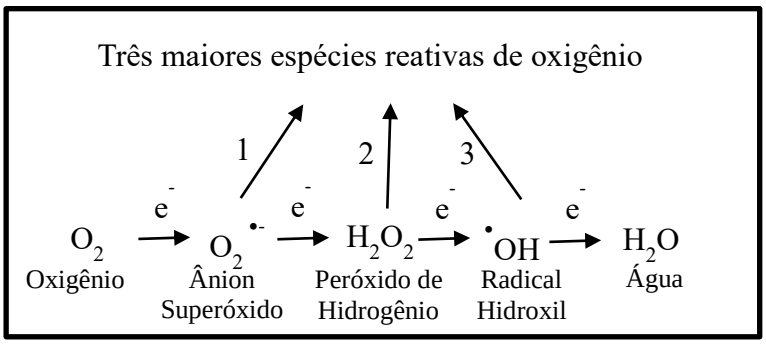
A cronicidade do processo em questão apresenta relevantes implicações sobre o processo etiológico de DCDNT, dentre as quais cita-se diabetes, obesidade, aterosclerose, transtornos neurodegenerativos e câncer (BARBOSA *et al*., 2010; HALLIWELL; GUTTERIDGE, 2007; MIROŃCZUK-CHODAKOWSKA; WITKOWSKA; ZUJKO, 2018; RESENDE, 2010; TREVISAN, 2008; VIZZOTTO, 2017).

2.1.1 Mecanismo de geração dos radicais livres

É de conhecimento que mais de 95% de todo o oxigênio consumido durante o processo de obtenção de energia é reduzido à agua, por meio da adição de 4 elétrons em reação catalisada pela citocromo oxidase (FIGURA 1). No entanto, cerca de 5% do oxigênio formarão espécies radicalares de oxigênio com os elétrons que escapam da cadeia de transporte de elétrons. Essa situação de vazamento de elétrons tem maior ocorrência quando há um aumento desproporcional no consumo mitocondrial de oxigênio (ANTUNES-NETO;

SILVA; MACEDO, 2005). Dentre esses radicais formados, citam-se o radical ânion superóxido ($O_2^{\bullet-}$), o peróxido de hidrogênio (H_2O_2) (que por não possuir um elétron desemparelhado na sua última camada eletrônica é considerado um aradical) e o radical hidroxil ($OH^{\bullet}$) (BARBOSA *et al*., 2010; SOUSA, 2013).

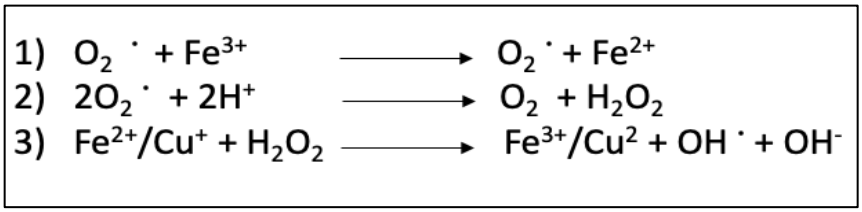
Figura 1 - Processo simplificado de formação de espécies reativas de oxigênio por meio da adição de 4 elétrons e 4 íons de hidrogênio, formando água e liberando energia.



Fonte: Adaptado de Scheibmeir *et al*. (2005).

Na reação de Haber-Weiss, demonstra-se a continuidade do processo de formação de EROs através do radical $O_2^{\bullet}$ (FIGURA 2):

Figura 2 - Formação de espécies reativas a partir do radical ânion superóxido.



Fonte: Antunes-Neto, Silva e Macedo (2005).

Quando apenas um elétron se adiciona à molécula de oxigênio, observa-se a produção de $O_2^{\bullet}$ (FIGURA 1; *reação 1*). A superóxidodismutase (SOD), uma enzima do sistema de defesa antioxidante, possibilita que duas moléculas de $O_2^{\bullet}$ se dismutem para a formação de H_2O_2, considerado um agente oxidante fraco (FIGURA 1; reação 2). Contudo, H_2O_2 tem a propriedade de atravessar membranas celulares e se unir com um elétron proveniente de metais de transição, Fe^{2+} ou Cu^{+}, fator que pode dar origem ao $OH^{\bullet}$ (FIGURA 1; reação 3). Esse radical é considerado o mais reativo e deletério em sistemas biológicos e a sua reação resultará no dano celular, por meio da oxidação de macromoléculas, como proteínas e lipídios. Como consequência, haverá a geração de outras espécies reativas (os chamados

produtos de oxidação) ampliando o dano celular, por meio de uma reação em cascata (ANTUNES-NETO; SILVA; MACEDO, 2005; MIROŃCZUK-CHODAKOWSKA; WITKOWSKA; ZUJKO, 2018; RESENDE, 2010; SOUSA, 2013; TELES *et al*., 2015; VIZZOTTO, 2017).

As enzimas NADPH oxidases, xantina oxidase, mieloperoxidases (usam cloro como substrato e H_2O_2 como cossubstrato para gerar espécies reativas) e óxido nítrico sintase (catalisa a oxidação de L-arginina à L-citrulina e óxido nítrico) são outra importante fonte geradora de radicais livres (BARBOSA *et al*., 2010; BERNARD; KRAUSE, 2007; SOUSA, 2013). A peroxidação lipídica (descrita no item 2.3) também é uma potente geradora de RLs e é provavelmente o evento citotóxico primário responsável por desencadear uma sequência de lesões celulares.

2.1.2 Principais espécies reativas de oxigênio e nitrogênio

As principais EROs e ERNs distribuem-se em dois grupos, os radicalares: hidroxila ($HO^{\cdot}$), $O2^{\cdot-}$, peroxila ($ROO^{\cdot}$) e alcoxila ($RO^{\cdot}$); e os não-radicalares: oxigênio (O_2), H_2O_2 e ácido hipocloroso (HClO). Abaixo, seguem informações sobre algumas dessas espécies:

a) Superóxido ($O_2^{\bullet-}$)

A respiração é a primeira fonte de ânion superóxido ($O_2^{-\bullet}$) que, em razão de meia- vida curta, é considerado não reativo em comparação aos outros RLs. O malefício principal da produção do $O_2^{\bullet-}$ é a capacidade de reação com H_2O_2 com consequente formação do radical $OH^{\bullet}$. As mitocôndrias são importantes fontes de $O_2^{\bullet-}$, no entanto, são ricas em superóxido dismutase (SOD), enzima esta que catalisa a dismutação de duas moléculas de $O_2^{\bullet-}$ em O_2 e H_2O_2. Este último, quando não eliminado do organismo pelas enzimas peroxidases e catalase, pode gerar radicais $OH^{\bullet}$ (como citado anteriormente). Como resultado, esse composto poderá danificar os componentes celulares essenciais, iniciar peroxidação lipídica e danificar o DNA (BARREIROS; DAVID; DAVID, 2006; RESENDE, 2010; VIZZOTTO, 2017).

b) Peróxido de hidrogênio (H_2O_2)

O H_2O_2 é considerado um não radical, por não possuir um elétron desemparelhado na sua última camada eletrônica. No entanto, por participar da geração da $OH^{\bullet}$ é uma espécie com alto potencial deletério. Diferentemente dos RLs que possuem meia-vida curta, o H_2O_2 tem vida longa sendo capaz de atravessar as membranas celulares, reagindo com um número

máximo de biomoléculas. O H_2O_2 apresenta a capacidade de alterar qualquer estrutura celular que se encontre próxima, no entanto, isoladamente é praticamente inócuo. Quando na presença de ferro, a sua toxidade pode ser aumentada em dez mil vezes. As principais enzimas eliminadoras de H_2O_2 nas plantas são a catalase (CAT), que está localizada nos peroxissomos/glioxissomos e ascorbato peroxidase (APX), que é primeiramente encontrada no citosol e nos cloroplastos (BARBOSA *et al.*, 2010; SCHNEIDER; OLIVEIRA, 2004; VIZZOTTO, 2017).

c) Radical hidroxila ($OH^{\bullet}$)

O radical hidroxila ($OH^{\bullet}$) é considerado o mais reativo oxidante na célula, pois, em razão de sua meia vida muito curta, dificilmente pode ser sequestrado *in vivo* por enzimas antioxidantes. Essa característica faz com que esse radical possa reagir rapidamente com quase todas as biomoléculas, tais como: carboidratos, lipídios, aminoácidos, DNA e ácidos orgânicos. Como resultado de sua atuação, haverá danos às moléculas por adição de insaturações e por abstração de hidrogênio, além do mais, o ataque frequente e intensivo desse radical, pode originar mutações no DNA podendo levar ao desenvolvimento de câncer. Não existe defesa antioxidante enzimática capaz de eliminá-lo, portanto, torna-se necessário inibir a sua formação e/ou reparar os danos causados por ele (BARREIROS; DAVID; DAVID, 2006; RESENDE, 2010).

d) Óxido nítrico ($NO^{\bullet}$)

O óxido nítrico ($NO^{\bullet}$) é um radical livre que possui sete elétrons do nitrogênio e oito do oxigênio, tendo um elétron desemparelhado. A sua produção nas células ocorre por dois processos: enzimáticos, que envolvem a atividade das enzimas óxido nítrico sintetase (NOS), a partir de arginina, oxigênio e NADPH; e não enzimáticos, que em pH ácido, o $NO^{\bullet}$ pode ser gerado a partir do dióxido de nitrogênio (NO_2^-) ou ácido nitroso (HNO^2) (VIZZOTTO, 2017).

Em condições biológicas e, na presença de O_2, o radical $NO^{\bullet}$ é susceptível à oxidação e à redução, podendo ser transformado em outras espécies reativas que compreendem: os radicais $^{\bullet}OH$ (originando o HNO_2); os radicais peroxil (gerando ROONO); os radicais tirosil; e os metais de transição (Fe^{2+}, Cu^{2+} e Zn^{2+}) (VIZZOTTO, 2017).

Em decorrência da toxidade dos radicais acima citados, bem como suas capacidades de provocarem malefícios à saúde, encontrar alternativas que consigam detectar a presença desses compostos, torna-se uma ferramenta necessária ao impedimento do desencadeamento

de danos decorrentes. Com essa finalidade, citam-se as metodologias analíticas que visam a identificar e quantificar o estresse oxidativo.

2.1.3 Avaliação do estresse oxidativo

O estresse oxidativo, como já citado, decorre do desbalanceamento na produção e eliminação dos RLs pelo organismo, sendo o principal responsável pela oxidação de biomoléculas, acarretando em perda das funções biológicas e/ou desequilíbrio homeostático. Quando essa situação já está instalada, haverá maior probabilidade do surgimento de problemas de saúde, como o enfraquecimento do sistema imunológico, envelhecimento e desenvolvimento de DCDNT (BARBOSA *et al*., 2008; FILIPPIN *et al*., 2008; HWANG; KIM, 2007).

A formação das DCDNT, por sua vez, apresenta relevante relação com os processos metabólicos e a utilização de biomarcadores do estresse oxidativo, que podem ser identificados e quantificados, torna-se um importante instrumento na elucidação dessas alterações biológicas (BARBOSA *et al*., 2008). Além do mais, a avaliação do estresse oxidativo pode fornecer uma relação entre o dano oxidativo, as biomoléculas e as patologias e dentre as principais técnicas utilizadas, inclui-se as mensurações de oxidação de DNA, lipídeos e proteínas (FILIPPIN *et al*., 2008; HWANG; KIM, 2007).

Em referência às alterações no DNA, é possível quantificar os níveis de danos oxidativos em uma célula isolada, por meio da técnica de eletroforese em gel. Esse teste, conhecido como ensaio do Cometa ou *Single Cell Gel Assay*, permite a medida dos níveis de estresse oxidativo em pequenos fragmentos de tecido, possibilitando o estudo comparativo entre células normais e alteradas. É um método sensível que é capaz de mensurar danos causados por câncer, radiações biológicas, toxicologia ambiental, dentre outros fatores (FILIPPIN *et al*., 2008; RIBEIRO *et al*., 2007).

A avaliação do produto final da oxidação da guanina por radicais hidroxil, o 8-hidroxidesoxiguanosina (8-OHdG), é outra metodologia realizada para verificar danos no DNA. Ele é um marcador de estresse oxidativo e sua presença no organismo indica oxidação das bases do DNA (FILIPPIN *et al*., 2008). Esse composto é produzido por todas as células do corpo, com intensidade variável, sendo, continuamente, liberado na corrente sanguínea e excretado na urina. Níveis aumentados de 8-OHdG têm sido encontrados em pacientes com diferentes patologias e doenças crônicas degenerativas não transmissíveis, entre as quais inclui-se diabetes, diferentes tipos de neoplasias e o Alzheimer (CUNHA *et al*., 2017b).

O estresse oxidativo também pode ser medido, pelas alterações nas moléculas de lipídeos (situação denominada peroxidação lipídica) e dependendo da via de oxidação e do produto final mensurado, pode ser investigado por diferentes formas. Quanto a isso, são várias as metodologias utilizadas, dentre as quais citam-se as detecções de 4-hidroxinonenal, isoprostanos, acroleína, dienos conjulgados, alaranjado de xileno, malonaldeído, dentre outros (LIMA; ABDALLA, 2001). A quantificação do malonaldeído (que é um produto secundário da peroxidação lipídica) é um dos mais utilizados e quanto a isso, as substâncias reativas ao ácido tiobarbitúrico (TBA) são as responsáveis por avaliar esse produto final. Um teste mais específico à formação do malondialdeído é a cromatografia líquida de alta eficiência (HPLC), na qual os componentes da amostra são separados e somente o malondialdeído é detectado (FILIPPIN *et al*., 2008).

Alterações em bases proteicas provocadas pelo estresse oxidativo também podem ser medidas e a avaliação do conteúdo carbonílico é uma medida amplamente utilizada como marcador de dano oxidativo em proteínas. Essa metodologia apresenta grande importância, pois com o dano oxidativo haverá fragmentação das cadeias e oxidação de quase todos os tipos de aminoácidos com produção freqüente de compostos carbonilados. Há diversas técnicas para se medir a presença de grupo carbonila em proteínas, no entanto, o método mais utilizado é o espectrofotométrico, utilizando-se a reação de ácido 5',5'-ditio-bis-(2-nitrobenzóico) (DTNB) com o grupo carbonila, que forma a hidrazona da proteína (FILIPPIN *et al*., 2008; LEVINE, 2002).

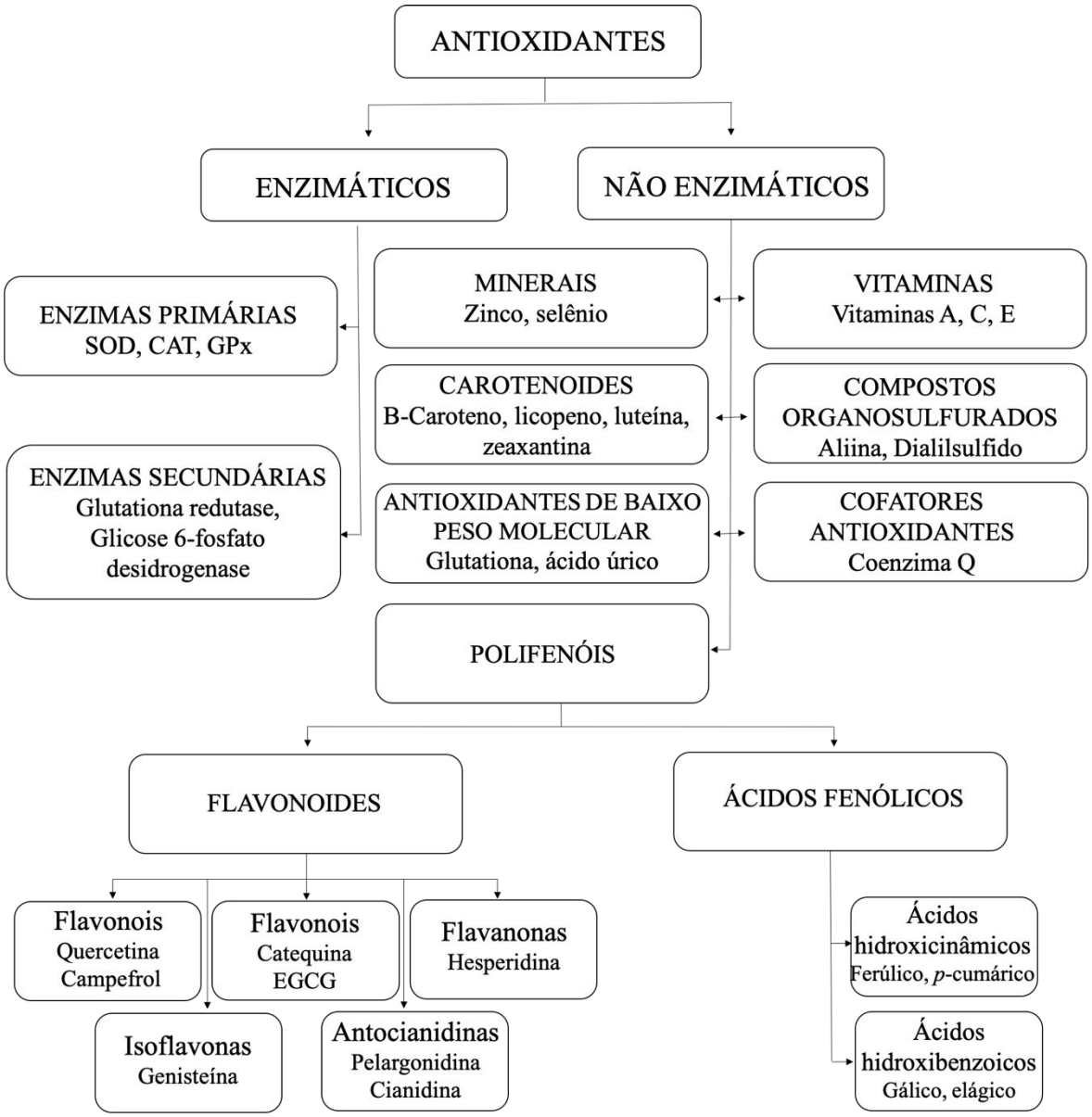
Apesar da relevante importância na detecção do estresse oxidativo em biomoléculas do organismo, já está comprovado que impedir o desenvolvimento dessa situação oxidativa é a maneira mais adequada para contribuir para a manutenção das estruturas biológicas.

2.2 Alternativas para redução do estresse oxidativo

Em razão da toxidade apresentada pelas EROs e ERNs, mecanismos de proteção enzimáticos (endógenos) e não enzimáticos (exógenos) (FIGURA 3) entram em ação para neutralizar os efeitos deletérios desses compostos. Esses mecanismos de proteção, também chamados de sistemas antioxidantes, podem contribuir na minimização desses agentes por diferentes mecanismos, tais como: sistema de prevenção (onde há o impedimento da formação dessas espécies tóxicas); sistemas varredores (por meio do bloqueio da ação desses compostos); e sistema de reparo (decorrente da reconstituição das estruturas biológicas

lesadas – algumas enzimas são responsáveis pela excisão das bases nitrogenadas danificadas e substituição por outras intactas) (BARBOSA *et al*., 2010).

Figura 3 - Classificação dos sistemas antioxidantes em enzimáticos e não enzimáticos.



Fonte: Adaptado de Ratnam *et al*. (2006). Legenda: SOD (superóxidodismutase), CAT (catalase), GPx (glutationa peroxidase), Catequina EGCG (epigalocatequina galato)

No sistema enzimático, encontram-se as enzimas produzidas, durante o metabolismo normal do corpo, capazes de bloquear a iniciação da oxidação, a citar: superóxido dismutase (SOD), glutationa redutase (GR), glutationa peroxidase (GPx) e catalase (CAT).

Já no sistema não enzimático estão presentes compostos capazes de manter o ambiente redutor, com capacidade de interagirem com as espécies radicalares, sendo consumidas

durante as reações. Podem ser incluídos os compostos com propriedade antioxidante, como polifenóis, vitamina E (α-tocoferol), vitamina C (ácido ascórbico), oligoelementos (zinco e selênio) e carotenóides. Pelo fato de o organismo não ser capaz de sintetizar essas substâncias, a alimentação torna-se um fator importante para a sua inserção das mesmas no organismo (ANGELO; JORGE, 2007; RESENDE, 2010).

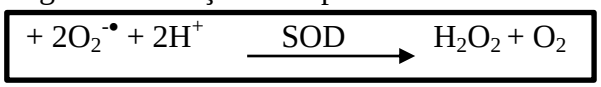
2.2.1 Sistema antioxidante enzimático

O sistema antioxidante enzimático relaciona-se a um grupo de antioxidantes que podem ser produzidos *in vivo*, como é o caso da superóxido dismutase, catalase, glutationa reduzida e glutationa peroxidase.

a) Superóxido Dismutase

A enzima superóxido dismutase (SOD) apresenta reconhecida importância nos sistemas biológicos, por ser a primeira enzima a atuar na defesa do organismo contra as espécies reativas, reduzindo, principalmente, o radical ânion superóxido ($O_2^{-\bullet}$). Isso ocorre, pois é capaz de converter (dismutar) dois $O_2^{-\bullet}$ em uma molécula de H_2O_2 e uma de O_2, no entanto, o H_2O_2 produzido é tóxico para as células podendo desencadear um efeito pró-oxidante. A fim de converter o H_2O_2 em uma molécula de H_2O e assim diminuir a sua reatividade, é essencial a presença de outros compostos antioxidantes, como as enzimas catalase e glutationa peroxidase (FIGURA 4) (ANTUNES-NETO; SILVA; MACEDO, 2005; PEREIRA, 2010; REIS, 2016).

Figura 4 – Reação da superóxido dismutase.



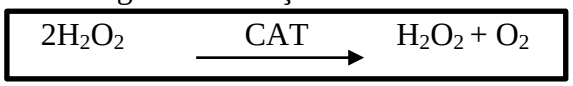
Fonte: Do autor (2019).

A SOD é considerada uma metaloproteína e pode ser classificada em três grupos, de acordo com o componente metálico presente em seu sítio ativo, tais como: cobre/zinco (Cu/Zn-SOD produzido no citosol), manganês (Mn-SOD produzido na mitocôndria) ou ferro (Fe-SOD produzido no retículo endoplasmático das células), sendo que as Cu/Zn-SOD são consideradas as mais abundantes em vegetais (MALLICK; MOHN, 2000; PEREIRA, 2010).

b) Catalase

A catalase (CAT) é uma das enzimas mais abundantes na natureza, encontrando-se, amplamente, distribuída no organismo humano, nas mitocôndrias e nos peroxissomos. Por conter um grupo porfirina de alto peso molecular, sua principal função no organismo é atuar na decomposição do H_2O_2 catalisando a dismutação de dois elétrons em oxigênio e água (FIGURA 5) (MALLICK; MOHN, 2000; PEREIRA, 2010; REIS, 2016).

Figura 5 – Reação da catalase.



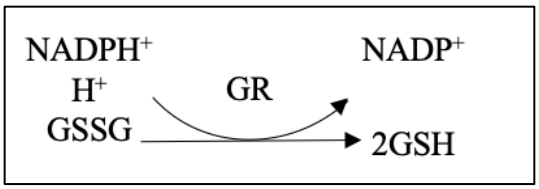
Fonte: Do autor (2019).

Por sua eficácia em reduzir esse composto, foi proposto que a CAT desempenhe um papel fundamental nos sistemas antioxidantes. Os nutrientes mais importantes coadjuvantes da catalase são o ferro e os tocoferóis (vitamina E), que estão distribuídos na membrana celular (na fase hidrofóbica) (MALLICK; MOHN, 2000; PEREIRA, 2010; REIS, 2016).

c) Glutationa

A glutationa é um tripeptídeo (g-L-glutamil-L-cisteinil-glicina) existente no organismo na forma reduzida (GSH) e oxidada (GSSG). Atua em processos biológicos, sendo responsável principalmente pela síntese de proteínas e proteção celular (por suas propriedades antioxidantes) (PEREIRA, 2010; REIS, 2016). É sintetizada nas células, a partir dos aminoácidos e geralmente é mantida na sua forma reduzida (GSH) por meio da enzima glutationa redutase (GR). Esta, por sua vez, catalisa a redução da GSSG para a forma tiol da glutationa (GSH) e as células utilizam a GSH na redução do H_2O_2 e de outros substratos oxidantes num ciclo catalítico acoplado a NADPH, como se vê na Figura 6:

Figura 6 – Reação da glutationa redutase.

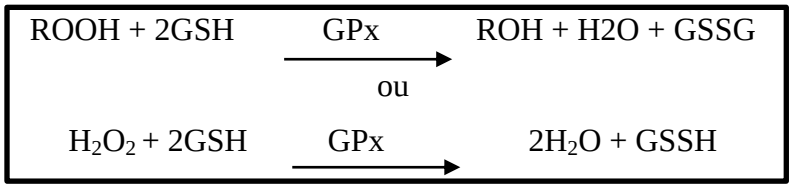


Fonte: Do autor (2019).

É capaz de reduzir outros metabólitos e, principalmente, de neutralizar os agentes oxidantes, sendo considerada, um dos antioxidantes celulares mais importantes (PEREIRA, 2010; REIS, 2016). Além do mais, pode reduzir ligações dissulfetos induzidas pelo estresse oxidativo e manter estáveis os grupos tióis das proteínas (PEREIRA, 2010).

Já a glutationa peroxidase (GPx) pertence a uma família de múltiplas isoenzimas, sendo responsável, principalmente, por reduzir a peroxidação lipídica. Existem, pelo menos, quatro tipos diferentes de GPx, sendo que a GPx 1 é mais abundante e muito eficiente no sequestro de H_2O_2 enquanto a GPx 4 é mais ativa, quando se trata de hidroperóxidos lipídicos (PEREIRA, 2010). A enzima GPx cataliza a redução de hidroperóxidos e de H_2O_2 em H_2O, à custa de glutationa (GSH), ou seja, pode ser responsável pela captação de água, catalisando a peroxidação da GSH, resultando como produto a glutationa na forma dissulfeto oxidada (GSSG) (FIGURA 7) (ANTUNES-NETO; SILVA; MACEDO, 2005).

Figura 7 – Reação da glutationa peroxidase.



Fonte: Do autor (2019).

2.2.2 Sistema antioxidante não enzimático

O sistema antioxidante não enzimático relaciona-se a um grupo de antioxidantes que podem ser obtidos principalmente da dieta, dentre os quais, citam-se compostos fenólicos, vitaminas E e C, carotenoides e oligoelementos (minerais).

a) Compostos fenólicos

Os compostos fenólicos, também chamados de polifenóis, são originados do metabolismo secundário das plantas e referem-se a um amplo grupo de moléculas encontradas em alimentos como frutas, cereais, hortaliças, cafés, chás, vinho, cacau, soja, dentre outras. Já foram contabilizados mais de 8000 tipos de polifenóis na natureza, logo, são os compostos mais abundantes no reino vegetal. Essas substâncias possuem funções específicas como fotoproteção, defesa contra microrganismos e insetos, estabilidade oxidativa, pigmentação, além de contribuir com algumas características sensoriais nos alimentos (CORY *et al*., 2018;

DINIZ, 2015). Maiores informações a respeito dos polifenóis podem ser encontradas no item no 2.5.1.1.

b) Vitamina E

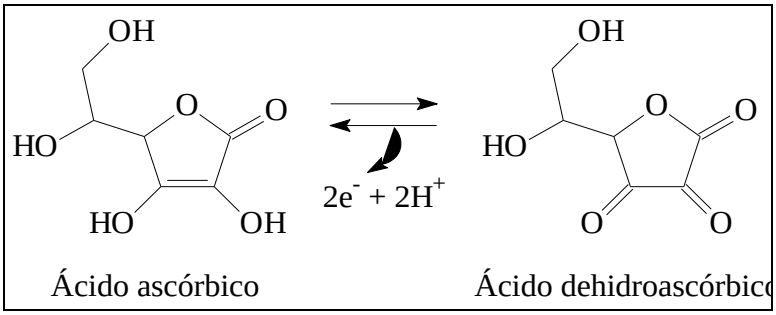
Os tocoferóis estão presentes em várias fontes de óleos vegetais, como milho e trigo, em frutas como coco e em oleaginosas, tais como amêndoas, nozes e algumas espécies de palmeiras. Apresentam-se como quatro compostos homólogos, denominados α, β, γ e δ - tocoferol, no entanto, o α-tocoferol é a forma mais abundante e a mais biologicamente ativa na natureza (COSTA; JORGE, 2011; DINIZ, 2015; LEMOS *et al*., 2016). Maiores informações a respeito dos tocoferóis podem ser encontradas no item no 2.5.1.2

c) Vitamina C

A vitamina C é um nutriente não sintetizado pelo organismo humano, sendo encontrada principalmente em frutas (laranja, limão, tangerina, kiwi, mamão, abacaxi, goiaba, morango e caju) e vegetais folhosos (agrião, espinafres, rúcula, couve-flor e brócolis) (DINIZ, 2015).

Na natureza, encontra-se sob duas formas: a forma reduzida (comumente designada como ácido ascórbico) e a forma oxidada (ácido desidroascórbico) (FIGURA 8). Cerca de 80% dessa vitamina é absorvida no trato gastrointestinal, mas essa taxa pode diminuir quando se aumenta a ingestão. Por ser facilmente excretada pela urina é necessário consumo diário (DINIZ, 2015; VANNUCCHI; ROCHA, 2012).

Figura 8 – Estrutura química do tocoferol e tocotrienol.



Fonte: Do autor (2019), adaptado de Angelo e Jorge (2007).

A vitamina C é considerada um antioxidante primário ou preventivo, pois reage com o oxigênio antes do início do processo oxidativo, protegendo, assim, as membranas lipídicas e as proteínas dos danos oxidativos. No organismo, encontra-se como ascorbato, um agente

redutor capaz de reduzir metais de transição como o ferro e cobre. Dentre as suas principais características, cita-se a sua capacidade de combater os radicais livres encontrados em meio aquoso e a sua aptidão em participar do sistema de regeneração da vitamina E, sendo, portanto, de fundamental importância para manter o potencial antioxidante do plasma (CATANIA; BARROS; FERREIRA, 2009; ROCHA; SARTORI; NAVARRO, 2016; VANNUCCHI; ROCHA, 2012).

Em razão da presença de metais como o Fe2+ e o Cu+, o ácido ascórbico pode atuar *in vivo* como pró-oxidante, por meio da formação dos radicais ascorbato e hidroxila, responsáveis por iniciar a peroxidação lipídica. A quantidade de metais de transição livre *in vivo* é, entretanto, muito baixa, pois normalmente se encontram eficazmente ligados a proteínas de transporte (PODSEDEK, 2007).

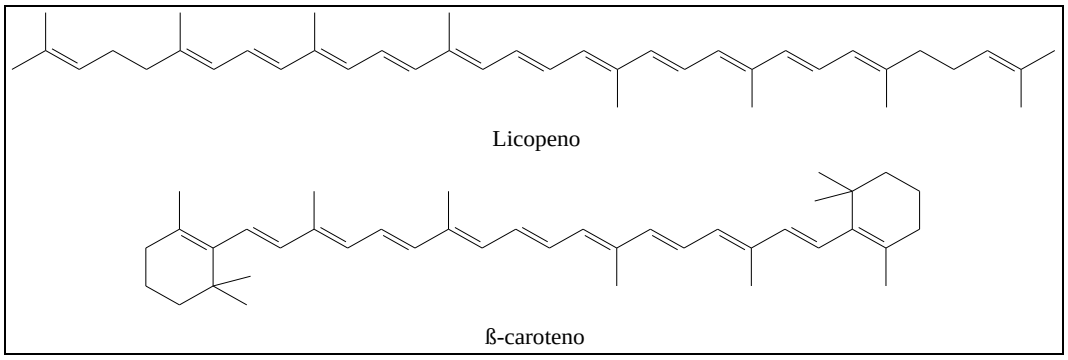
d) Carotenóides

Carotenóides são uma classe de pigmentos sintetizados por plantas, algas e bactérias fotossintetizantes com atividade biológica na saúde. Esses compostos são responsáveis pela coloração amarela, avermelhada ou laranja de frutas e vegetais, crustáceos gema do ovo e alguns peixes. Apesar de terem sido identificados mais de 700 tipos de carotenóides, apenas 50 podem ser absorvidos e metabolizados pelo corpo, estando estes presentes na dieta humana. Desses 50, apenas seis foram identificados no sangue humano, dentre os quais, citam-se β-caroteno, β-criptoxantina, α-caroteno, licopeno, luteína e zeaxantina (DINIZ, 2015; RODRIGUEZ-AMAYA, 2008).

Os carotenóides podem ser classificados em carotenos (que são hidrocarbonetos poliênicos com variados graus de instauração) ou xantofilas (que são sintetizadas a partir dos carotenos, por meio de reações de hidroxilação e epoxidação). O β-caroteno e o licopeno são exemplos de carotenos, enquanto a luteína e a zeaxantina são xantofilas (AMBRÓSIO; CAMPOS; FARO, 2006; COSTA; ORTEGA-FLORES; PENTEADO, 2002).

Os carotenóides podem ser definidos, quimicamente, como tetraterpenóides onde há a união de oito unidades isoprenóides (C5), com cinco átomos de carbono, resultado na formação de uma cadeia carbônica com quarenta átomos de carbono. Alguns carotenoides apresentam um ou dois anéis β-ionona nas extremidades, conferindo a essas moléculas a sua atividade vitamínica (AMBRÓSIO; CAMPOS; FARO, 2006; LIMA, 2003). Quanto maior o número de duplas ligações conjugadas na molécula, maior será a sua eficácia, sendo o licopeno mais eficiente que o β-caroteno (SOUSA, 2013; TAPIERO; TOWNSEND; TEW, 2004) (FIGURA 9).

Figura 9 – Estrutura química do licopeno e Beta-caroteno.



Fonte: Do autor (2019).

Os carotenoides são as substâncias mais eficientes na inativação do oxigênio singlete (além de auxiliar na remoção dos já formados) e também são responsáveis por sequestrarem os radicais peroxila. Os carotenoides podem atuar de duas formas diferentes: via física, que ocorre pela transferência de energia das moléculas de oxigênio singlete aos carotenóides; e via química, que constitui não mais que 0.5% do total da inativação do oxigênio singlete (PODSEDEK, 2007). No entanto, sob elevada pressão de oxigênio e na presença de íons de ferro, os carotenoides podem atuar como agentes pró-oxidantes, sendo necessárias outras moléculas com atividade coantioxidantes (SOUSA, 2013; TAPIERO; TOWNSEND; TEW, 2004).

Os carotenoides são compostos lipofílicos, hidrofóbicos e insolúveis em água e em decorrência da presença das insaturações, os carotenóides são sensíveis à temperatura, luz, acidez, bem como reações de oxidação. No entanto, são as insaturações que proporcionam atividade antioxidante a essas moléculas (principalmente ao β-caroteno) (AMBRÓSIO; CAMPOS; FARO, 2006; RODRIGUEZ-AMAYA, 2008) podendo ocorrer, por meio da adição de radicais à cadeia do carotenoide, pelo isolamento do hidrogênio e/ou pela transferência de elétrons. De modo geral, essas moléculas agem diminuindo a formação do oxigênio singlet e ajudam a remover aqueles já formados (SOUSA, 2013; STAHL; SIES, 1992).

e) Oligoelementos (Minerais)

Os oligoelementos (minerais) não atuam diretamente no sistema antioxidante, mas são necessários à produção de enzimas antioxidantes, essenciais no combate do processo de oxidação. Dentre os principais minerais que atuam como cofatores antioxidantes, citam-se o zinco e o selênio.

Zinco: O zinco é um dos nutrientes mais eficaz no funcionamento dos sistemas antioxidantes e por estar envolvido em diversos processos metabólicos, protege a membrana da ação lesiva das EROs e ERNs. Logo, esse mineral é essencial para a integridade e funcionalidade das membranas celulares (CRUZ; SOARES, 2011). O zinco está presente no citoplasma das células e é o componente estrutural e catalítico da enzima SOD, que, por sua vez, é capaz de reduzir a toxicidade das espécies reativas, transformando-as em espécie menos danosa às células. Além do mais, o zinco é capaz de inibir a NADPH-oxidase, uma enzima que está envolvida na produção de radicais livres. Também participa diretamente da neutralização do radical livre hidroxila e induz a produção de metalotioninas (substâncias que atuam na remoção desse radical) (CRUZ; SOARES, 2011; PRASAD, 2008).

Selênio: O selênio é um micronutriente essencial que, quando incorporado às selenoproteínas, exerce importantes funções no organismo. Dentre essas funções, cita-se a sua participação na defesa antioxidante, no sistema imune e na regulação da tireoidiana. A sua atividade antioxidante ocorre por ser um componente essencial ao funcionamento da glutationa peroxidase (GSH-Px), enzima esta responsável pelo combate do estresse oxidativo. Essa ação ocorre quando a GSH-Px atua sobre hidroperóxidos e liporóxidos, impedindo que esses compostos causem danos a célula. Como resultado, haverá a redução dos compostos tóxicos, tais como H_2O_2 e peróxidos orgânicos, em compostos atóxicos, como água e alcoóis, sendo estes últimos, não reativos às células (AVANZO *et al*., 2001; RAYMAN, 2000).

2.3 Metabolismo dos lipídeos, hipercolesterolemia e lipotoxicidade

Os lipídios são moléculas orgânicas, solúveis em solventes orgânicos e insolúveis em água, formados, principalmente, por ligações carbono-hidrogênio não polares. São considerados essenciais ao funcionamento do corpo humano e encontram-se distribuídos em todos os tecidos, sendo responsáveis por exercerem funções biológicas importantes. Desempenham uma variedade de funções celulares e são a principal forma de armazenamento de energia na maioria dos organismos. Atuam na formação da estrutura básica das membranas celulares, são precursores dos hormônios esteróides, dos ácidos biliares e da vitamina D, proporcionando fluidez das membranas celulares e ativação de enzimas (FALUDI *et al*., 2017; REIS, 2016; SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2007).

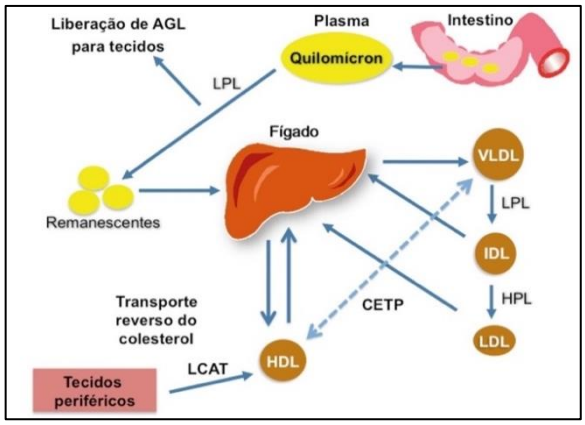
Os lipídeos são representados pelos ácidos graxos (AG), colesterol, triacilgliceróis (TAG) e fosfolipídios e são transportados e solubilizados na forma de complexos macromoleculares conhecidos como lipoproteínas ou apoliproteínas (Apo; pela associação

entre proteínas e lipídeos). Essas moléculas são formadas no fígado, intestino ou no plasma por transformação de lipoproteínas pré-existentes. Possuem um núcleo apolar hidrofóbico de colesterol esterificado e TAG, sendo rodeado por uma camada polar hidrófila de fosfolipídios, colesterol livre, Apo e antioxidantes lipossolúveis (FALUDI *et al*., 2017; REIS, 2016).

Entre as diferentes classificações propostas para as lipoproteínas, aquela, relacionada à sua densidade, tem sido a mais utilizada, dependendo, principalmente, da proporção de lipídeos e de proteínas em sua estrutura (REIS, 2016). Quanto a isso, podem ser separadas em dois grandes grupos: a) as ricas em TGA, maiores e menos densas, representadas pelos quilomícrons, de origem intestinal, e pelas lipoproteínas de densidade muito baixa (VLDL), de origem hepática, e b) as ricas em colesterol, formando partículas de lipoproteínas de baixa densidade (LDL) e lipoproteínas de alta densidade (HDL) (FALUDI *et al*., 2017; SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2007).

As lipoproteínas participam de três ciclos básicos de transporte de lipídeos no plasma denominados ciclo exógeno, endógeno e reverso (FIGURA 10). No ciclo exógeno, as gorduras advindas da dieta e da circulação entero-hepática, são absorvidas no intestino e chegam ao plasma, sob a forma de quilomícrons, após degradação pela enzima lipase lipoproteica (LPL) no fígado ou tecidos periféricos. No ciclo endógeno, as VLDL, ricas em TAG, são hidrolisadas pela enzima lipase lipoproteica originando as lipoproteínas de densidade intermediária (IDL), as quais são rapidamente removidas do plasma. A enzima lipase hepática dá continuidade a esse processo de catabolismo, originando às LDL que possuem o remanescente de TAG e a apo B100 como conteúdo proteico. As partículas de LDL são removidas do plasma pela ação de seus receptores, cuja expressão é um dos principais determinantes da concentração dessa lipoproteína no sangue. No ciclo reverso, as partículas de HDL são formadas no fígado, no intestino e na circulação e seu conteúdo proteico é representado pelas apo A-1 e A-2. Essas partículas são capazes de receber os ésteres de colesterol livre em suas membranas celulares, realizar o transporte reverso de colesterol, remover os lipídios oxidados da LDL, inibir a fixação de moléculas de adesão e monócitos ao endotélio, além de estimular a liberação de óxido nítrico. Esse mecanismo é considerado a base de proteção do organismo contra a deposição de quantidades excessivas de colesterol nos tecidos (FALUDI *et al*., 2017; SOUSA, 2013).

Figura 10 - Ciclos de transporte de lipídeos no plasma.



Fonte: Faludi *et al*. (2017).

Em condições normais, essas vias atuam no controle da síntese e do catabolismo das lipoproteínas, sendo responsáveis, portanto, pela manutenção dos níveis séricos de lipídios, tais como colesterol total e TAG, dentro dos limites desejáveis (REIS, 2016; SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2013). No entanto, em decorrência de fatores genéticos e estilo de vida desbalanceados, caracterizados, principalmente, pela alimentação rica em alimentos industrializados, observa-se que a maior parte do colesterol está ligada ao LDL-c. Ressalta-se que, quando em excesso, essas lipoproteínas são depositadas nas paredes das artérias e a progressividade e acumulação dessas moléculas podem ocasionar uma restrição ou até à obstrução total do fluxo sanguíneo, desenvolvendo acidentes cardiovasculares (FALUDI *et al*., 2017; REIS, 2016). Esse quadro pode ser agravado, especialmente, quando essas moléculas estão na sua forma oxidada, consequência de uma inflamação que se estabelece no organismo. Além do mais, o endotélio (canal onde circula o sangue) possui células sensíveis que podem ser agredidas, por pequenas quantidades de nicotina, açúcares e colesterol (FALUDI *et al*., 2017).

Por outro lado, o HDL-c é responsável por retirar o LDL-c da parede das artérias, transportando-o para ser metabolizado no fígado, sendo então associado aos baixos índices de doenças cardiovasculares. Embora esta seja uma das ações mais conhecidas dessa lipoproteína, é importante enfatizar que as HDL possuem outras ações que também contribuem para a proteção do leito vascular, tais como inibição da fixação de moléculas de adesão e monócitos ao endotélio e estimulação da liberação de óxido nítrico (LEANÇA *et al*., 2010; REIS, 2016).

Quando as lipoproteínas plasmáticas apresentam distúrbios caracterizados por anormalidades quantitativas e ou qualitativas, há a instalação de um processo denominado

hipercolesterolemia (ou dislipidemia). Segundo dados da Sociedade Brasileira de Cardiologia (2007), essas alterações podem se apresentar de quatro maneiras bem descritas. Hipercolesterolemia isolada (níveis séricos elevados de colesterol total), hipertrigliceridemia isolada (níveis séricos elevados de triacilglicerois), hiperlipidemia mista (níveis séricos elevados de colesterol total e de triacilglicerois) e, por último, diminuição isolada de HDL ou associada a um aumento de LDL e/ou triacilglicerois (SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2007).

Embora os efeitos da hipercolesterolemia não sejam percebidos pelo indivíduo, em longo prazo, o excesso de lipídeos, bem como o aumento dos níveis séricos de colesterol total, LDL-c, TAG e AG, assim como as concentrações reduzidas de HDL-c, podem contribuir, significativamente, para o desenvolvimento de efeitos tóxicos no organismo. Esse processo resultará em implicações patológicas em diversos tecidos ou órgãos e pode desencadear uma situação denominada lipoperoxidação (processo também conhecido por lipotoxicidade ou peroxidação dos lipídeos), responsável pelo desenvolvimento de DCDNT (SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2007).

A lipoperoxidação é, provavelmente, o evento citotóxico primário responsável por desencadear uma sequência de lesões celulares. Tal processo inicia-se com a retirada de um átomo de H (hidrogênio) dos ácidos graxos poli-insaturados (presentes na membrana celular dos fosfolipídeos) pelo radical $OH^{\bullet}$, produzindo um radical de lipídeo ($L^{\bullet}$) que, ao reagir com o oxigênio molecular forma o radical peroxila ($LOO^{\bullet}$). Na propagação, o $LOO^{\bullet}$ retira hidrogênio de outro lipídeo, formando o hidroperóxido de lipídeo (LOOH) e $L^{\bullet}$ e, assim. sucessivamente. Os íons de ferro catalisarão essa reação e serão os responsáveis pela conversão de LOOH em radicais $L^{\bullet}$ e $LOO^{\bullet}$ que, por serem potencialmente reativos, iniciam uma nova cadeia de reações representadas pelas etapas de iniciação, propagação e terminação. O término da lipoperoxidação ocorre quando os radicais ($L^{\bullet}$ e $LOO^{\bullet}$) produzidos nas etapas anteriores propagam-se até a eliminação de si mesmos (BARBOSA *et al.*, 2010; BARREIROS; DAVID; DAVID, 2006; HALLIWELL, 2000; LEMOS, 2012; SOUSA, 2013).

Como resultado desse processo, haverá a produção de compostos reativos, tais como: hidrocarbonetos de cadeia curta (etano, pentano), aldeídos (como o malondialdeído), ésteres, cetonas, alcoois e até mesmo hidrocarbonetos provenientes da clivagem de hidroperóxidos. Esses compostos, por sua vez, possuem capacidade de reagir com macromoléculas (ex.: DNA, RNA e proteínas), causando disfunções celulares e mitocondriais, modificação do fluxo de íons (resultando na perda da seletividade para a entrada e/ou saída de nutrientes e substâncias tóxicas à célula), alterações do DNA, oxidação do LDL-c, comprometimento dos

componentes da matriz intra e extracelular, estresse do retículo endoplasmático, acúmulo de ceramidas, exposição aumentada a EROs, lesões potencialmente mutagênicas e morte celular (BARBOSA *et al*., 2010; BARREIROS; DAVID; DAVID, 2006; PÔRTO, 2001; TELES *et al*., 2015). Logo, os efeitos tóxicos da lipotoxicidade podem ser associados aos principais mecanismos que levam ao desenvolvimento de DCDNT.

2.4 Inflamação

A inflamação pode ser definida como a reação do tecido vivo vascularizado em resposta a estímulos endógenos e exógenos e tem por objetivo livrar o organismo da causa inicial da lesão celular e das posteriores consequências. Esse processo pode ser agudo, desencadeado por infecções ou lesão tecidual, ou crônico, observado em doenças autoimunes ou causado por estímulos exógenos que podem levar a um dano tecidual. É um componente importante da resposta imune que atua na proteção contra um determinado tipo de agressão, por meio de mediadores que se tornam ativos, formando um complexo regulatório. Dentre os principais fatores que a desencadeiam, está o ataque oxidativo aos componentes essenciais das células pelas EROs (KATERJI; FILIPPOVA; DUERKSEN-HUGHES, 2019; MARTINS *et al*., 2016; MIROŃCZUK-CHODAKOWSKA; WITKOWSKA; ZUJKO, 2018; SILVA, 2018; VIZZOTTO, 2017).

Quando o processo inflamatório está instalado, haverá a estimulação do sistema de defesa imunológico, com posterior ativação dos fosfolipídeos da membrana. Esses compostos serão clivados e gerarão o ácido araquidônico, que é responsável pela formação dos prostanóides (protaglandinas, prostacilinas e tromboxanas) e leucotrienos. Esses metabólitos, conhecidos como eicosanóides, serão responsáveis pela modulação da resposta inflamatória e desencadearão a produção de neutrófilos e macrófagos. Estes, por sua vez, ao serem ativados, irão liberar os mediadores químicos, tais como: os metabólitos reativos (radicais livres), moléculas de adesão, proteases, quimiocinas, enzimas hidrolíticas e citocinas inflamatórias (BARBALHO *et al*., 2011; BEAVES; BRINKLEY; NICKLOS, 2010; SOARES *et al*., 2015a).

Dentre os principais responsáveis pela formação dos eicosanóides, citam-se as enzimas *ciclooxigenase* (*Cox*) e *lipoxigenase* (*Lox*). Referente a *Cox*, existem dois principais tipos, a *ciclooxigenase-1* (*Cox-1*) e a *cicooxigenase-2* (*Cox-2*), embora, tenha sido identificada uma terceira isoforma, a *ciclooxigenase-3* (*Cox-3*). A *Cox-1* é uma enzima constitutiva, expressa em muitos tecidos e, sob condições fisiológicas, produz PGs necessárias

à modulação das funções gastrintestinais, renais e a homeostase vascular. A *Cox-2*, descrita em 1992, está presente, principalmente, no cérebro e medula espinhal. É induzida, em células inflamatórias, tais como: fibroblastos, macrófagos, monócitos e células sinoviais, quando elas são ativadas. É considerada enzima que produz os mediadores da inflamação da classe dos prostanóides. O gene da *Cox-2* é expresso em resposta a vários agentes pró-inflamatórios, citocinas, endotoxinas, fatores de crescimento e promotores de tumor (PIRES; PANCOTE; TOLEDO, 2017; YAQUB *et al*., 2008).

Após a formação desses elementos serão produzidas substâncias pró-inflamatórias, dentre as quais citam-se: interleucinas (IL) 1, IL-6, IL-12, e IL-18 (produzidas no local da inflamação), fator de necrose tumoral α (TNF-α) e proteína C reativa (PCR). No entanto, para que haja efeito inflamatório e os leucócitos e neutrófilos possam migrar até o sítio, é necessário que os fatores de transcrição (tal como o fator nuclear kappa Beta - NF-KB) e a proteína ativadora (AP) - que atua induzindo a produção de interleucinas pró-inflamatórias -, estejam ativados (BARBALHO *et al*., 2011; BEAVES; BRINKLEY; NICKLOS, 2010; SOARES *et al*., 2015a).

A via do NF-kB pode ser ativada, por meio da ligação de um ligante no receptor de superfície celular como o TNF-R (receptor para o TNF- α) e o do tipo *Toll* (receptor para TLR4). Esses receptores, por sua vez, podem ser ativados tanto por ácidos graxos saturados como por LPS. Após esses estímulos, a IKK-b é ativada e promove a fosforilação do inibidor do kB (IKB)-a, o que favorece a sua poliubiquitinação e subsequente degradação pelo proteassoma 26S, localizado no citosol. Após essa etapa, o NF-kB é liberado e translocado para o núcleo da célula, o que promove a ativação de genes que codificam proteínas envolvidas nas respostas imune e inflamatória (ROGERO; CALDER, 2018; SILVA, 2018).

Dentre as principais consequências envolvendo a ativação de vias de sinalização está a formação da metainflamação, que é a inflamação associada à doença metabólica que pode ser do dano oxidativo a biomoléculas e situações que impedem que danos sistêmicos irreparáveis ocorram, evitando, assim, o desenvolvimento de DCDNT (ROS, 2015).

Dentre as principais medidas preventivas, que busquem impedir a instalação da incidência das DCDNT detectada em numerosos tecidos envolvidos na regulação de nutrientes (ROGERO; CALDER, 2018; SILVA, 2018). Logo, controlar a incidência desses eventos é uma condição importante, pois estudos suportam a hipótese de que a inflamação crônica provocada, principalmente pelo dano oxidativo no DNA, pode contribuir para o desenvolvimento de DCDNT (KATERJI; FILIPPOVA; DUERKSEN-HUGHES, 2019;

MARTINS *et al*., 2016; MIROŃCZUK-CHODAKOWSKA; WITKOWSKA; ZUJKO, 2018; VIZZOTTO, 2017).

Diante do exposto, minimizar alterações no organismo, tais como estresse oxidativo, hipercolesterolemia e inflamação, torna-se essencial para a manutenção do estado de saúde em geral. Mais especificamente, o controle desses eventos patológicos irá proporcionar maior desses eventos patológicos, destacam-se o consumo de substâncias biologicamente ativas presentes nos alimentos. A amêndoa de baru (*Dipteryx alata* Vog.), em razão de suas características nutricionais e bioativas, destaca-se por apresentar alto potencial na inibição da inflamação e há indícios de que essa oleaginosa possa desempenhar efeitos positivos na minimização integridade das células endoteliais e tecidos, equilíbrio homeostático, prevenção.

2.5 Baru (Dipteryx alata Vog.)

O baru (*Dipteryx alata* Vog.) é uma espécie nativa do Cerrado brasileiro e está entre as dez mais promissoras para o cultivo. A árvore (barueiro) possui grande porte, chegando a medir até 25 m de altura e 70 cm de diâmetro (FIGURA 11 A), com 60 anos de vida útil, em média. É encontrado, principalmente, nos estados de Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais e Tocantins. Além disso, essa espécie pode ser encontrada também no Paraguai e nas cercanias do complexo do Pantanal (LEMOS *et al*., 2012; PAGLIARINI *et al*., 2012; SANO; RIBEIRO; BRITO, 2004).

Figura 11 – A: Árvore do barueiro; B: Fruto do barueiro; C: Amêndoa do baru.

Fonte: Central do Cerrado (2008).

O barueiro, também conhecido como cumaru, cumbaru, barujó, castanha-de-ferro, castanha-de-burro, coco-feijão, emburena-brava, feijão-coco, fruta-de-macaco, entre outros,

inicia a sua produção frutífera, a partir de seis anos (fase adulta), sendo uma árvore altamente prolífera. Apresenta frutos (FIGURA 11 B) do tipo drupa, ovóides, levemente achatados e de coloração marrom, com uma única semente comestível, a amêndoa de baru. (FIGURA 11 C). Os frutos pesam em média 25 gramas, dentre os quais 65% correspondem ao endocarpo lenhoso, 30% a polpa e 5% a amêndoa, no entanto, em função das condições de solo, água e genética da planta, esse tamanho pode variar entre as regiões. O fruto baru é considerado um importante recurso para a fauna silvestre, pois é consumido, principalmente, por macacos, pacas, cotias, morcegos e pelo gado, que acabam atuando também como dispersores de suas sementes (LEMOS *et al*., 2012; SANO; RIBEIRO; BRITO, 2004).

A cultura do barueiro, além de apresentar grande importância social e ecológica, faz parte de um patrimônio genético abundante sob o ponto de vista medicinal, porém pouco estudado. A manutenção e preservação de baruzeiros no Cerrado são de fundamental importância, pois esta é uma espécie que pode ser utilizada como alternativa de uso múltiplo e, além disso, pode contribuir para o aumento da renda e qualidade de vida de famílias e comunidades rurais. Quanto a isso, apresenta diversas possibilidades no âmbito alimentício (fabricação de geleias, doces, licores, farinhas, óleos e outros), farmacêutico (óleos e cosmética), medicinal (propriedades antirreumáticas e reguladoras hormonais), forrageiro (recobrimento de pastagens principalmente por ser produzido no período da seca), madeireiro (uso na construção civil), paisagístico (recuperação de áreas degradadas), dentre outras (LEMOS *et al*., 2012; REIS, 2016; SANO; RIBEIRO; BRITO, 2004).

Dentre os diversos usos do barueiro mencionados acima, é possível que a sua utilização, no segmento agroalimentar se enquadre como uma das alternativas mais viáveis e promissoras. Mais especificamente, cita-se a utilização da amêndoa de baru que, em razão da sua composição nutricional superior (descrito adiante), pode ser empregada na manutenção do estado de saúde.

2.5.1 Amêndoa de Baru (*Dipteryx alata* Vog.)

A amêndoa do baru é classificada como uma semente comestível, oriunda do fruto da família das leguminosas. É considerada grande, elipsóide, lisa, hilo branco, com sua cor variando de castanho-escuro ao castanho ou amarelo esverdeado. Apresenta comprimento de 1 a 2.6 cm, largura de 0.9 a 1.3 cm, espessura de 0.7 a 1.0 cm e massa de 0.9 a 1.6 g (FREITAS; NAVES, 2010). Por ser fonte significativa de nutrientes benéficos à saúde, recomenda-se a sua utilização, na alimentação humana, desde que não haja compostos tóxicos

ou alergênicos. A amêndoa torrada tem sabor semelhante ao amendoim, com características sensoriais agradáveis e pode ser aproveitada no enriquecimento de pães, bolos, sorvetes, doces, barras de cereais e também na fabricação de licor. No entanto, antes de ser consumida, recomenda-se que a mesma seja submetida ao processo de torrefação, a fim de eliminar a presença de compostos antinutricionais responsáveis por reduzir a biodisponibilidade de nutrientes (FERNANDES *et al*., 2010; LEMOS *et al*., 2012; REIS *et al*., 2015).

Dentre os elementos presentes na composição da amêndoa de baru, citam-se elevados teores de macro e micronutrientes necessários e fundamentais para a manutenção do organismo, proporcionando-lhe alto potencial nutricional. Quanto a isso, menciona-se que essa oleaginosa é fonte expressiva de fibras alimentares (com predominância de fibras insolúveis) e proteínas. Também apresenta quantidades consideráveis de lipídios e, em decorrência desse fato, constitui-se como uma adequada fonte energética. Referente à composição mineral, contém elementos como potássio, fósforo, magnésio, manganês, ferro, zinco e cobre, possuindo valores Tocoferóis (Vitamina E) acima dos preconizados pela Ingestão Diária Recomendada (IDR) (BRASIL, 2005; CAMPIDELLI *et al*., 2019; LEMOS *et al*., 2012; REIS, 2016). De modo geral, esses compostos são responsáveis por desempenharem funções específicas no organismo e por não serem sintetizados pelo mesmo, devem ser obtidos, por meio da alimentação. Logo, a ingestão da amêndoa de baru é recomendada.

Além de sua composição rica em macronutrientes, a amêndoa de baru também apresenta elevados teores de substâncias bioativas, tais como os compostos fenólicos, tocoferóis, ácidos graxos e fitoesteróis (CAMPIDELLI *et al*., 2020; FARIA *et al*., 2015; LEMOS *et al*., 2012, 2016; SANO; RIBEIRO; BRITO, 2014).

Abaixo, são descritas as principais características dos compostos bioativos presentes na amêndoa de baru.

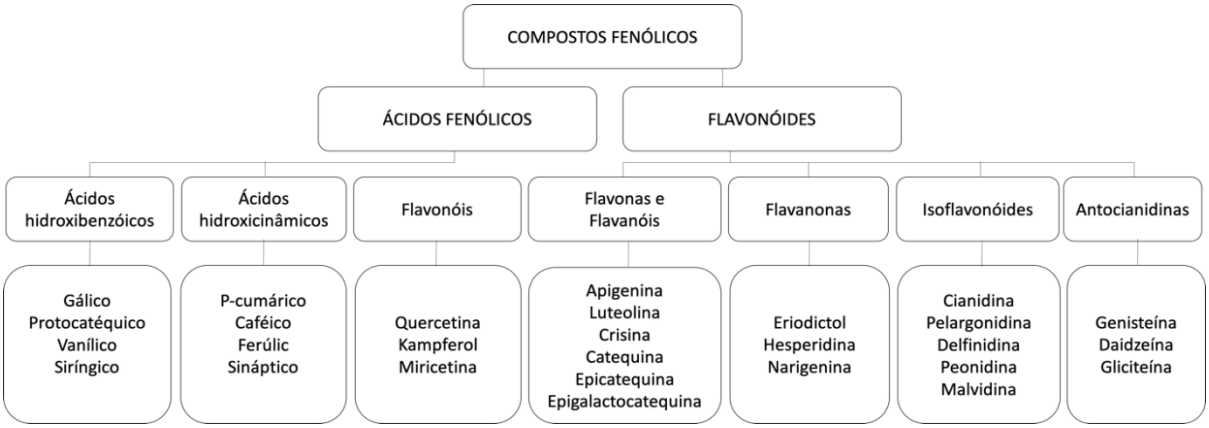
2.5.1.1 Compostos fenólicos

Os compostos fenólicos, também chamados de polifenóis, são originados do metabolismo secundário das plantas e referem-se a um amplo grupo de moléculas encontradas em alimentos como frutas, cereais, hortaliças, cafés, chás, vinho, cacau, soja, dentre outras. Já foram contabilizados mais de 8000 tipos diferentes de polifenóis, logo, são os compostos mais abundantes no reino vegetal. Essas substâncias possuem funções específicas como fotoproteção, defesa contra microrganismos e insetos, estabilidade oxidativa, pigmentação,

além de contribuir com algumas características sensoriais nos alimentos (CORY *et al*., 2018; DINIZ, 2015).

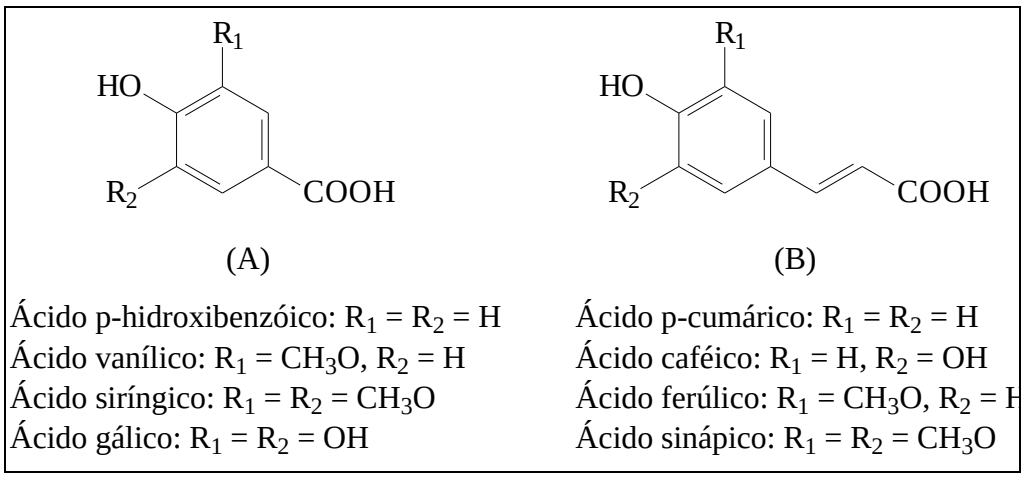
Esses compostos podem ser divididos em dois grupos: os flavonoides e os não flavonoides (ácidos fenólicos) (FIGURA 12). Os denominados flavonoides são os que apresentam estrutura química descrita como C6-C3-C6. Já os denominados de não flavonoides possuem estrutura química C6-C1 (ácidos gálico), C6-C3 (ácido caféico) ou C6-C2-C6 (resveratrol) (COSTA; JORGE, 2011; HUBER; HOFFMANN-RIBANI; RODRIGUEZ-AMAYA, 2009; RODRIGUEZ-AMAYA; KIMURA; AMAYA-FARFAN, 2008). Na Figura 13, estão representadas as estruturas químicas de alguns ácidos fenólicos:

Figura 12 - Classificação dos compostos polifenólicos.



Fonte: Adaptado de Karakaya (2004).

Figura 13 - Estruturas químicas dos principais ácidos fenólicos derivados do ácido benzoico (A) e ácido cinâmico (B).



Fonte: Do autor (2019), adaptado de Costa e Jorge (2011).

Os compostos fenólicos podem ser definidos quimicamente como substâncias que possuem um fenol ou um anel aromático, contendo um ou mais grupo hidroxila. Estão ligados por uma ligação de três carbonos para formar uma unidade heterocíclica com seis unidades no anel e incluem-se os seus grupos funcionais, tais como: ésteres, ésteres metílicos e glicosídios (COSTA; JORGE, 2011).

O grau de hidroxilação e a posição dos grupos hidroxila na molécula dos compostos fenólicos são os mais importantes fatores que determinam sua atividade antioxidante (LIMA, 2008). Isso decorre do poder redutor do grupo hidroxila que reduz radicais livres reativos e produz o radical fenoxila (que se decompõem posteriormente) (DINIZ, 2015). Dentre as principais espécies reativas eliminadas pelos polifenóis, cita-se superóxido ($O2^{\bullet-}$), alquilperoxila ($ROO^{\bullet}$), hidroxila ($HO^{\bullet}$), alcoxila ($RO^{\bullet}$), óxido nítrico ($NO^{\bullet}$) e o oxidante peroxinitrito (ONOO/ONOOH) (DINIZ, 2015; SOUSA, 2013). Os polifenóis também podem quelar metais, como ferro e cobre e, dessa forma, inibir a formação de radicais livres por meio da reação de Haber–Weiss/Fenton (SOUSA, 2013).

Além do efeito protetor, esses compostos desempenham diversas atividades biológicas, proporcionando benefícios fisiológicos na saúde. No entanto, os possíveis benefícios dependem da sua absorção e metabolismo e, quanto a isso, em alguns casos, apresentam baixa biodisponibilidade. Isso ocorre, em decorrência da baixa absorção pelo trato digestivo (por serem muito hidrofílicos para travessarem a parede intestinal) e também por serem rapidamente excretados (CORY *et al*., 2018; DINIZ, 2015).

À seguir, são descritos os principais compostos fenólicos, bem como às suas principais propriedades biológicas.

a) Ácido gálico

O ácido gálico que possui forte capacidade de captação de radicais livres (KARAMAC; KOSINSKA; PEGG, 2005) é eficaz na minimização de doenças e pesquisas indicam que essas substâncias são capazes de induzirem a apoptose e desempenhar efeito citotóxico e antiproliferativo entre diferentes linhagens de células tumorais (GUIMARÃES *et al*., 2007). Magalhães *et al*. (2014), revelaram que 10 μg/mL de ácido gálico foi capaz de alterar o comportamento fenotípico das células imortalizadas de carcinoma epidermóide de língua, resultando em uma redução da proliferação, migração e invasão celular. Outros relatos indicam a sua ação antifúngica, antiviral e antioxidante (KUBOLA; SIRIAMORNPUN, 2008).

b) Ácido caféico

O ácido caféico apresenta capacidade antioxidante, pois é responsável por neutralizar os radicais livres responsáveis por danos oxidativos nas membranas celulares e DNA. Esse ácido também auxilia na prevenção de danos às células causados pela luz ultravioleta, além de apresentar poder anti-hipertensivo, antifibrótico, antiviral e antitumorigênico (PRASAD *et al*., 2011).

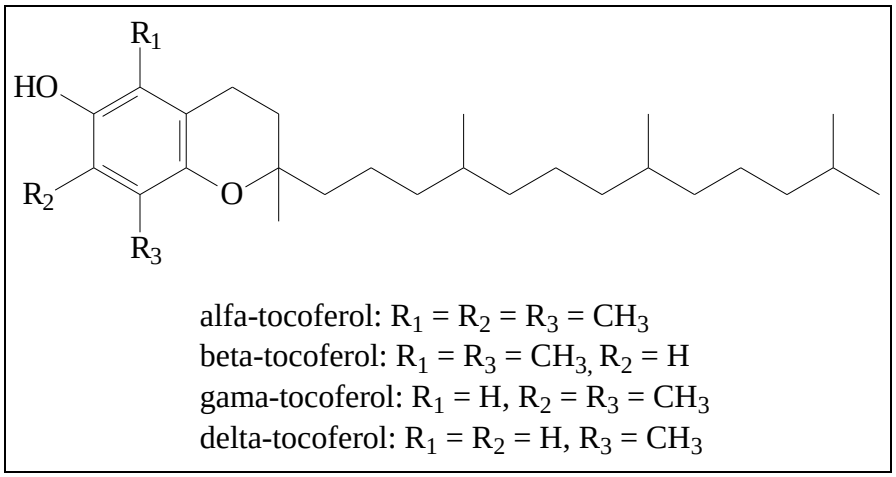
c) Taninos

Os taninos representam um dos grupos mais importante de polifenóis e pelo fato da amêndoa de baru possuir elevados teores dessas substâncias, também se constitui como fonte majoritária desses compostos (CAMPIDELLI *et al*., 2020). Apesar da sua bioatividade, os taninos apresentam a desvantagem de serem considerados compostos antinutricionais. Como prejuízo, o consumo *in natura* dessa oleaginosa, poderá reduzir a biodisponibilidade de nutrientes, tais como minerais e proteínas, podendo provocar efeitos fisiológicos adversos. Visando a inativar esses compostos e minimizar os efeitos deletérios, é necessária a utilização de processos tecnológicos, tais como a torrefação, lixiviação, maceração e/ou germinação (SANO; RIBEIRO; BRITO, 2014).

2.5.1.2 Tocoferóis (Vitamina E)

Os tocoferóis, conhecidos, genericamente, como vitamina E, consistem de um núcleo básico constituído por dois anéis, um fenólico e outro heterocíclico, ligados a uma cadeia lateral saturada formada por 16 carbonos. Dependendo do número e posição de grupos metila ligados ao anel aromático, os tocoferóis apresentam-se como quatro compostos homólogos, denominados α, β, γ e δ - tocoferol (FIGURA 14) (COSTA; JORGE, 2011). O α-tocoferol é o mais abundante nos tecidos, plasma e colesterol e apresenta a mais alta atividade biológica e capacidade antioxidante (COSTA; JORGE, 2011; DINIZ, 2015).

Figura 14 – Estrutura química do tocoferol.



Fonte: Do autor (2019), adaptado de Angelo e Jorge (2007).

Os tocoferóis são os constituintes químicos principais dos óleos vegetais (girassol, milho, gérmen de trigo e soja), frutos secos (amêndoas, nozes e avelãs) e também estão nos vegetais folhosos (espinafres e brócolis). Para que essa vitamina seja absorvida no organismo, é necessária a ação dos sais biliares, formação das micelas, incorporação nos quilomícrons, para então serem transportadas e excretadas nas fezes para sua eliminação (DINIZ, 2015).

Estudos reportam que o α-tocoferol inativo reage com o oxigênio singlet protegendo a membrana celular contra essa espécie. Durante a sua ação antioxidante, haverá a destruição da cadeia de lipoperoxidação (por meio da modificação da estrutura da membrana), sendo consumido e convertido em forma de radical (SERRACARBASSA, 2006). O responsável por essa atividade é o grupo hidroxila livre no anel, sendo os hidrogênios fenólicos doados aos radicais livres lipídicos, formando produtos estáveis e interrompendo a propagação em cadeia da oxidação lipídica (COSTA; JORGE, 2011; RAMALHO; JORGE, 2006).

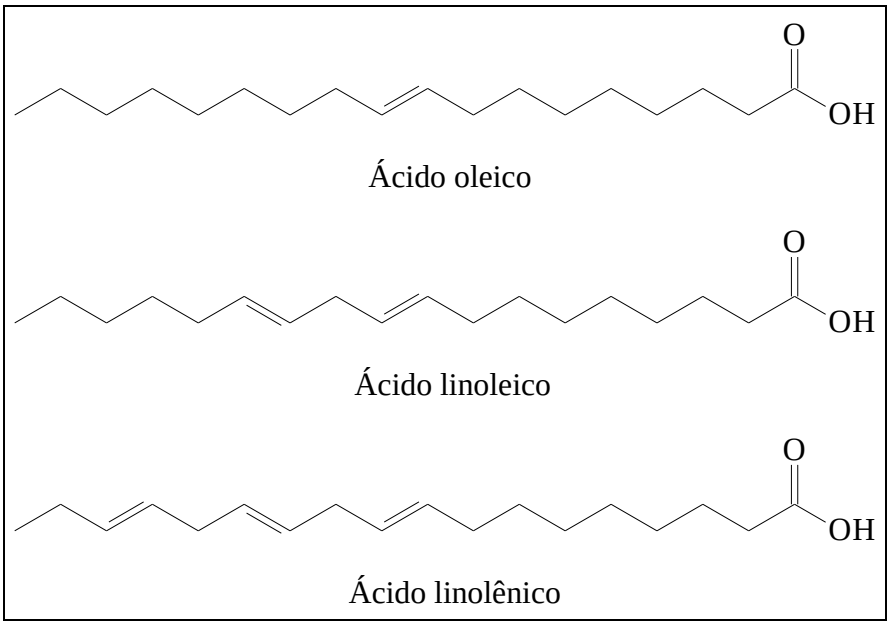
Os tocoferóis também são capazes de inativarem os efeitos deletérios de outras espécies reativas, ao cederem átomos de hidrogênio a radicais como alquilperoxila (ROO•). No entanto, para maior eficiência na eliminação desses compostos e para evitar o efeito pró-oxidante, é necessária a presença de outros coantioxidantes, como o ácido ascórbico. Com isso, podem dempenhar benefícios, dentre os quais, citam-se a inibição da peroxidação lipídica *in vivo* e das lipoproteínas de baixa densidade (LDL) (BARREIROS; DAVID; DAVID, 2006; DINIZ, 2015).

2.5.1.3 Ácidos graxos

Os componentes lipídicos, especialmente os ácidos graxos (AG), são responsáveis, principalmente, pelo armazenamento de energia no organismo. Desempenham relevantes funções nos processos metabólicos, sendo encarregados de atuarem em diferentes funções celulares, além de transportarem vitaminas lipossolúveis e serem precursores de hormônios. Encontram-se distribuídos em todos os tecidos, principalmente nas membranas celulares e células de gordura sob a forma de triacilgliceróis (PERINI; STEVANATO; SARGI, 2010).

Os AG são classificados, conforme a presença de duplas ligações (insaturações) na cadeia hidrocarbonada, sendo a ausência de insaturações um fator prejudicial quanto às funções biológicas desses componentes. Esses compostos podem ser denominados em ácidos graxos saturados (AGS), que são ausentes de duplas ligações e ácidos graxos insaturados (AGI), em razão da presença de uma ou mais insaturações. Dentre o grupo de AGI, estão presentes os ácidos graxos monoinsaturados (MUFAs) e ácidos graxos poli-insaturados (PUFAs). Esses compostos pertencem a diferentes séries, que são definidas pela localização da primeira dupla ligação na cadeia de carbono, a partir do grupo metil. Além disso, são identificados pela letra ω (ômega) e classificados nas séries ω-3, ω-6 e ω-9 (PERINI; STEVANATO; SARGI, 2010; SETE; FIGUEREDO, 2013) (FIGURA 15).

Figura 15 – Estrutura química dos principais ácidos graxos insaturados.



Fonte: Do autor (2019), adaptado de Lopez *et al*. (2010).

a) Ácidos graxos monoinsaturados (MUFAs)

Os MUFAs, que são representados, principalmente, pelo ácido oleico (18:1), apresentam apenas uma insaturação na cadeia hidrocarbônica e são classificados em ácidos graxos da série ω-9, em decorrência da ligação dupla na posição do carbono 9 (FIGURA 14). Os MUFAs são encontrados, principalmente, no azeite de oliva, oleaginosas e óleos vegetais, tais como canola, girassol, amendoim e cártamo (LOPEZ *et al*., 2010; LOTTENBERG, 2009).

O ácido oleico é o ácido graxo mais abundante encontrado nos lipídeos das membranas e dentre as suas principais atribuições, está a sua atividade na produção de energia celular, regulação de funções metabólicas, reduções da LDL-c e da síntese endógena do colesterol (LOPEZ *et al*., 2010; LOTTENBER, 2009). É responsável por reduzir o risco de doenças cardíacas e auxiliar na prevenção do desenvolvimento de câncer. Também foi comprovado que esse composto desempenha funções cerebrais, pois pode ser transportado do sangue até as células endoteliais dos microvasos do cérebro (pela ligação com ácidos graxos e proteínas de transporte). Em razão dos benefícios que apresentam, as recomendações alimentares atuais sugerem que o conteúdo de ácido oleico em uma dieta saudável seja em cerca de 20% da energia total (LOPEZ *et al*., 2010).

Em relação aos efeitos moleculares desempenhados pelo ácido oleico, está a sua capacidade de modular a produção de mediadores inflamatórios, incluindo interleucina (IL) IL-1β, IL-6 e CINC-2αβ (citocina quimioatraente induzida de neutrófilos em macrófagos). Também é responsável por reduzir a resposta inflamatória, por meio da regulação da expressão de COX-2 e da prevenção da ativação de quinase p38, em diferentes tipos de células como miócitos cardíacos e hepatócitos de camundongo (CARDOSO *et al*., 2011; MAGDALON *et al*., 2012).

Kastorini *et al*. (2011) realizaram uma revisão sistemática de estudos epidemiológicos, para avaliar os efeitos da dieta do Mediterrâneo (conhecida pelos altos teores de ácido oleico) no organismo humano. Como conclusão, foi verificado que esse padrão alimentar está associado ao aumento dos níveis de HDL-c e reduções de colesterol e triacilgliceróis.

b) Ácidos graxos poli-insaturados (PUFAs)

Os PUFAs são caracterizados por conterem mais de uma insaturação na cadeia hidrocarbônica. Os principais representantes desse grupo de ácidos graxos são os ácidos linoleico (18:2) e linolênico (18:3), classificados, respectivamente, como AGI da série ω-6 e

ω-3. São frequentemente encontrados em peixes, oleaginosas e óleos vegetais (MARTIN *et al*., 2006) (FIGURA 14).

No que diz respeito às funções dos PUFAs no organismo, menciona-se a sua capacidade de influenciar processos biológicos, como síntese de mediadores inflamatórios, incluindo a formação de eicosanoides (responsáveis por regularem a função imune e inflamatória) (WAITZBERG, 2006). Esses ácidos graxos também participam da transferência do oxigênio atmosférico para o plasma sanguíneo, da síntese da hemoglobina, da divisão celular e são responsáveis pelas funções cerebrais e a transmissão de impulsos nervosos (MARTIN *et al*., 2006).

Quanto à atividade molecular dos PUFAs, estes atuam na modulação de diversos genes envolvidos nos processos oxidativos, aumentando, por exemplo, a expressão de PPARs (*peroxisome proliferator-activated receptors*). Também são responsáveis por bloquearem os genes relacionados à lipogênese que, em excesso, trazem prejuízos à saúde por serem responsáveis pelo armazenamento de lipídeos no fígado e no tecido adiposo. Além do mais, minimizam a expressão dos SREBP (*Sterol regulatory element-binding proteins*), que são fatores de transcrição ligados à biossíntese de ácidos graxos (HANNA *et al*., 2001; LOTTENBERG, 2009).

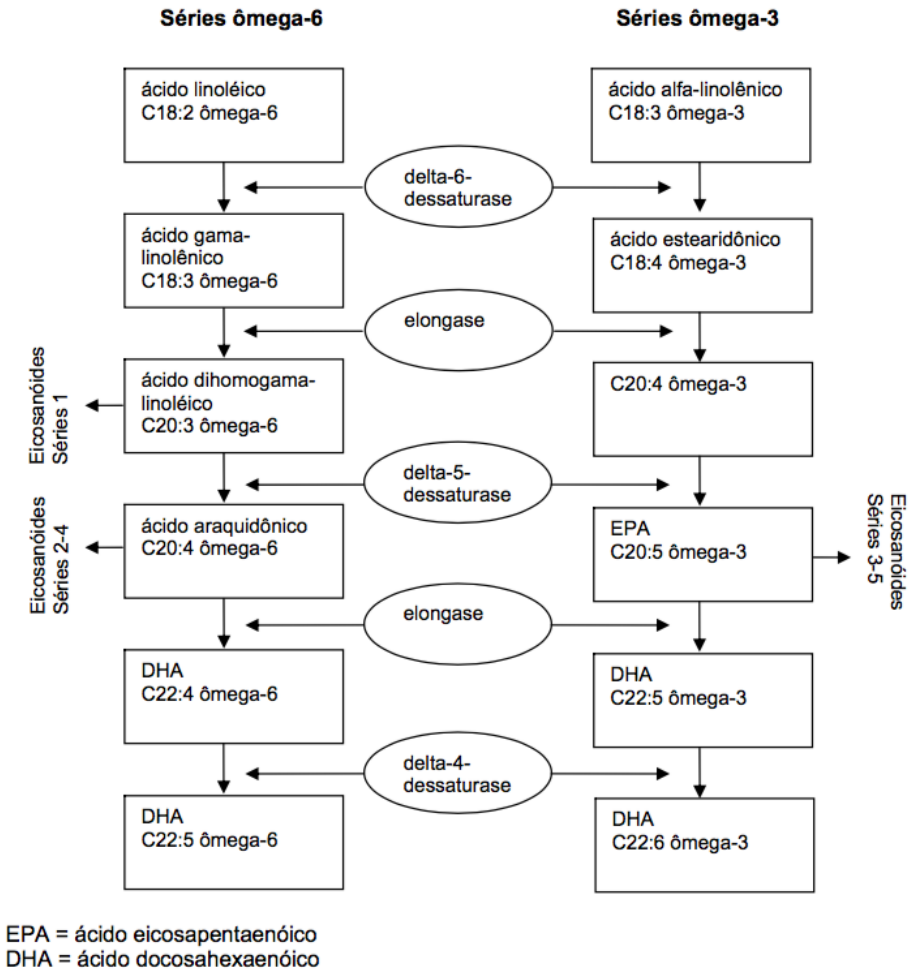
Especificamente, os dois tipos de PUFAs, tal como o da série ω-3 e o da série ω-6, possuem diferentes efeitos sobre a resposta imune e inflamatória. Logo, o balanço na ingestão desses ácidos graxos e, consequentemente, a incorporação na membrana das células imunes, é importante para determinar a ação no processo inflamatório. Os PUFAs da série ômega-3 possuem efeitos supressores, como inibição da proliferação de linfócitos, produção de anticorpos e citocinas, expressão de moléculas de adesão e ativação das células *Natural Killers* (NK) (PERINI; STEVANATO; SARGI, 2010). Os PUFAs da série ômega-6 possuem ambos os efeitos, tanto inibitório quanto estimulatório da resposta imune, embora quando consumidos em grandes quantidades possam proporcionar efeito deletério, tal como desencadear efeito pró-inflamatório (PERINI; STEVANATO; SARGI, 2010).

Dentre os prejuízos causados pelo alto consumo de ômega 6, cita-se a redução da HDL-c, o aumento da suscetibilidade das LDL-c à oxidação e a atuação como precursores de mediadores pró-inflamatórios (responsáveis por promovem vasoconstrição e ação pró-agregatória) (LOPEZ *et al*., 2010; LOTTENBERG, 2009). Quanto a isso, as recomendações sugerem que a ingestão de PUFAs seja mantido em cerca de 5 % da energia total (LOPEZ *et al*., 2010).

c) *Obtenção dos MUFAs e PUFAs*

Os mamíferos utilizam os AG obtidos de sua dieta diária para exercerem funções específicas no organismo e nessas condições são denominados essenciais (não são produzidos *in vivo*). No entanto, quando necessário, são capazes de sintetizá-los e dentre os AG incluídos nesse segmento, citam-se os saturados e os monoinsaturados, que são denominados não essenciais. Referente à essa síntese, essa propriedade ocorre, por meio de reações entre a glicose e os aminoácidos, em decorrência ação das enzimas de alongamento (que adicionam unidades de dois carbonos) e dessaturação (que são responsáveis por adicionarem uma dupla ligação antes do nono carbono, a partir da extremidade metil) (FIGURA 15). A atividade de dessaturação é estimulada pela insulina e inibida pela glicose, pela adrenalina e pelo glucagon (hormônio produzido pelo pâncreas que atua como antagonista da insulina aumentando os níveis plasmáticos de glicose e ácidos graxos) (LOPEZ *et al*., 2010; LOTTENBERG, 2009).

Figura 16 - Formação de novos ácidos graxos poli-insaturado tipo ômega-6 e ômega-3 derivados dos ácidos graxos linoléico e linolênico.



Fonte: Waitzberg (2006).

Dentre as enzimas dessaturases, citam-se a delta-9 e delta-15, que são responsáveis por transformarem o ácido oléico (18:1 ω-9) em ácido linoléico (18:2 ω-6) e ácido linolênico (18:3 ω-3). Mais especificamente, o ácido linoleico pode ser convertido (por meio das enzimas) em ácidos ϒ-linolênico (18:3 ω-6), em seguida, em dihomo-ϒ-linolênico (20:3ω-6) e, finalmente, em ácido araquidônico (AA, 20:4 ω-6). Já o ácido linolênico pode ser convertido em ácido eicosapentaenoico (EPA, 20:5 ω-3) e tanto o AA quanto o EPA podem ser novamente metabolizados, dando origem ao ácido docosapentaenoico (DPA, 22:5 ω-3) e ao ácido docosahexaenoico (DHA, 22:6 ω-3) (SETE; FIGUEREDO, 2013; WAITZBERG, 2006).

Em relação às duas rotas citadas na Figura 15, aquela proveniente do metabolismo de PUFAs tipo ômega-6, sintetizados a partir de AA, são potentes mediadores pró-inflamatórios. É válido constatar, que os eicosanóides provenientes do AA são biologicamente ativos em pequenas quantidades e, se formados em elevadas quantidades contribuem para formação de trombos e ateromas. Referente aos eicosanóides sintetizados a partir de EPA e DHA (da série ômega-3), salienta-se que estes possuem atividade anti-inflamatória, logo, a produção de citocinas pró-inflamatórias podem ser atenuadas e outros processos celulares modulados beneficamente (LOPEZ *et al*., 2010; WAITZBERG, 2006).

Como as duas séries necessitam das mesmas enzimas para serem sintetizadas, existe uma competição de ambos por ela, no entanto, essa competição geralmente só acontece quando há um desbalanceamento na ingestão de ômega 3 e 6. Aumentar a ingestão de EPA e, consequentemente, diminuir a ingestão de AA, resulta na produção de compostos menos inflamatórios (AI, 2016; PERINI; STEVANATO; SARGI, 2010; WAITZBERG, 2006). Logo, pode-se afirmar que é possível modular o processo de formação do AG, pois quando o indivíduo ingere AG n-3, os ácidos graxos EPA e DHA provenientes da dieta substituem parcialmente os AG n-6, principalmente o AA (nas membranas e células do fígado), modificando a composição dos AGPI nos tecidos lipídicos em relação aos eicosanóides. Portanto, o metabolismo dos eicosanóides provenientes do AA é alterado, favorecendo a formação de eicosanóides anti-inflamatórios (PERINI; STEVANATO; SARGI, 2010; WAITZBERG, 2006).

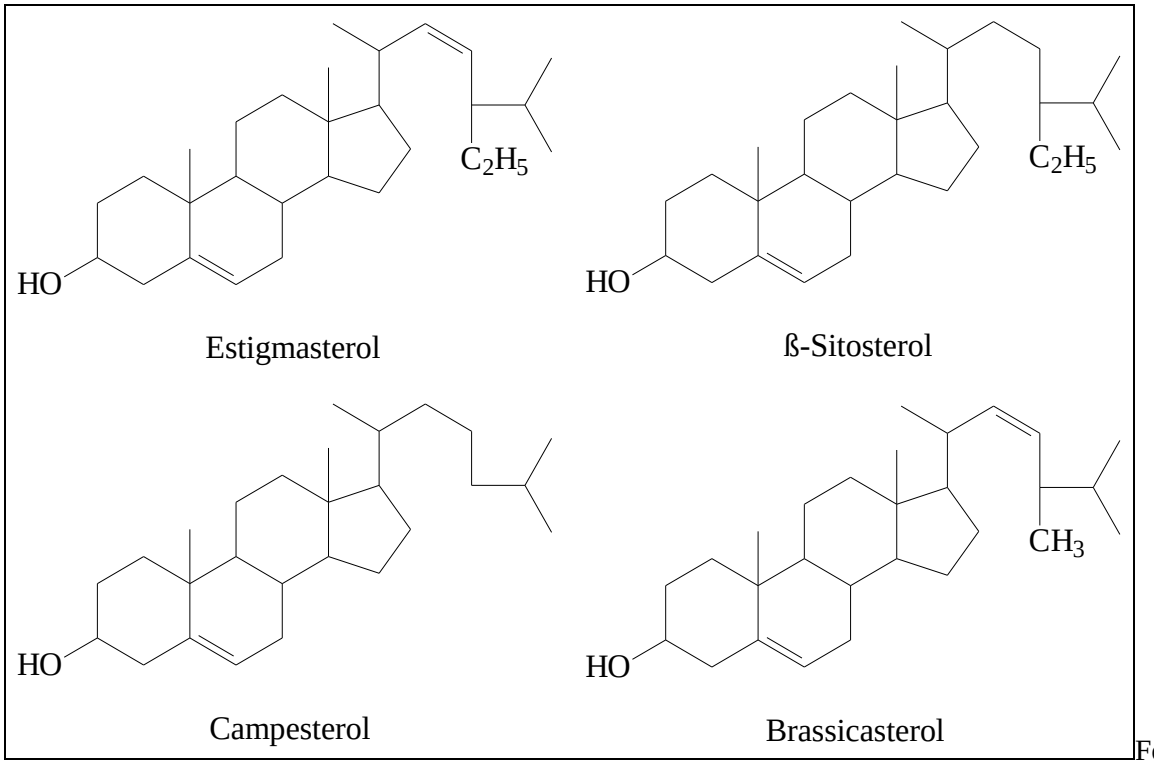
Apesar da amêndoa de baru possuir teores mais elevados de ácidos graxos ômega 6 em relação ao ômega 3, não é inferido a essa oleaginosa a capacidade inflamatória, haja vista, que a mesma possui teores elevados de ômega 9. Estes, por sua vez, podem agir como antagonistas de PPAR (receptor de ativação de proliferação de peroxissomas), cuja ativação

exerce efeitos anti-inflamatórios e estabilizam o complexo NFkB (factor nuclear kappa B) / IkB (complexo enzimático envolvido na propagação da resposta celular à inflamação). Diante disso, há uma supressão da ativação de genes envolvidos no processo inflamatório (LOPEZ *et al*., 2010; PERINI; STEVANATO; SARGI, 2010). Outras informações a respeito do perfil lipídico da amêndoa de baru são descritas mais adiante (item 2.5.2.1).

2.5.1.4 Fitoesteróis

Os fitoesteróis, também chamados de esteróis vegetais, são componentes naturais dos óleos vegetais comestíveis como os óleos de girassol e soja, seguidos pelos cereais e oleaginosas (COSTA; JORGE, 2011). A literatura descreve mais de 40 tipos diferentes de fitoesteróis, no entanto, os mais abundantes são o β-sitosterol, o campesterol, o estigmasterol e o brassicasterol (FIGURA 17). Dentre as principais características desses compostos, cita-se a sua similaridade estrutural com o colesterol, embora não sejam absorvidos pelo intestino. Além do mais, podem ocorrer tanto na forma cristalina quanto esterificada a ácidos graxos livres, ácidos fenólicos ou açúcares (COSTA; JORGE, 2011; HOVENKAMP *et al*., 2008; MATSUOKA *et al*., 2010).

Figura 17 – Estrutura química dos principais fitoesteróis.



Fonte: Do autor (2019), adaptado de Xu *et al*. (2005).

Os fitoesteróis possuem estrutura química composta por 28 ou 29 carbonos, dispostos em um anel tetracíclico e uma cadeia lateral no carbono C17, diferindo-se do colesterol. Este, por sua vez, apresenta 27 carbonos em sua estrutura e não possui um grupamento metil, etil ou insaturação em sua cadeia lateral (MOREAU; WHITAKER; HICKS, 2002). O anel esterol é comum a todos os esteróis e as diferenças encontram-se na cadeia lateral (COSTA; JORGE, 2011; HOVENKAMP *et al*., 2008).

O mecanismo de atuação dos fitoesteróis baseia-se na sua similaridade estrutural com o colesterol. No intestino, o colesterol e os fitoesteróis competem pela incorporação dentro das micelas e, por isso, apresentam absorção e taxas metabólicas distintas: as células intestinais têm a capacidade seletiva para diferenciar os compostos, tendo mais afinidade com o colesterol (a relação de absorção fitoesterol/colesterol é de cerca de 1/10) (BERNE; LEVY, 2000).

Mesmo com essa baixa absorção, os fitoesteróis têm a capacidade de bloquear a absorção e diminuir em partes a recirculação endógena do colesterol: em quantidades adequadas, os fitoesteróis podem deslocar até 50% do colesterol que seria absorvido pelas micelas (BERNE; LEVY, 2000). Após se ligarem às micelas e impedir em partes a absorção do colesterol, ambos compostos não absorvidos são excretados pelas fezes (MATSUOKA *et al*., 2010).

Uma dieta habitual fornece em média 100 à 400 mg/dia de fitoesteróis e a sua eficiência na redução da colesterolemia foi observada com o consumo de 0.8 à 3 g/dia, quantidade suficiente para reduzir o colesterol plasmático entre 10 e 15% (PATEL; THOMPSON, 2006). Uma dieta hipercolesterolêmica suplementada com esses compostos, a diminuição no colesterol LDL é de 30% (JENKINS *et al*., 2006).

2.5.2 Principais efeitos fisiológicos da amêndoa de baru

Estudos envolvendo respostas metabólicas frente à ingestão de amêndoas de baru em modelo animal e humano, bem como relacionados à caracterização química e bioativa das mesmas, demonstram a eficiência de seu consumo. Isso se deve, em grande parte, à capacidade dos componentes fitoquímicos atuarem como agentes protetores em diferentes desordens metabólicas e de origem inflamatória e por modularem os biomarcadores envolvidos na patogênese dessas enfermidades (LEMOS *et al*., 2016; REIS *et al*., 2015). Diante disso, são esperadas algumas propriedades como o controle do perfil lipídico, reduções do estresse oxidativo, da inflamação e de doenças neurodegenerativas.

2.5.2.1 Controle do perfil lipídico

O consumo de ácidos graxos poliinsaturados (PUFA) e de ácidos graxos monoinsaturados (MUFA) tem sido recomendado para melhorar o perfil lipídico em relação aos SFAs (SOUZA *et al*., 2018). No entanto, um efeito indesejável que o alto consumo de PUFA promove é a maior suscetibilidade da LDL-c sofrer peroxidação lipídica (em razão do elevado número de insaturações) com consequente oxidação e redução dos níveis da fração HDL-c (SOUZA *et al*., 2018).

Diante desse fato, há uma tendência em recomendar a ingestão de MUFA e dentre as oleaginosas, a amêndoa de baru enquadra-se como sendo fonte desse composto. Há evidências de que o efeito hipotensor dessa oleaginosa esteja associado à alta concentração de ácido oleico presente (aproximadamente 50%) (CAMPIDELLI *et al*., 2020; SOUZA *et al*., 2018) que, por sua vez, proporciona aumento dos níveis de HDL-c e redução das frações de LDL-c e triacilgliceróis (FREITAS; NAVES, 2010).

O HDL-c possui a função biológica de extrair o excesso de colesterol das células periféricas (efluxo de colesterol) e levá-lo ao fígado para maior metabolismo e excreção (SOUZA *et al*., 2018). Quanto o LDL-c, esse ácido graxo e, consequentemente, essa amêndoa, também são capazes de reduzirem significativamente as modificações oxidativas dessa lipoproteína. A constituição da amêndoa de baru rica em α-tocoferol (1.97 $mg.kg^{-1}$), apoA-I e apoA-II (que possuem capacidade antioxidante) e, principalmente, paraoxonase (uma enzima que catalisa a hidrólise de ácidos carboxílicos aromáticos e compostos organofosforados) é a principal responsável pela redução dessa lipoproteína (CAMPIDELLI *et al*., 2020; SOUZA *et al*., 2018). Esse processo pode resultar, em teoria, na maior sobrevida das células saudáveis no organismo, proporcionando, consequentemente, inibição do processo aterogênico, já que é amplamente aceito que a molécula de LDL-c oxidada tem relevante papel na inflamação vascular, na disfunção do endotélio e na formação de células espumosas na parede intimal (SANTOS *et al*., 2013).

O aumento do consumo de ácidos graxos monoinsaturados também está diretamente relacionado ao aumento da adiponectina plasmática, tornando-se um fator favorável quanto à melhora do perfil lipídico. Esse hormônio atua, principalmente, na redução da glicose, triacilgliceróis, metabolismo energético dos ácidos graxos saturados, minimizando principalmente patologias como diabetes, aterosclerose, obesidade, síndrome metabólica, entre outras (SOUZA *et al*., 2018).

Os fitoesteróis presentes na amêndoa de baru também podem contribuir positivamente com perfil lipídico, já que esses compostos reduzem a absorção intestinal de CT-c e LDL-c. Ao que tudo indica, essas moléculas competem com as de CT-c pela incorporação nas micelas mistas no trato intestinal (CARVALHO *et al*., 2012; MARANGONI, 2013).

Os ensaios clínicos com indivíduos dislipidêmicos demonstraram um efeito positivo do consumo de diferentes tipos de oleaginosas em parâmetros bioquímicos. O conteúdo de lipídeos foi aumentado a cada 4 semanas, por meio da suplementação com amêndoas. A dieta com maior porcentagem de lipídios (39%), contendo 68 g de amêndoas produziu os seguintes resultados: redução de 8.6% no CT-c, redução de 8% na apo B e aumento de 4% na apo A (SABATÉ *et al*., 2003).

Em outro estudo, foi demonstrado que a suplementação com aproximadamente 77 g de amendoins, por 4 semanas reduziram as concentrações de CT-c, LDL-c, índice aterogênico plasmático e pressão arterial. Além disso, aumentou os níveis de HDL-c e a concentração sérica total da capacidade antioxidante (NOURAN *et al*., 2009).

2.5.2.2 Redução do estresse oxidativo

Com base na composição química da amêndoa de baru, rica em compostos bioativos e ácidos graxos monoinsaturados, conforme citado anteriormente, é possível inferir a esta oleaginosa a propriedade de reduzir o estresse oxidativo. Essa redução ocorre, porque essas substâncias são capazes de sequestrar os radicais livres e metais pró-oxidantes, responsáveis por danos oxidativos nas membranas celulares e DNA.

Dentre os compostos presentes, o ácido oleico atua na redução intracelular das EROs, por meio da remoção dos radicais mais reativos como o ânion superóxido ($O_2^{-\bullet}$). Os fitoesteróis (beta-sitosterol) por sua vez, aumentam a atividade enzimática da superóxido dismutase (SOD) contribuindo também para a redução de $O_2^{-\bullet}$.

Aos tocoferóis são atribuídas atividades antioxidantes relacionadas a mecanismos de proteção das membranas celulares dos compostos oxidáveis do citoplasma celular. Sua estrutura e facilidade de interação fazem dessa substância um antioxidante lipofílico muito mais eficiente em relação aos hidrossolúveis, contribuindo eficazmente no combate ao estresse oxidativo (CARVALHO *et al*., 2012; PEREIRA, 2010). Também interrompem a peroxidação lipídica, doando o hidrogênio fenólico para o radical peroxil (LOOH), formando o radical tocoferoxil, que não é reativo e é incapaz de continuar a cadeia de oxidação (BURTON *et al*., 2008; FARIA *et al*., 2015).

As altas concentrações de substâncias polifenólicas, tais como o ácido gálico, o ácido caféico e a rutina (que são os compostos majoritários na amêndoa de baru) podem atuar na redução do estresse oxidativo de várias formas, a citar: combatendo os radicais livres por meio da doação de um átomo de hidrogênio de um grupo hidroxila (OH) da sua estrutura aromática; quelando metais de transição, como o Fe^{2+} e o Cu^{+}; interrompendo a reação de propagação dos radicais livres na oxidação lipídica; modificando o potencial redox do meio; reparando a lesão de moléculas atacadas por radicais livres; impedindo que as EROs ativem o sistema NF-kB (fator nuclear kappa Beta) impossibilitando-o de se translocar para o núcleo e se ligar ao DNA; e reduzindo a biossíntese de dieno conjugado (produzidos durante a peroxidação lipídica), em decorrência da ação antioxidante (CAMPIDELLI *et al*., 2020; HERBELLO-HERMELO *et al*., 2018; LEMOS *et al*., 2012; MARANGONI, 2013; PERONA; CABELLO-MORUNO; RUIZ-GUTIERREZ, 2006; PODSEDEK, 2007).

O elevado conteúdo de zinco da amêndoa de baru (CAMPIDELLI *et al*., 2019) também é um fator relevante no que diz respeito à redução do estresse oxidativo. Esse mineral não age diretamente sobre os radicais livres, porém, é importante na sua prevenção e formação, pois inibe principalmente NADPH oxidase (responsável por produzir o radical oxigênio *singlet),* além de fazer parte da enzima SOD, que converte o $O_2^{\bullet}$ em H_2O_2 (CATANIA; BARROS; FERREIRA, 2009; PRASAD, 2011). Prasad *et al*. (2007), constataram que após a suplementação por seis meses com 45 mg de zinco (4 vezes o recomendado para ingestão diária) foi observada uma redução dos níveis plasmáticos de malondialdeído e 8-hidroxi-2'-deoxiguanosina, que constituem marcadores importantes na avaliação da oxidação de lipídios e do dano oxidativo ao DNA, respectivamente.

Estudos com adultos saudáveis mostraram que a suplementação com uma mistura contendo 15 g de nozes comum, 7.5 g de amêndoas e 7.5 g de avelãs por 12 semanas foram suficientes para melhorar os níveis de várias substâncias oxidativa e também reduzir os danos ao DNA, que foram avaliados com base na redução do biomarcador 8-oxo-2′-desoxigguanosina (LÓPEZ-URIARTE *et al*., 2010).

2.5.2.3 Redução da inflamação

Oleaginosas, de modo geral, podem ser incluídas no *hall* de alimentos que são eficazes na redução do processo inflamatório e atenção especial é dada à amêndoa de baru. Isso ocorre, porque o elevado conteúdo de tocoferóis presentes na mesma pode inibir a ativação do fator de transcrição Nf-kB, o qual é responsável pela regulação de genes que irão codificar

citocinas pró-inflamatórias, moléculas de adesão, fatores de crescimento, enzima *Cox-2* e enzima óxido nítrico sintase induzível (*iNOS*), que estão envolvidos na resposta inflamatória e, desse modo, ligados ao desenvolvimento de diferentes doenças (WU *et al*., 2008).

A elevada presença de compostos antioxidantes, nesse alimento também é um elemento favorável quanto a esse perfil, visto que essas moléculas também são capazes de modularem a atividade de várias proteínas e fatores de transcrição, os quais estão envolvidos em vias metabólicas relacionadas à síntese de citocinas, quimiocinas e moléculas de adesão (SOUSA; DE ANGELIS, 2013).

O ácido oleico presente na amêndoa de baru, também pode ser um dos responsáveis pela atividade anti-inflamatória da mesma. Em estudo publicado por Song *et al*. (2019), foi detectado que o ácido oleico livre (30 mg/kg intraperitoneal) pode ativar o PPAR-γ (Peroxisome Proliferator-Activated Receptor Gamma) e reduzir acentuadamente a expressão de mediadores inflamatórios pós-isquêmicos, incluindo *Cox-2*, *iNOS* e *TNF-α* no cérebro. Também foi demonstrado por Moraes *et al*. (2018), que o tratamento com ácido oleico está associado ao aumento dos níveis da citocina anti-inflamatória *IL-10* e à diminuição dos níveis das citocinas pró-inflamatórias *TNF-α* e *IL-1β*.

2.5.2.4 Redução das doenças neurodegenerativas

Estudos epidemiológicos têm demonstrado que o consumo regular de alimentos ricos em compostos bioativos diminuem a predisposição ao desenvolvimento de doenças neurodegenerativas, tal como a doença de Alzheimer (DA) (CORREIA *et al*., 2015). Esta, por sua vez, pode ser desencadeada por múltiplos fatores patogenéticos, incluindo agregados de beta-amilóide, emaranhados neurofibrilares, disfunção colinérgica e estresse oxidativo (BABRI *et al*., 2014). Por possuir teores elevados de compostos bioativos (CAMPIDELLI *et al*., 2020) e, consequentemente, diferentes mecanismos terapêuticos, a amêndoa de baru se enquadra como uma opção eficaz à minimização dessa patologia.

Os compostos fenólicos por promoverem uma interação entre peptídeos e proteínas (tal como βeta-Amilóide), conseguem inibir a formação desses oligômeros, apresentando atividade antiami--loidogênicas. Além de minimizar esses compostos nocivos, também podem proteger contra a neurotoxicidade provocada pelo peptídeo (MORZELLE *et al*., 2016). Entre os compostos fenólicos, o ácido gálico, que também é o fenol majoritário na amêndoa de baru, se destaca por sua ação neuroproteteora. Alguns estudos demonstram que a administração desse composto pode melhorar os défices cognitivos, após danos cerebrais,

pode diminuir os níveis de EROs (pela redução do MDA e aumento das enzimas antioxidantes, tais como: SOD, GPx e CAT) no hipocampo e no córtex cerebral, pode impedir a morte neural apoptótica (induzida pela βeta-amilóide), dentre outras propriedades (KORANI *et al*., 2014).

A vitamina C é um dos compostos presentes na amêndoa de baru (CAMPIDELLI *et al*., 2020) e estudos comprovam que portadores da DA possuem baixos níveis plasmáticos dessa vitamina (CORREIA *et al*., 2015). A redução dessa substância no tecido cerebral dos portadores da doença é algo prejudicial, visto que a mesma possui função protetora contra o declínio cognitivo relacionado à idade. Dentre os principais mecanismos pelo qual a vitamina C pode desempenhar benefícios, está o seu papel na síntese de neurotransmissores (como a dopamina e noradrenalina), além de seu desempenho na proteção dos neurônios contra o estresse oxidativo. Também atua como coantioxidante auxiliando a vitamina E na sua função de prevenção da oxidação (CORREIA *et al*., 2015; MOHAJERI; TROESCH; WEBER, 2015)

No que diz respeito à vitamina E, que também está presente em níveis elevados na amêndoa de baru (conforme já citado), a sua importância na prevenção de doenças neurodegenerativas se dá pelo fato de esta ser constituinte das membranas dos neurônios, logo, sua presença, torna-se essencial para o funcionamento correto dos mesmos. Além do mais, pelo fato de as membranas das células cerebrais serem ricas em ácidos graxos ômega 3 e estarem sujeitas à sofrer oxidação pelos radicais livres, a ação antioxidante da vitamina E torna-se fator essencial para a manutenção da integridade dessas células. No entanto, a deficiência dessa vitamina pode desencadear disfunções neurológicas, tais como: ataxia (perda da coordenação muscular) e disartria (distúrbio da articulação da fala) contribuindo para maior incidência de demência e de DA (CORREIA *et al*., 2015; MOHAJERI; TROESCH; WEBER, 2015; MULLER *et al*., 2010). Um fator limitante aos benefícios propostos pela suplementação de vitamina E, relaciona-se à dose necessária para impedir essas disfunções, haja vista que o consumo de teores excessivos pode causar hemorragia (CORREIA *et al*., 2015).

Um ensaio clínico randomizado duplamente cego com 613 indivíduos com DA (com grau leve à moderado), mostrou que a ingestão de α-tocoferol (2000UI/dia) juntamente com os inibidores da acetilcolinesterase (já administrados pelos pacientes), resultou no atraso da progressão do declínio funcional. Como resultado, o grupo tratado teve um atraso de 19%, por ano, na progressão da doença e menor necessidade de apoio por parte dos cuidadores, comparativamente, com o grupo placebo (DISKEN *et al*., 2014).

A alta ingestão de ácidos graxos monoinsaturados (MUFA) também está inversamente associada ao risco de declínio cognitivo relacionado à idade. O ácido oleico, por ser um dos principais encarregados pela manutenção da integridade da estrutura das membranas neurais, pode ser um dos compostos responsáveis por essa propriedade. Além disso, é demonstrada a relação desse ácido graxo e o funcionamento adequado do cérebro, ocorrendo, principalmente, por esse composto ser utilizado na síntese de fosfolipídios da mielina, tornando os impulsos nervosos mais rápidos e eficientes. Também atua como um fator neurotrófico, promovendo o crescimento axonal e dendrítico, melhorando a migração e agregação neuronal e facilitando a formação de sinapses (POLO-HERNÁNDEZ *et al*., 2014). Mitchell e Hatch (2011), ao investigarem a presença de ácido oleico no plasma de pacientes com DA, detectou uma redução de 80% desses compostos, em comparação com aqueles sem comprometimento cognitivo. Além do mais, foi encontrado por Amtul *et al*. (2010) que camundongos transgênicos com DA suplementados com ácido oleico, tiveram uma redução considerável nos níveis da proteína βeta-amiloide.

Foi demonstrada, por meio de pesquisas, uma relação entre indivíduos adeptos à dieta mediterrânea (caracterizada pela alta presença desse composto) e a menor incidência de transtornos neurodegenerativos. Valls-Pedret *et al*. (2015) fizeram uma investigação com 334 idosos (idade média de 66.9 anos) e demonstraram que, após 4 anos consumindo a dieta mediterrânea, tiveram os escores de memória, cognição frontal e escores cognitivos globais diminuídos significativamente. Em outro estudo realizado por Martınez-Lapiscina (2013), que envolveu 522 participantes com alto risco vascular em uma intervenção nutricional, comparou a dieta mediterrânea suplementada com azeite de oliva e castanhas, com uma dieta controle com baixo teor de gordura. Após 6.5 anos de intervenção nutricional, os participantes que receberam azeite de oliva e castanhas apresentaram melhor cognição e menor comprometimento cognitivo em comparação com o grupo controle. O mesmo foi observado em pesquisa realizada por Scarmeas *et al*. (2006) onde foi constatado que os indivíduos com DA adeptos à dieta mediterrânea possuem menor risco de mortalidade.

Scarmeas *et al*. (2006) realizaram uma investigação observacional, mostrando que a aderência à dieta mediterrânea está associada a uma redução de 40% na chance de indivíduos desenvolverem DA. Os autores postularam que as propriedades antioxidantes dos fenóis e dos ácidos graxos monoinsaturados presentes nessa dieta podem reduzir o estresse oxidativo e a inflamação que ocorrem nos pacientes com essa patologia.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 – IMPACT OF THE DRYING PROCESS ON THE QUALITY AND PHYSICO-CHEMICAL AND MINERAL COMPOSITION OF BARU ALMONDS (*Dipteryx alata* VOG.)

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Impact of the drying process on the quality and physico-chemical and mineral composition of baru almonds (*Dipteryx alata* vog.)

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Abstract

The present work aims to investigate the impact of different drying processes on baru almonds. It was found that the drying processes (65°C and 105°C for 30 min) did not change the physicochemical, mineral and quality composition of baru almonds (except moisture and acidity indexes that decreased and increased, respectively, the use of temperature of 105°C for 30 min). All treatments are sources of protein, fiber, lipids, boron, copper, manganese, iron, sulfur, calcium and magne- sium. All treatments are below the maximum limits established by the inspection bodies for the presence of acidity and peroxides, indicating the quality of these products.

Keywords: Brazilian Cerrado; Food Composition; Food Drying; Micro and macro nutrients; Oilseeds.

Introduction

Drying is one of the oldest processes used by man for preserving food. Due to reduced water activity, there is a minimization of microbial growth, which enables the increase of the useful life of the raw materials and can facilitate and reduce costs of packaging, transport and storage, in addition to bringing improvements in sensory aspects, inhibit the factors antinutritional and provide the raw materials throughout the year (Calín-Sánchez et al., 2012; Igual et al., 2012). Moreover, it is responsible for minimizing possible points of contamination of some foods, especially those that have low levels of technology and present poor management conditions that pose a risk to consumer health.

Despite the benefits, it is estimated that drying may contribute to the reduction of the presence of macro and micro nutrients and may negatively influence food quality parameters (Fujita et al., 2013; Azevêdo et al., 2014; Li et al., 2017). Favoring this scenario, there is a high incidence of producers employ this technique improperly in its raw materials. As a

result, there is a distribution of products with functional activity compromised and between the food, the almonds are the most injured by being the ones that employ this method of conservation.

In this context, the baru almond (*Dipteryx alata* Vog.), despite having limited information, stands out for its superiority in nutritional composition (Fraguas et al., 2014; Siqueira et al., 2015; Lemos et al., 2016). Recent studies make reference to the use of this almonds as a viable alternative of incorporation of phytochemical components with potentiality in the prevention of certain diseases. Therefore, the study with this food should be boosted since the almond in question presents high nutritional value, due to the presence of substances that are able to assure the maintenance of the vital functions of the organism (Siqueira et al., 2015).

However, the use of high drying temperatures, which often result in the roasting of food, play significant declines in food properties, mainly because these matrices are thermally unstable (Paraginski et al., 2015). As the current trend is focused on the supply of nutritional deficiencies through food consumption, the maintenance of these characteristics must be preserved and laboratory investigations executed, in order to verify the impacts of high temperatures on the constitution of the foods, executed (Mello; Santos, 2001; Loguercio, 2005).

Therefore, in order to extend the knowledge about the potential and investigate possible changes resulting from the processing, it is attributed to this work is an investigation that aimed to identify the impacts of the application of different drying processes (65 °C and 105 °C for 30 minutes) on the quality and physico-chemical and mineral constitution of the almond in question.

Materials and Methods

Materials

The baru almonds were obtained from the Cerrado Biome region-raw, located in the city of Barra do Garças-MT, during the harvest season (between August and September) and forwarded to the laboratory of Post-harvesting of Fruit and Vegetables at the Federal University of Lavras. In order to assure the microbiological quality of the almond, the presence of toxigenic fungi was analyzed (Samson et al., 2004) and the absence of the fungi was detected.

Methods

Drying process of almonds

Temperature variations on drying process of almond baru are listed in Table 1 and were chosen because they are the most used by the producers of this oilseed. A total of 100 grams of almonds (by experimental repetition) were submitted to milling (20 mesh - in an experimental mill, Viti Molinos, VG 2000i, Itajaí, Brazil), packed in stainless steel trays and added (after reaching the desired temperature) in a forced air circulation oven (Marconi, MA0351, Piracicaba, Brazil), where they remained for 30 minutes. They were then packed in transparent polyethylene containers and stored at 25 ° C (oven model Eletrolab, EL202) for further use.

Physical Chemical Composition

Centesimal composition

Analyses of moisture (65 °C for 1 hour per 5 days), ether extract (lipids), protein (N x 6.25), ash (the incineration 550 ºC), total carbohydrates and dietary fiber (soluble and insoluble) content were determined in accordance with the Aoac (2005).

Mineral composition

Minerals were described by the Sarruge method and Haag (1974) and was used in digestion with nitric acid perchloric 50 ºC for 10 to 15 minutes and 100 ºC to digest all the material. Then, the determination in atomic absorption spectrophotometer (Perkim Elmer, 3110) with reading at 248 nm. Boron, sulphur, potassium, calcium, magnesium, copper, manganese and iron were the quantified minerals.

Quality parameters

Analyses of pH, total soluble solids, total titratable acidity and *ratio* were determined according to Instituto Adolfo Lutz (2005). Total sugars were evaluated by spectrophotometry at 620 nm the antrona (Yemm; Willis, 1954).

The peroxide index was determined according to the method described by Instituto Adolfo Lutz (2008) based capacity of oxidation of potassium iodide by peroxides present in the sample, followed by iodometric titration of iodine formed using sodium thiosulfate as titrant and starch as an indicator.

The acid index of each sample, expressed as the percentage of oleic acid was determined according to the method described by Instituto Adolfo Lutz (2008). To almonds,

added a neutral solution of ethyl ether, ethanol and two drops of the indicator fenolftaleina 1%. This mixture was titrated with 0.1 N KOH solution to the predominance of a rosy coloring. The acid value was determined as the *ratio* of the volume of base required by mass of the sample analysed.

To quantify the peroxide index and acidity, it was necessary to carry out the extraction of the oil present in the baru almond, which occurred as follows: The baru almonds (Tin, T65 and T105) were pressed into digital Extractor Oster (yoda 60 Hz model) with the following specifications; nominal power - 400W; turbo - electric heating mode of heat; way of understanding; axle pressure. Subsequently, the oil was transferred to amber jars and kept under refrigeration equipment (4 ºC).

Statistical Analysis

The results were submitted to analysis of variance (ANOVA) and test for comparison of averages (Tukey 5% the probability of error). Statistical calculations were performed using the program R® version 5.0. The differences were considered significant when $p \leq 0.05$.

Results and Discussion

Physical-Chemical Composition

To determine the composition of macro and micronutrients of baru almonds submitted to different drying processes (Tin, T65 and T105), moisture content, ashes, proteins, lipids, carbohydrates, dietary fibers and minerals were determined (Table 2). These data were also related with Recommended Daily Intake (RDI) (Figure 1) which is the level of daily intake of a nutrient that is considered sufficient to meet the nutritional requirements of most individuals and groups of a healthy population (Brazil, 2005).

The moisture contents detected in the three treatments differed significantly (P <0.05) and as the drying temperature increased (T65 and T105), these levels were reduced. This fact is explained because the dehydration is a process that consists of the elimination of water of a product by evaporation, with transfer of heat and mass, thus, the higher the temperature used, the lower the humidity index. Lemos et al. (2016) found moisture contents of 5.8 and 9.9 $g.100g^{-1}$ in roasted and raw barren almonds, respectively, as Pineli et al. (2015) detected 3.63 $g.100g^{-1}$ in defatted baru almond flour.

The lipid content of the in natura and submitted to different drying processes (T65 and T105) varied from 31.73 to 34.16 g.100g-1, without statistical difference (P> 0.05), proving that the heating interference. Martins (2006), observed a similar behavior when analyzing

baru raw and roasted almonds (35.8 g.100g^{-1}). Lemos et al. (2016) that found a higher content (48.6 and 46.4 g.100g^{-1} respectively for raw and roasted almond), found a reduction of lipids after heating. It is believed that the fat detected in the baru almond, because it is abundant, can be a viable alternative to the substitution of olive oil, which according to Pestana-Bauer et al. (2011), presents only 19.8% of lipids inferring in a lower yield. Moreover, Lemos et al. (2016) detected the presence of tocopherols in baru (2.7 mg.100g^{-1}) almond oil and that, because it is an antioxidant substance, it can avoid that when heated, this oil has its oxidized molecules (which can confer pro-inflammatory properties detrimental to health) thus having their beneficial characteristics protected.

When the lipid content was related to the RDI, a supply of 48.8, 50.13 and 52.55% of the same (through consumption of 100 g) was observed (Figure 1). Considering that baru almond has approximately 80% unsaturated lipids (Lemos et al., 2016), the consumption of this almond may be effective in different metabolic disorders (Herbello-Hermelo et al., 2018), more specifically, of inflammatory origin. In addition, ingestion of these fats is highly recommended by experts as there is scientific evidence that they can reduce the risk of cardiovascular disease, be effective in weight loss and contribute to lowering LDL blood levels without affecting HDL levels (beneficial to health) (Mattes et al., 2008; Lottenberg, 2009).

The values of 1.55, 1.87 and 1.77 g.100g^{-1} of ash respectively for Tin, T65 and T105 were found in the baru almonds. There was no statistical difference between the samples ($P>0.05$) that heating did not interfere with the amount of fixed mineral residue present. According to the Food Composition Table (Nepa, 2011), the almond (*Amygadalus Communis* L) toast presents 1.5g.100g^{-1} of ash with levels lower than the baru almond. However, in the cultivar studied by Fragua et al. (2014), mean values higher than those found in the present study were found for freeze-dried baru almond (2.73 g.100g^{-1}) and toast (2.65 g.100g^{-1}).

Drying also did not have a significant effect ($P>0.05$) on the protein content, being between 22.96 and 24.23 g.100g^{-1}. This observation is similar to that of De Pilli et al. (2008), that when working with extruded baru almond flour found an average corresponding to 22.24 g.100g^{-1}.When compared to Brazil nuts, pine nuts, pecans and hazelnuts, which have 16.30, 13.0, 9.0 and 14.5 g.100g^{-1} of proteins respectively (Freitas, 2008; Yang, 2013), baru almonds can be distinguished by their values higher. Considering that Brazilian legislation recommends the consumption of 50 grams of protein per day (Brasil, 2005), the treatments Tin, T65 and T105 represent, respectively, 45.6, 45.3 and 46.3% of this orientation (values obtained by the consumption of 100 grams of almonds) (Figure 1). These results present high

merit, since the proteins contribute in an effective way to the development of the structure of the organism and aid in the nutrition.

Regarding the presence of carbohydrates, in this study we found averages of 37.13, 36.28 and 37.20 g.100g^{-1} for Tin, T65 and T105, respectively, with no statistical difference (P>0.05). These results are similar to that found by Takemoto et al. (2001), who reported a mean of 31% of carbohydrates for baru almond. On the other hand, Nucleus of Studies and researches in Food (Nepa, 2011) reports lower values for cashew nuts, Brazil nuts and walnuts that have respectively 29.1, 15.1 and 18.4 g.100g^{-1} carbohydrates. Because it is a nutrient source of immediate energy for the body and for referencing the presence of natural sugars, high carbohydrate levels become a viable option among the almonds. The main role of this nutrient is to provide energy to the cells, particularly the brain, which is the only carbohydrate-dependent organ in the body (Trumbo, 2002). The RDI was established at 300 g/day (Brasil, 2005) (Figure 1), so the consumption of 100 grams of baru almond may represent 12.37, 12.09 and 12.4% of this guidance. Despite the relevance of this nutrient in the functioning of the body, it is important to note that excessive consumption has been associated with a number of adverse effects.

In the case of total dietary fiber, the levels found were 14.44, 14.22 and 15.22 g.100g^{-1} respectively for Tin, T65 and T105. As for the literature, Takemoto et al. (2001) reported similar values (13.4g.100g^{-1}) to those found in the present study, however, higher values (38.8 g.100g^{-1}) were reported by Pinelli et al. (2015). Freitas (2008) detected 10.97, 6.48, and 12.53 g.100g^{-1} of total dietary fiber, respectively, for peanuts, cashews and Brazil nuts, which are lower than those found in the present study. According to the Brazilian Resolution, the fiber RDI is 30 grams per day (Brasil, 2016), so the consumption of baru almonds (100 grams) corresponding to Tin, T65 and T105 respectively, 48.1, 47.4 and 50.7% of the daily recommendation and can be considered as food sources of fiber (Figure 1). The daily consumption of baru almonds should be boosted since the fibers are important agents that influence the intestinal flora and positively modulate the microbiota preventing the onset of diseases.

Mineral Composition

Table 2 and Figure 1 show the determination of the mineral composition of boron, sulfur, potassium, calcium, magnesium, copper, manganese and iron present in barn almonds submitted to different drying processes (Tin, T65 and T105), as the RDI of the same.

As for minerals analyzed, it was detected that the drying process did not cause changes in these elements (P>0.05) and inorganic elements, thermal stability is a peculiar feature of the same. It was possible to detect that the baru almond presented high content of phosphorus, potassium, magnesium, sulfur, manganese, zinc, boron, copper and iron because, according to Brazilian legislation, they satisfy at least 30% of the reference RDI. In addition, because it represents 15% of the RDI, it is a source of calcium. It has been found that the consumption of 100 grams of baru almond represents more than 100% of the RDI for magnesium and almost 3 times the RDI for manganese and copper (Brasil, 2005).

This composition in minerals is relevant for health, since they are catalysts of biochemical reactions, for example, zinc, which acts as a cofactor of antioxidant enzymes, preventing and/or controlling the formation of free radicals and non-radical species involved in oxidative stress (Siqueira et al., 2015). Already copper because it is a component of several enzymes, is involved in the production of cellular energy and in the formation of connective tissues, besides having antioxidant character. The physiological role of magnesium is also of considerable importance as it is involved in the doubling of nucleic acids, in neural excitability and in the transmission of nervous influx, acting on the ionic exchanges of the cellular membrane (Abe-Matsumoto et al., 2015). Regarding the main benefits provided by manganese, we mention the actions on the metabolism of fats, protection of liver cells, implication in the metabolism of neurotransmitters, as well as protection of cells against free radicals (Cozzolino, 2007). It is thus established that the consumption of baru almond must be stimulated since it supplies the RDI of different minerals helping to maintain health.

In a study with toasted and freeze-dried baru almonds by Fraguas et al. (2014), higher levels of phosphorus, magnesium, zinc and iron were detected. However, the levels of potassium, calcium, manganese, sulfur and copper were lower when compared to this research. This author justifies that the differences between the mineral contents can be associated to factors such as soil composition, degree of seed maturation, harvest season, among others.

Quality Parameters

The pH, total soluble solids, total titratable acidity, ratio, total sugars, peroxide index and acidity index contents are described in Table 3.

The pH of the baru almonds ranged from 6.08 to 6.24 and showed no difference between treatments (P>0.05), confirming that the application of different drying processes did not interfere in this attribute. These results are in agreement with the one detected by Martins

(2006) that carried out physical-chemical analysis of baru almonds and reported pH values of 6.09.

As for the total soluble solids parameters expressed as °Brix and total sugars, which represent the water-soluble compounds, these were considered high and did not differ statistically ($P>0.05$), evidencing also that the drying processes did not alter the sugar content present in the almond. Moreover, the presence of soluble solids in food is directly related to the profitability of food processing companies, since the amount of sugar to be added in the products is reduced. Thus, it is concluded that the baru almond presents attributes of sweetness favorable to the development of food products.

The titratable acidity of the baru almonds was also stable ($P>0.05$) with the use of drying processes and according to the authors Chitarra and Chitarra (2005), the acidity of a food is given by the presence of the organic acids that serve as substrates for respiration and the variation of this parameter may influence the quality characteristics. Lemos et al. (2016), when evaluating the acidity of the baru almond, verified a significant increase ($P<0.05$) in the acidity after the roasting process and attributed this result to the occurrence of intracellular hydrolytic reactions.

The *ratio*, consisting of the sugar/acid *ratio*, presented values of 90.7, 92.7 and 80.9 respectively for Tin, T65 and T105, and did not differ statistically ($P>0.05$). This relationship is an important characteristic in determining the taste and texture of the food. It is also a sensorial and commercial indicator of maturity because with the development of the food, the sugars are degraded and the sugar/acid *ratio* reaches a higher value.

As to the contents of peroxides, no difference was detected ($P>0.05$) between the oils extracted from the baru almonds, which showed an average of 13.60 meq. Kg^{-1}. This result indicates that the drying process does not influence this parameter, besides, the values obtained are lower than the maximum limit established by the ANVISA which is of 15 $meq.kg^{-1}$ (Brasil, 2005). The peroxides are the first compounds formed during the process of oxidative degradation of oils, when the unsaturated fatty acids react with oxygen atmospheric. Their determination reflects, therefore, the state of conservation of the oil (Machado et al., 2006).

With respect to the acid index, the levels obtained ranged from 1.75 to 3.52 $mg.KOH.g^{-1,}$ of oil, below the values established by the Commission Codex Alimentarium (2006), which determines how quality parameter for crude oils, maximum acidity of 4.0 $mg.KOH.g^{-1}$. Almond oil of baru who was exposed to the largest drying process (T105) suffered greater changes in relation to acidity, while the oil that has not been subjected to heat

(Tin) had the smallest detected concentrations. However, it can be said, all treatments meet the standards guaranteeing the quality of the product in question.

Conclusion

In the experimental conditions of the present study, the results showed that the application of different drying processes (65 °C and 105 °C for 30 minutes) did not alter the physico-chemical, mineral and quality composition of baru almonds (except moisture and acidity indexes that decreased and increased respectively with the use of the temperature of 105 ºC for 30 minutes). It has been found that all treatments are sources of proteins, dietary fiber, lipids, boron, copper, manganese, iron, sulfur, calcium and magnesium and present approximately 50% of the daily recommendation. It was also detected that all treatments are below the maximum limits established by the inspection bodies for the presence of acidity and peroxides, indicating the quality of these products. As sources of micro and macro nutrients, it is estimated that regular consumption of baru almonds (regardless of the drying process) can contribute to a balanced diet that meets the needs of consumers.

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Disclosure statement

These is no conflict of interest in this work.

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Tables and Figure

Table 1: Treatments evaluated in the study

	Tin	T65	T105
Treatments [1]	"In natura"	65 °C per 30 minutes	105 °C per 30 minutes

[1] Treatments: Tin (almond "in natura"), T65 (almond submitted to 65 ºC for 30 minutes) and T105 (almond submitted to 105 ºC for 30 minutes).

Table 2: Values of moisture, lipids, ashes, proteins, carbohydrates and total dietary fiber present in baru almonds submitted to different drying processes [1]

	Treatments [2]			
Macronutrients ($g.100g^{-1}$)	Tin	T65	T105	*P*
Moisture	6.63±2,31[a]	5.10±0,40[b]	2.64±0,47[c]	0.0001
Lipids	31.73±2,09	32.59±1,26	34.16±2,12	0.2635
Ash	1.55±0,30	1.87±0,12	1.77±0,10	0.4791
Proteins	22.96±0,32	24.16±2,64	24.23±2,22	0.6970
Carbohydrates [3]	37.13±0,54	36.28±0,22	37.20±0,32	0.3934
Total fibers	14.44±0,98	14.22±0,24	15.22±0,52	0.2453
Micronutrientes ($mg.100g^{-1}$)	Tin	T65	T105	*P*
Boron	2.4±0.87	2.6±0.75	2.3±1.34	0.0727
Copper	2.8±0.64	2.7±0.39	2.6±1.98	0.4219
Maganese	6.4±0.97	6.5±0.78	6.1±0.69	0.2315
Iron	6,5±0.10	6.4±0.76	6.1±0.15	0.1105
Sulphur	380±0.30	370±0.87	360±1.7	0.2102
Potassium	1810±0.69	1810±0.98	1620±0.39	0.3001
Calcium	240±0.49	230±0.10	210±1.10	0.4029
Magnesium	330±0.67	340±0.76	330±1.91	0.2471

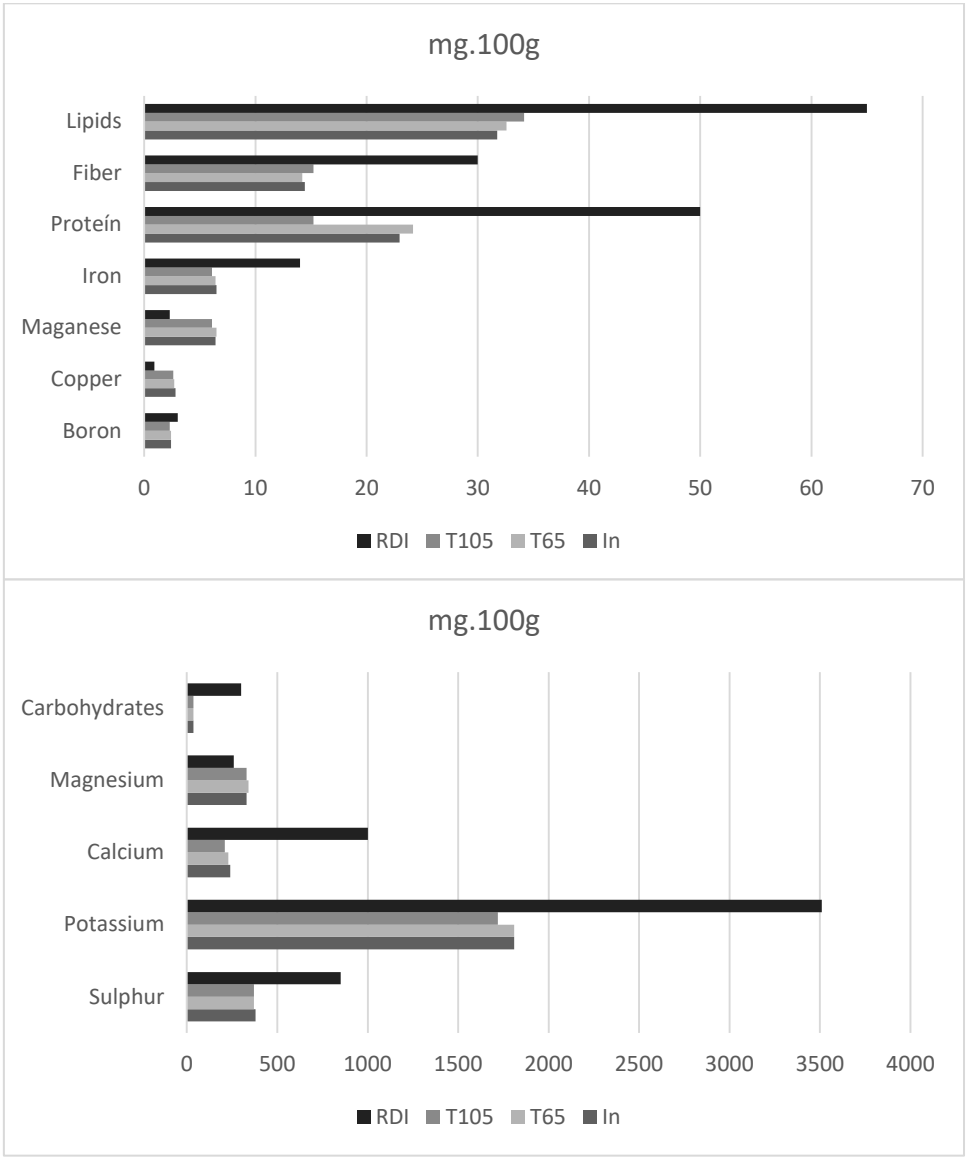
Note: Mean values with distinct letters in the same column differ from each other ($P < 0.05$). [1] Mean ± standard deviation; [2] Treatments: Tin (almond "in natura"), T65 (almond submitted to 65 ºC for 30 minutes) and T105 (almond submitted to 105 ºC for 30 minutes); [3] Calculated from the difference to 100 of the sum of ash, lipids, protein and moisture. *P*: p-value.

Table 3: Values of pH, total soluble solids, total titratable acidity, *ratio*, total sugar, peroxide value and acid value present in baru almonds submitted to different drying processes[1]

Treatments [2]	pH	Total soluble solids [3]	Total titratable acidity [4] ($g.100g^{-1}$)	*Ratio* [5] ($g.100g^{-1}$)	Total sugar ($g.100g^{-1}$)	Peroxide index ($meq.kg^{-1}$)	Acid index ($mg.KOH.g^{-1}$)
Tin	6.17±0.13	45.33±1.16	0.56±0.37	80.95±2.30	9.70±1.39	13.20±1,.39	1.75±1.39[a]
T65	6.24±0.04	46.00±2.00	0.49±0.68	92.75±1.04	10.09±2.45	13.36±0.45	2.95±2.45[b]
T105	6.08±0.08	47.33±1.16	0.52±0.87	90.67±2.21	8.85±1.19	14.23±1.19	3.52±1.19[c]
P	0.1819	0.3170	0.1952	0.1072	0.6961	0.304	0.0001

Note: Mean values with distinct letters in the same column differ from each other ($P < 0.05$). [1] Mean ± standard deviation; [2] Treatments: Tin (almond "in natura"), T65 (almond submitted to 65 ºC for 30 minutes) and T105 (almond submitted to 105 ºC for 30 minutes); [3] Expressed in ºBrix; [4] total titratable acidity expressed as % oleic acid; [5] Relationship between total soluble solids/total titratable acidity; *P*: p-value.

Fig. 1: Relationship between the composition of micro and macro nutrients of baru almonds submitted to different drying processes and the Recommended Daily Intake



Note: Treatments: Tin (Almond "in natura"), T65 (Almond subjected to drying at 65 °C per 30 minutes) and T105 (Almond subjected to drying at 105 ºC per 30 minutes); [3] RDI (BRASIL, 2005); Values in mg.100g.

ARTIGO 2 – FATTY ACIDS, PHENOLIC PROFILE, TOCOPHEROLS AND ANTIOXIDANTS ACTIVITY OF BARU ALMONDS (*Dipteryx alata* VOG.): EFFECTS OF DRYING PROCESS

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Fatty acids, phenolic profile, tocopherols and antioxidants activity of baru almonds (*Dipteryx alata* Vog.): effects of drying process

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SUMMARY: This study carried out a chromatographic and spectrophotometric characterization in the presence of bioactive compounds, antioxidants, phenolics, tocopherols, sterols and fatty acids in Baru almonds *in natura* and submitted to drying processes. It was detected in Baru *in natura* almond presented high levels of phenolic compounds, vitamin C, antioxidants, phenolics, sterols, total monounsaturated fatty acids and low thrombogenic, and atherogenic indexes. During the process of drying it at 65 °C for 30 minutes, a decrease was noted in the levels of caffeic acid, chlorogenic acid, anthocyanins, p-coumaric acid, ferulic acid, o-coumaric acid, quercetin, polyunsaturated fatty acids. The same condition resulted in an increase in the levels of gallic acid, rutin, catechin, trans-cinnamic acid, vanillin, m-coumaric acid, tocopherols, monounsaturated fatty acids and antioxidant activity (ORAC and DPPH•). When submitted to a temperature of 105 ° C for 30 minutes it presented the same behaviour, however it influenced the reduction of vitamin C and ORAC content and increased the presence of flavonoids.

KEYWORDS*: Antioxidant activity; Bioactive compounds; Brazilian Cerrado; High-Performance Liquid Chromatography; Oilseeds.*

RESUMEN: Este estudio realizó una caracterización cromatográfica y espectrofotométrica de la presencia de compuestos bioactivos, antioxidantes, fenólicos, tocoferoles, esteroles y ácidos grasos en almendras del tipo baru *in natura* y sometidos a procesos de secado. Se detectó, en la almendra de baru *in natura*, altos contenidos de compuestos fenólicos, vitamina C, antioxidantes, fenólicos, esteroles, ácidos grasos monoinsaturados totales y bajos índices de trombogénicos y aterogénicos. Durante el proceso de secado a 65 °C durante 30 minutos, se observó una disminución en los niveles de ácido cafeíco, ácido clorogénico, antocianinas, ácido *p*-cumárico, ácido ferúlico, ácido *o*-cumárico, quercetina, ácidos grasos poliinsaturados. La misma condición resultó en un aumento en los niveles de ácido gálico, rutina, catequina, ácido trans-cinámico, vanilina, ácido *m*-cumárico, tocoferoles, ácidos grasos monoinsaturados y actividad antioxidante (ORAC y DPPH•). Cuando se sometió a una temperatura de 105 °C durante 30 minutos, presentó el mismo comportamiento, sin embargo, influyó en la reducción del contenido de vitamina C y ORAC y aumentó la presencia de flavonoides.

PALABRAS CLAVE*:* Actividad antioxidante; Cerrado Brasileño; Compuestos bioactivos; Cromatografía líquida de alto rendimiento; Semillas oleaginosas.

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1. INTRODUCTION

Drying is one of the oldest processes used by man for preserving food. Due to reduced water activity, there is a minimization of microbial growth that enables the increase the shelf life of raw materials. It can facilitate and reduce costs of packaging, transport and storage, in addition to bringing improvements in sensory aspects (especially when it comes to oleaginous seeds since the majority, when in natura, have a bitter and astringent taste due to the presence of tannins), inhibiting the antinutritional factors and providing raw material throughout the year (Igual *et al*., 2012).

Despite the benefits, it is estimated that drying can contribute to the reduction of the functional activity of the food, because the vast majority of bioactive compounds exhibit heat-sensitive behaviour (Lemos *et al*., 2012; Lemos *et al*., 2016). Favoring this scenario, there is a high incidence of producers that improperly employ this technique in their raw materials. As a result, there is a distribution of products with compromised functional activity, and among food, the oil seeds are the most affected, as these employ this method of conservation.

In order to maintain the nutritional quality of food, verification of bioactive compounds changes during the drying process must be checked. This finding is based on the fact that recent research has linked daily consumption of oilseeds with reducing the incidence of chronic non-communicable diseases, which in turn is provided by the presence of biologically active substances (Liu *et al*., 2019).

In this context, the baru almond (*Dipteryx alata* Vog.), despite having limited information, stands out for its superiority in nutritional composition (Fraguas *et al*., 2014; Lemos *et al*., 2016). Its contains high levels of lipids, proteins, amino acids, features efficient digestibility, has high content of minerals (such as calcium, iron, magnesium, potassium and zinc) and dietary fiber (Fraguas *et al*., 2014). Besides, being found in regions with high sun exposure, plant defense system promotes the protection of the same through the biosynthesis of secondary compounds that present bioactive characteristics. These substances have the ability to minimize the action of free radicals by

interception of active oxygen (responsible for oxidative damage in cell membranes and DNA), and they can assist in the prevention of chronic non-communicable diseases (Lemos *et al*., 2012).

Although the aforementioned benefits, the research is limited to investigate specific compounds that exhibit characteristics desired in minimizing chronic non-communicable diseases, such as, profiles of fatty acids and phenolics and tocopherols compounds. Thus, analyzing the possibility of a complete and efficient use of baru almond and craving to meet new functional properties of the same, it is necessary a detailed research on the presence of different bioactive compounds, such as phenolic metabolites, fatty acids, tocopherols, among others. “In addition, it is necessary to check the effectiveness of the two drying processes (65 and 105 ° C for 30 minutes) which are the most used by the producers of this food, mainly because the vast majority of these foods reach the end consumer in this condition”.

Therefore, in order to extend the knowledge about the functional potential of compounds and investigate possible changes resulting from the processing, the present study sought to carry out a chromatographic and spectrophotometric characterization regarding the presence of bioactive compounds, antioxidants, sterols and phenolic profiles, tocopherols and fatty acids present in baru almond *in natura* and subject to processes of dryings.

2. MATERIALS AND METHODS

2.1. Reagents, materials and experimental design

All chemicals, reagents and solvents used were of analytical grade or HPLC and obtained from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (> 18 MΩ.cm) was obtained from a Milli-Q system (Millipore, Brussels, Belgium).

The almonds of baru were obtained from the Cerrado Biome region, located in the city of Barra do Garças-MT, during the harvest season (between August and September 2016). The baru

almond has the following centesimal composition (g/100g): 6.63 moisture, 22.96 protein, 31.73 lipid, 14.44 dietary fiber and 1.55 ashes grams.

For this work, we used the completely randomized experimental design with three replications. Three treatments were evaluated: baru almonds *in natura* (T1 – which was the control of the drying experiment), baru almonds submitted to drying at 65 °C for 30 min (T2) and baru almonds subjected to drying at 105 °C for 30 min (T3). The temperatures were chosen because they are the most used by the producers of these oleaginous plants, necessitating an investigation on the effectiveness of this drying process. In addition, it is known that the roasting process compromises part of the nutritional and bioactive value of the food, and therefore to use lower temperatures and that are effective in drying the almond baru and that preserve the nutrients, can become a new form of use and consumption of this food.

2.2. Drying process of almonds

The drying of the baru almond occurred in forced air circulation oven (Marconi, MA0351, Piracicaba, Brazil), then it underwent the grind (20 mesh - in experimental mill, Viti Molinos, VG 2000i, Itajaí, Brazil), packed in polyethylene containers and stored at 25 °C (oven model Eletrolab, EL202). During the drying processes (time and temperature) were chosen because there are still no investigations using them. The moisture contents of the almonds were determined after the drying process (AOAC, 1990) and the averages were 6.63, 5.10 and 2.64 $g.100g^{-1}$ respectively for T1 (corresponds to the moisture of the almond in the natural state) T2 and T3. All results of the analytical determinations described below (including moisture) were expressed on a wet basis.

2.3 Determination of bioactive compounds

We evaluated the content of total phenolic compounds, anthocyanins, flavonoids, tannins and vitamin C. The hydroalcoholic extract was prepared according to methodology adapted from Milardovic *et al.* (2006).

Total phenolics were determined by the *Folin–Ciocalteu* reagent method, using gallic acid as the standard for the calibration curve. The absorbance was measured at 765 nm in a spectrophotometer (UV-Visible 50 Probe-Cary) and results were expressed in mg of gallic acid equivalent (GAE) 100 g^{-1} (Lemos, 2012).

The total phenolic compounds were also evaluated by the use of diazonium salt Fast Blue BB, using standard gallic acid for the calibration curve. The absorbance was measured at 420 nm in spectrophotometer (UV-Visible 50 Probe-Cary) and results were expressed in milligrams gallic acid equivalent (GAE) per 100 g^{-1} (Palombini *et al*., 2016).

Monomeric anthocyanins were determined by differential pH method described by Giusti and Wrolstad (2001), using spectrophotometer (UV-Visible 50 Probe-Cary) for absorbance measurements of samples (510 and 700 nm) and the results were calculated as malvidin-3,5-diglucoside per 100 g^{-1}.

Flavonoids were performed following the methodology described by Fraguas *et al.* (2014). The reading was held at 415 nm spectrophotometer (UV-Visible 50 Probe-Cary) using 2% solution of aluminum chloride in methanol. Total flavonoids values were expressed as equivalent of catechin per 100 g^{-1}.

Tannins were measured by colorimetric method according to Association of Official Analytical Chemists (AOAC, 1990). The method was based on the intensity of blue color produced in reducing the *Folin-Denis* reagent for phenols, and was then measured in spectrophotometer (UV-Visible 50 Probe-Cary) to 760 nm expressed as equivalent of catechin per 100 g^{-1}.

Vitamin C was performed by colorimetric method using 2,4-dinitrophenylhydrazine and the results have been read on spectrophotometer (UV-Visible 50 Probe-Cary) to 520 nm and expressed in equivalent of ascorbic acid per 100 g^{-1} (Milardovic *et al.*, 2006).

2.4 Individual identification of phenolic compounds by HPLC-DAD

Individual identification of phenolic compounds was performed following the methodology described by Ramaiya *et al*., (2013). Quantification and identification of these phenols compounds was performed in liquid chromatography (HPLC-DAD/UV-Vis) model Shimadzu (Shimadzu Corp., Kyoto, Japan) equipped with a gradient pump (2487 Serie), a valve injector with a loop of 50 µL, a degasser (Waters 200 Series) and an integrator-plotter with a software (Total Chrom, Waters). Phenolic compounds was separated in C:18 reversed-phase column (150 mm × 4.6 mm I.D., 5 µM), (Phenomenex, CA, USA) with a C:18 (20 mm × 4.6 mm I.D.) pre-column cartridge. The mobile phase consisted of 2% (v/v) acetic acid in deionized water (mobile Phase A) and 70:28:2 (v/v) methanol/water/acetic acid (mobile phase B), and phenolics were detected at 280 nm. Phenolic compounds were identified by comparison of retention times with standards (gallic acid, catechin, chlorogenic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, *o*-coumaric acid, trans-cinnamic acid, quercetin and rutin). The results were expressed as mg of phenolic compound in 100 g^{-1} of fresh weight.

2.5 Screening of antioxidant activity

Test of elimination of free radicals (DPPH•)

The antioxidant activity was evaluate by DPPH• scavenging actitivy ability of antioxidant activity. Absorbance was performed using UV-Visible spectrophotometer (50 Probe–Cary) at 517 nm expressed as % of free radical sequestration. Trolox was used to construct the standard curve (Milardovic *et al*., 1943).

Antioxidant activity via β-carotene/linoleic acid System

Antioxidant activity determination by β-carotene/linoleic acid system was conducted according to the methodology described by Miller (1971) and absorbance was measured to 470 nm

with spectrophotometer (UV-Visible 50 Probe-Cary). The results were expressed as percentage of inhibition of β-carotene oxidation.

Oxygen Radical Antioxidant Capacity (ORAC) assay

The ORAC method used, with fluorescein (FL) as the "fluorescent probe", was performed as described by Aazza *et al.* (2011).

2.6 Determination of total sterols, tocopherols and fatty acids

We evaluated the levels of total sterols, tocopherols and fatty acids present in almond of baru. To carry out these analyses, it was necessary to extract the oil present in the almond baru.

2.6.1 Process of almond oil extraction baru

To perform the analyses mentioned above, it was necessary to carry out the almond oil extraction from baru. For this procedure was used a Extractor Oster (yoda model 60 Hz) with the following specifications: rated power-400W, Turbo-electric heating mode heat. Subsequently, the oil was transferred to amber jars and kept in refrigeration equipment (4 °C). Because it is a method that extracts lipids in cold, this was the chosen one.

Total sterols

For the reading of the samples were added 0.8 mL of reagent of *Lieberman-Burchard*, 0.1 g of the sample and 3.1 mL^{-1} of chloroform, leaving to stand for 12 min. The solution was ready in the 625 nm spectrophotometer (UV-Visible 50 Probe-Cary), using chloroform as white (Kenny, 1952).

Identification of tocopherols by HPLC-DAD

For the determination of tocopherols (α and γ), (0.08 g) of the obtained oil was dissolved in 4.0 mL of 2-propanol. The analysis was performed by liquid chromatography (HPLC-DAD/UV-Vis) model Shimadzu (Shimadzu Corp., Kyoto, Japan) equipped with a gradient pump (2487 Series), a valve injector with a loop of 50 µL, a degasser (Waters 200 Series) and an integrator-plotter with a software (Total Chrom, Waters). Vitamin E was separated at C:18 reversed-phase column (150 mm × 4.6 mm I.D., 5 µM), (Phenomenex, CA, USA) with a C:18 (20 mm × 4.6 mm I.D.) pre-column cartridge. The mobile phase consisted of a mixture of methanol (96%) and water (4%) using the isocratic solvent and system with a flow of 1.0 mL min. Detection was made at 292 nm. Quantification of α and γ tocopherol was performed using external standard method. The results were expressed in mg of tocopherol 100 g^{-1} (Freitas *et al*., 2008).

Profile of fatty acids by CG-FID

For the analysis of fatty acid profile, the lipids were extracted according to the procedures described by Folch *et al*., (1957). The analysis was performed by gas chromatography on a Shimatzu CG 2010 chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with a flame ionization detector, split injection at the rate of 1:50 and capillary column SPTM-2560 Supelco, 100 m × 0.25 mm × 0.20 µm (Supelco Inc., Bellefonte, PA, USA). The initial temperature of the column was of 140 °C, maintained for 5 min, changing to 240 °C with increment of 4 °C, maintained by 30 min for a total of 60 min. The injector and detector were kept at the temperature of 260 °C and used the helium as carrier. The acids identified fat compared to the retention times presented by chromatographic pattern SupelcoTM37 pattern FAME Mix (Supelco Inc., Bellefonte, PA, USA) and are expressed in percentage (%) of the total fatty acids. Later were grouped them in: saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The atherogenic index (AI) and thrombogenic index (TI) were determined in accordance with the specified by Ulbricht and Southgate (1991), as the Eq. (1 and 2):

$$AI: [(C12:0+(4\times C14:0)+C16:0)]/(\Sigma MUFAs+\Sigma\Omega 6+\Sigma\Omega 3) \quad (1)$$

$$TI:(C14:0+C16:0+C18:0)/[(0.5\times\Sigma MUFAs)+(0.5\times\Sigma\Omega6)+(3\times\Sigma\Omega3)+(\Sigma\Omega3/\Sigma\Omega\text{-}6)] \quad (2)$$

The *ratio* of fatty acids hypocholesterolemic and hypercholesterolemic (h/H) was calculated according to the formula described by Santos-Silva (2002) and shown in Eq. (3):

$$h/H:(C18:1+C18:2+C20:4+C18:3+C20:5+C22:5+C22:6)/(C14:0+C16:0) \quad (3)$$

2.7 Statistical Analysis

The results were submitted to analysis of variance (ANOVA) and test for comparison of averages (Tukey 5% the probability of error). Statistical calculations were performed using the program R® version 5.0. The differences were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Determination of bioactive compounds present in baru almonds

The results of the determinations of total phenolics, monomeric anthocyanins, flavonoids, tannins and vitamin C of baru almonds are displayed in Table 1.

Table 1. Average values of total phenolics (measured by *Folin Ciocalteau* methods and Fast Blue BB), monomeric anthocyanins, total flavonoids, tannins, vitamin C, ORAC, DPPH and β-carotene/linoleic acid present in baru almonds submitted to different drying processes [1]

	Treatments [(2)]			
Analytical Determinations	**T1**	**T2**	**T3**	**P**
TPC (*Folin Ciocalteau*) GAE [3] (mg 100 g^{-1})	1254.12±39.71	1175.23±35.15	1306.34±33.18	0.6229
TPC (Fast Blue BB) GAE [3] (mg 100 g^{-1})	179.14±1.54	167.85±1.42	186.56±1.26	0.5698
Monomeric Anthocyanins (mg 100 g^{-1})	0.38±0.02^{a}	0.32±0.01^{b}	0.25±0.02^{c}	0.0006
Total Flavonoids QE [4] (mg 100 g^{-1})	9.17±0.45^{b}	8.01±1.46^{b}	15.73±0.45^{a}	0.0001
Tannins CE [5] (g 100 g^{-1})	1.51±0.87	1.62±0.65	1.67±0.89	0.1567
Vitamin C [6] (mg 100 g^{-1})	39.14±0.43^{a}	37.85±0.79^{a}	35.23±0.31^{b}	0.0004
ORAC [7] (uM g^{-1})	4.06±0.76^{a}	3.43±0.98^{b}	2.96±0.45^{c}	0.0029
DPPH• (% SRL)	69.02±2.86^{b}	79.68±3.78^{b}	84.38±3.98^{a}	0.0056
β-carotene/linoleic acid system (% protection)	91.72±3.35	89.94±10.52	86.10±1.42	0.5738

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p < 0.05$); [2] Treatments: T1 (Almond *in natura* T2 (Almond subjected to drying at 65 °C per 30 minutes) and T3 (Almond subjected to drying at 105 °C per 30 minutes); [3] TPC: Total Phenolics Compounds; GAE: Gallic acid equivalent; [4] Equivalent of rutin; [5] Equivalent of catechin; [6] Expressed in mg of ascorbic acid; [7] Equivalent of trolox; *p: p*-value.

It was observed that the drying processes have not altered the total phenolics and tannins because these compounds remained unchanged ($p > 0.05$), thus showing thermal stability to these temperatures. When applied to the process of 65 °C for 30 min (T2), a significant reduction ($p < 0.05$) was detected only in the presence of monomeric anthocyanins, which proves these molecules are sensitive to such drying process. When using 105 °C for 30 min (T3) were reduced ($p < 0.05$) in addition to the anthocyanins and vitamin C, although the level of flavonoids has increased ($p < 0.05$).

In reference to the means obtained on determination of phenolic compounds, it is demonstrated the predominance of these substances in baru almonds, which in turn, exceed the values reported to other nuts (with an average between 32 to 420 mg 100 g^{-1} for macadamias, cashews, walnuts, hazelnuts and peanuts) (Kornsteiner *et al*., 2006). The processes of dryings is found not to influence this content and this behavior can be associated with the absence of oxidative reactions by reactive oxygen species. Discrepant behavior was found by Lin *et al.*, (2016), who studying the effect of drying on almonds (150 °C for 20 min) have found that the phenolic compounds were reduced. Pasqualone *et al*. (2018), when studying the drying process of almond by-products, also found a significant reduction in the presence of phenolic compounds in these foods.

On the other hand, the use of Fast Blue BB method provided a reduction in the detection of these phenolic substances, demonstrating difference between the two processes. This evidence is attributed to the fact that this procedure is specific and unique in the quantification of phenols, for reagents used do not complex with other substances, such as proteins, sugars and other compounds like ascorbic acid reducers (as occurs in the *Folin Ciocalteau's* method) (Naczk and Shahidi, 2004). Soon, it is noted that this methodology presents greater precision for determining these substances.

As regards the quantification of monomeric anthocyanins in baru almonds, a considerable reduction ($p < 0.05$) was observed (15.78% and 34.21% respectively in T2 and T3). It is observed that the higher the temperature of the drying process, the greater the degradation of these substances. A negative coexistence between the temperature/oxygen interaction is evidenced, being that combination detrimental to the maintenance of these molecules.

Higher flavonoids content was found in treatment T3 presented when compared to T1 and T2, indicating that the process of drying at 105 °C per 30 min increased ($p < 0.05$) 72% in the presence of these molecules. The increase of bioactive compounds, such as flavonoids, may be related not only to the degradation of polymeric polyphenols and flavonoids glycosylated hydrolysis (Lemos *et al*., 2012) but also to the *Maillard* products reaction (Liu, Kitts, 2011). Besides, the intracellular water evaporation can change the lignocellulosic structure, in addition to promoting the denaturation of proteins, resulting in increased availability of compounds active in the array (Lemos *et al*., 2012). Similar behavior was found by Lin *et al.*, (2016), who concluded that the roasting process (200 °C for 20 min) can contribute to the increase of 124% of the content of flavonoids in almonds.

With respect to tannins, considerable presence was observed in baru almonds and the drying processes were found not to promote a significant reduction ($p > 0.05$) thereof. The abundant consumption of this substances should be controlled, because the exploitation of minerals and proteins causes antinutritional effects. However, as the consumption of *in natura* baru almonds is not a practice mostly due to the sensory aspect it presents in this condition (bitter and astringent), the application of heat becomes an important feature that aims to improve the sensory aspects and inactivate the antinutritional substances. Despite this information, recent researches indicate that these substances present antimutagenic potential, which, in turn, are related to their antioxidant potential, being effective in protection from oxidative damage (Macáková *et al*., 2014).

While in the concentration of vitamin C, the drying process at 105 °C per 30 min was found to cause a decrease ($p < 0.05$) of 9.98% in T3 that can be assigned to a likely oxidation of this vitamin, which by its biologically active nature, is unstable and reversibly oxidized to Ldehidroascórbic acid. Even with the reduction occurred, it was found that the treatments correspond to a high content of vitamins. According to the Dietary Guidelines for Americans (McGuire, 2011), the recommendation of daily vitamin C intake is 75 mg for women and 90 mg for men over 30 years old. These levels are based on their physiological functions and antioxidants needed to benefits the physiological organism. In this regard, when consumed 100 g of baru almonds, treatments T1, T2 and T3 may represent respectively 52.2, 46.9 and 50.5% of the recommendation for women, and, 41.4, 43.5 and 39.2% for

men. It is found that this almond is a food with high levels of this nutrient. Important to note that Fatin and Azrina (2017) have found lower results of vitamin C in fresh lime (27.78 mg 100 g^{-1}), who are one of the referenced foods concerning this vitamin, allowing in this way that there is favoritism in baru almonds consumption for the supply of possible vitamin deficiencies.

3.2 Screening of antioxidant activity

Test of elimination of free radicals (DPPH•)

The results obtained in the total antioxidant levels analyzed by DPPH• method were respectively 69.02, 79.68 and 84.38% free radical scavenging capacity for T1, T2 and T3 (Table 1). Showing that the drying process at 105 °C per 30 min promoted a significant reduction ($p < 0.05$) in T3, decreasing 36.05% of DPPH• radical scavenging capacity in comparison with baru almonds *in natura*. However, almond T2 remained stable when compared to (T1) control, therefore, it is reasonable to conclude that the drying process held at 65 °C for 30 min have not reduced the presence of antioxidants in almonds. It is found that more intense heat treatments can be responsible for the loss of antioxidants. Lemos *et al*. (2012), explain that during the heating part of the moisture is lost through evaporation and *maillard* reaction occurring increases the antioxidant capacity of the almond. It is appropriate to point out that higher values of free radical scavenging capacity indicate a lower antioxidant activity. Some compounds do not react facing the DPPH• free radical, because they are lipophilic and, therefore, there is a need to carry out different methodologies that seek to characterize such components.

Antioxidant activity via β-carotene/linoleic acid System

The total antioxidant activity was also measured by β-carotene/linoleic acid system and the average value obtained among treatments was 89.24% protection without statistical difference ($p > 0.05$) (Table 1). These results indicated that the baru almonds presented high levels of these

components and heat-resistant behavior. These data present a recognized importance because oxidative processes can be avoided through the use of antioxidants with prevention or decrease in triggering oxidative reactions. Moreover, this method is suitable for the investigation of lipophilic antioxidants and fits to the constitution of baru almonds especially due to the high lipid content.

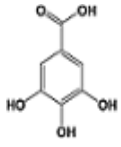
Oxygen radical activity capacity (ORAC) assay

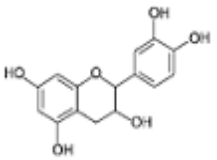
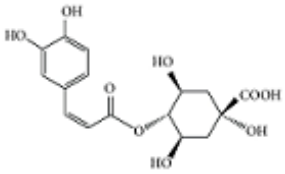
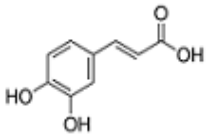
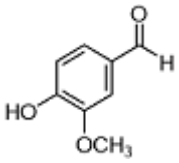
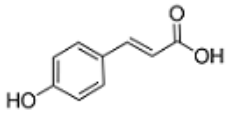
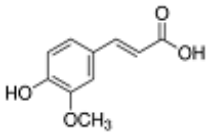
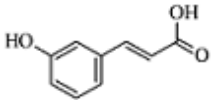
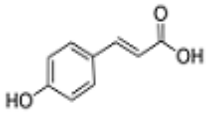
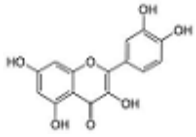
In the ORAC method it was possible to observe that the baru almond *in natura* showed to be significantly ($p > 0.05$) more effective as an antioxidant when compared to the other treatments (Table 1). As they are heat-sensitive substances, processes of dryings have contributed to the reduction of the same. As this essay is based on hydrogen atoms transfer reaction using a free radical source predominant in human biology (peroxyl radical), the ORAC test is relevant to express the antioxidant capacity of a given substance under *in vivo* conditions.

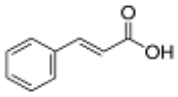
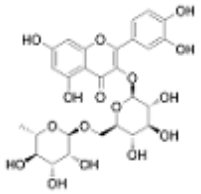
3.3 Individual identification of phenolic compounds by HPLC-DAD

A total of twelve phenolic compounds were detected in the three treatments, and the results associated with the identification and quantification of these molecules are shown in Table 2.

Table 2. Identification and quantification via HPLC-DAD/UV-Vis of phenolic compounds present in baru almonds submitted to different drying processes [1]

		Treatments (mg 100 g^{-1}) [2]			
Phenolic Compound	**Chemical Structure**	**T1**	**T2**	**T3**	**P**
Gallic Acid		45.83±0.69^{c}	47.18±1.16^{b}	48.90±1.28^{a}	0.0001

Catechin		9.01±1.40[c]	9.98±0.52[b]	11.06±0.57[a]	0.0001
Chlorogenic Acid		7.33±0.87[a]	7.23±0.62[b]	6.80±0.98[c]	0.0001
Caffeic Acid		19.89±1.17[a]	11.94±1.09[b]	11.68±1.62[c]	0.0001
Vanillin		7.56±0.01[c]	9.18±0.33[b]	10.17±0.50[a]	0.0001
P-coumaric		0.43±0.23[a]	0.32±0.22[b]	0.30±0.02[c]	0.0001
Ferulic Acid		1.17±0.62[a]	0.98±0.31[b]	0.30±0.02[c]	0.0001
M-coumaric		0.86±1.09[c]	0.93±1.22[b]	1.01±1.35[a]	0.0002
O-coumaric		9.24±2.01[a]	8.78±0.98[b]	8.54±0.20[c]	0.0001
Quercetin		1.60±0.25[a]	1.48±0.19[b]	1.45±0.22[b]	0.0002

Trans-Cinnamic Acid		9.12±0.27[c]	9.41±1.44[b]	10.12±1.70[a]	0.0001
Rutin		17.81±1.40[c]	17.89±1.04[b]	18.40±0.74[a]	0.0001

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p < 0.05$); [2] Treatments: T1 (Almond *in natura*), T2 (Almond submitted to 65 °C per 30 minutes) and T3 (Almond submitted to 105 °C per 30 minutes); *p*: *p*-value.

Between phenols identified by high-performance liquid chromatography, gallic acid was the main phenolic compound detected in baru almond. It should be noted that the high content of these substances in food, more specifically in almonds, are desired because they provide health benefits. Gallic acid, which has a strong capacity of free radicals, is effective in reducing disease, and research indicates that it is able to induce apoptosis and cytotoxic and antiproliferative effect between different strains of tumor cells (Guimarães *et al*., 2007). As the drying process has increased these substances (which was an increase of 1.07 times), we can infer that the benefits to be achieved through the ingestion of these molecules can be enhanced. Lin *et al*., (2016) have also observed a significant increase in the presence of gallic acid in almonds dried at 150 °C for 30 min. However, this increase was 13 times bigger (when compared with the raw almond) and the end content of this substance was 52 mg 100 g^{-1}.

It was found that other components, such as catechin, vanillin, trans-cinnamic acid, *m*-coumaric acid and rutin, increased ($p < 0.05$) with the drying process (when compared with the *in natura* treatment), and this increase was respectively 1.22, 1.34, 1.19, 1.17 and 1.03%. One possible explanation for this increase is associated with the changes that might have occurred on the protein of

phenolic compounds, causing your exposure and the consequent increased availability (Lemos *et al*., 2012). The thermal processing is responsible for increasing the phenolic content due to the increase in the number of free phenolic groups resulting from hydrolysis of glycosylated flavonoids that are released from the phenolic cell walls (D'archivio *et al*., 2010). Rodríguez-Bencomo *et al.* (2015), when studying the effect of roasting process (160 °C for 20 min) in pistachios, have found an increase of 17, 79 and 81% in the presence of chlorogenic acid, rutin and catechin. It was also found that the drying (when compared to T1 with T3) promoted reduction ($p < 0.05$) of the presence of chlorogenic acid, caffeic, *p*-coumaric acid, quercetin, ferulic and *o*-coumaric acid in respectively 7.2, 41.3, 30.2, 9.4, 74.3 and 7.57%. The decrease of these substances can be attributed to the effects of heat, which may have caused possible protein denaturation, resulting from the disruption of covalent links (Shahidi and Yeo, 2016).

3.4 Determination of total sterols, tocopherols and fatty acids

Total sterols

Determination of contents of baru almond total sterols subjected to different processes of drying are shown in the Table 3.

Table 3. Average of total sterols and tocopherols present in the baru almond submitted to different drying processes [1]

	Treatments [(2)]			
Analytical Determinations	T1	T2	T3	P
Total sterols (mg 100 g^{-1})	427.34±14.27	432.76±13.59	439.94±24.68	0.7227

α- tocopherol (mg kg^{-1})	0.50±0.02[c]	0.82±0.23[b]	0.94±0.04[a]	0.0001
γ- tocopherol (mg kg^{-1})	1.47±0.32[b]	1.33±0.02[b]	2.33±1.75[a]	0.0025
∑ α + γ tocopherol (mg kg^{-1})	1.97±0.23[c]	2.15±0.35[b]	3.27±0.10[a]	0.0001

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p < 0.05$); [2] Treatments: T1 (Almond *in natura*), T2 (Almond submitted to 65 °C per 30 minutes) and T3 (Almond submitted to 105 °C per 30 minutes); p: *p*-value.

The total sterol content of baru almond received an average of 432.66 mg of 100 g^{-1} without statistical difference ($p > 0.05$) between the treatments, and these results may demonstrate that the drying process does not influence these molecules. For bringing health benefits, it is recommended to eat between 1 to 2 g of plant sterols a day. In this regard, the ingestion of 100 g of baru almond can meet more than 40% of this recommendation (considering the consumption of 1 g / day). This result is favorable, since these components have different physiological effects mainly in the treatment of hypercholesterolemia, as well as in the control of cholesterol by secondary causes in diabetics and patients with metabolic syndrome (Lemos *et al*., 2016). Besides, the baru almonds present total sterols values greater than other oilseeds considering that Brazil's almonds, walnuts, cashews, hazelnuts, macadamia nuts, pistachios and walnuts have average total sterols of 192, 160, 154, 132, 105, 189 and 197 mg 100 g^{-1} respectively (Miraliakbari and Shahidi, 2008).

Identification of tocopherols by HPLC-DAD

The total content of tocopherols (α and γ) (Table 3) obtained between treatments showed significant difference ($p < 0.05$) indicating that the drying process (65 and 105 °C per 30 min) can contribute to an increase of 8.12 and 63.95% of these compounds, respectively. When evaluated

individually, the tocopherols also presented the same behavior, and these data indicate that these substances may be potentialized through the use of heat. γ-tocopherol was the most abundant, with a corresponding value for T1, T2 and T3 of 74.6, 71.2 and 61.9%, of total tocopherol content. While α-tocopherol corresponded to 25.3, 38.1 and 43.7% for the same treatments. Factors such as the composition in fatty acids, the presence of compounds with antioxidant activity (especially those from lipophilic), variety, degree of ripeness and care in almond production can be responsible for the maintenance of these molecules (Lemos *et al*., 2016). The quantification of the levels of tocopherols, specifically in food, becomes necessary due to action of this vitamin in preventing oxidative damage to DNA, which act as a antioxidant structures and assists on peroxidation inhibition lipid (Lemos *et al*., 2016).

Profile of fatty acids by CG-FID

Table 4 presents the identifications, the quantification and relations between the saturated, polyunsaturated and unsaturated fatty acids present in baru almond subjected to different drying temperatures, as well as the atherogenic index (AI) and thrombogenic index (TI), and the hypocholesterolemic and hypercholesterolemic fatty acids (h/H).

Table 4. Fatty acid profile of the almonds of baru submitted to different drying temperatures [1]

		Treatments[2]			
Fatty Acids (g 100 g^{-1})	Chemical Structure	T1	T2	T3	P
Methyl Hexanoate	C6:0	0.03±0.00[a]	0.03±0.00[a]	0.01±0.01[b]	0.0271
Methyl Octanoate	C8:0	0.03±0.00[b]	0.03±0.00[b]	0.06±0.17[a]	0.0001
Methyl Decanoate	C10:0	0.03±0.02[a]	0.02±0.07[a]	0.00±0.09[b]	0.0001

Methyl Laurate	C12:0	0.24±0.06[a]	0.13±0.01[b]	0.08±0.06[c]	0.0001
Methyl Myristale	C14:0	0.16±0.03[a]	0.11±0.23[b]	0.05±0.02[c]	0.0001
Methyl Pentadecanoate	C15:0	0.22±0.03[a]	0.18±0.21[b]	0.18±0.06[b]	0.0001
Methyl Palmitate	C16:0	6.59±0.04[a]	6.28±0.03[b]	6.16±0.01[c]	0.0001
Methyl Heptadecanoate	C17:0	0.09±0.03[a]	0.08±0.13[b]	0.08±0.01[b]	0.0076
Methyl Stearate	C18:0	4.39±0.21[a]	5.32±0.08[a]	5.22±0.12[b]	0.0001
Methyl Arachidate	C20:0	1.06±0.07[c]	1.18±0.09[b]	1.21±0.16[a]	0.0001
Methyl Behenate	22:00	3.47±0.25[c]	3.67±0.54[b]	3.73±0.43[c]	0.0001
∑ SFA[3]	-	16.32±0.71[c]	17.04±0.53[a]	16.79±0.65[b]	0.0001
Cis-10-pentadecanoic acid methyl ester	C15:1	0.031±0.01[a]	0.07±0.03[b]	0.02±0.01[b]	0.0041
Methyl Palmitoleate	C16:1	0.08.±0.01[a]	0.07±0.01[b]	0.07±0.02[b]	0.0012
Cis-10-heptadecanoic acid methyl ester	C17:1	0.15±0.02[a]	0.13±0.76[b]	0.13±0.16[c]	0.0001
Cis-9-oleic acid methyl ester	C18:1Ω9	48.99±0.07[c]	50.15±1,98[b]	51.01±0.62[a]	0.0001
Methyl cis-11-Eicosenoate	C20:1	2.40±0.87[c]	2.52±0.54[b]	2.56±0.19[a]	0.0001
Methyl Erucate	22:1Ω9	0.26±0.06[c]	0.28±0.01[b]	0.29±0.05[a]	0.0001
∑ MUFA[4]	-	51.91±1.98[c]	53.18±1.34[b]	54.08±1.54[a]	0.0001
Methyl Linoleate	C18:2Ω6	27.28±0.23[c]	26.46±0.35[b]	26.89±0.04[a]	0.0001
Methyl Linolenate	C18:3Ω3	0.14±0.01[a]	0.13±0.03[b]	0.12±0.05[c]	0.0001

Cis-11,14-eicosadienoic acid methyl ester	C20:2Ω6	0.08±0.05[a]	0.06±0.07[b]	0.05±0.22[c]	0.0001
Methyl cis-5,8,11,14,17-eicosapentaenoate	C20:5Ω3	0.07±0.09[a]	0.06±0.06[b]	0.05±0.32[c]	0.0004
Cis-13,16-docasadienoic acid methyl ester	C22:2	3.93±0.35[c]	4.04±1.78[b]	4.13±0.97[a]	0.0001
∑ PUFA[5]	-	31.50±1.04[a]	30.74±0.75[c]	31.25±0.69[b]	0.0001
PUFA/SFA[6]	-	1.93±0.14[a]	1.80±0.45[b]	1.82±0.76[b]	0.0001
MUFA/SFA[7]	-	3.180±0.53[a]	3.12±0.25[b]	1.87±0.34[c]	0.0001
TI[8]	-	0.28±0.21[b]	0.29±0.08[a]	0.28±0.21[b]	0.0003
AI[9]	-	0.09±0.09[a]	0.08±0.76[b]	0.08±0.34[c]	0.0001
h/H[10]	-	11.32±0.24[c]	12.02±1.12[b]	12.59±0.43[a]	0.0001

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p < 0.05$); [2] Treatments: T1 (Almond *in natura*), T2 (Almond submitted to 65 °C per 30 minutes) and T3 (Almond submitted to 105 °C per 30 minutes); [3] Total saturated fatty acids; [4] Total unsaturated fatty acids; [5] Total of polyunsaturated fatty acids; [6] Relationship between saturated and polyunsaturated fatty acids; [7] Relationship between saturated and unsaturated fatty acids; [8] Thrombogenic index; [9] Atherogenic index; [10] hypocholestrolemic/ hypercholesterolemic potential; *p*: *p*-value.

It was found that the unsaturated fatty acids, including oleic acids-linoleic, were predominant. The high proportion of monounsaturated fatty acids and polyunsaturated has also been observed. The Dietary Guidelines for Americans (AHA, 2016) states that the daily intake of PUFAs is 20 g^{-1} and, in this sense, it is recommended to ingest 63.5, 65.1 and 64.1 g^{-1} baru almonds corresponding to T1, T2 and T3 to provide respectively 100% of this recommendation.

On the presence of saturated fatty acids, palmitic acid was the majority representative in all treatments. The relation between PUFA/SFA was higher than the 1.9, 1.8 and 1.8 respectively for T1, T2 e T3, and T2 and T3 are statistically similar ($p < 0.05$). For a balanced intake of these elements, which are essential to health, this value must be at least 0.45. In relation to this profile (low levels of SFA and high of MUFAs), it is estimated that the baru almonds can be effective in the control of the traditional risk factors for atherosclerotic cardiovascular disease. This is because the presence of polyunsaturated fatty acids may promote a hypocholesterolemic effect.

As to the atherogenic index (AI) and thrombogenic index (TI) and the hypocholesterolemic fatty acids and hypercholesterolemic (h/H), which indicate the potential for stimulating aggregation platelet and coronary artery disease, the values obtained for T1, T2 and T3 were respectively 0.28, 0.09, 11.31, 0.29, 0.08, 12.01, and 0.27, 0.07, 12.58 with statistical difference ($p < 0.05$) between the treatments. Although there is no established parameter for these indices, the smaller the result for AI and TI and greater for h/H, the less likely are the changes as mentioned above and the healthier the food is. This is due to the greater concentration of anti-atherogenic fatty acids (Ω3 and Ω6) (Turan *et al*., 2007). Foods such as cheeses and meats that are significant sources of fatty acids, have indexes of AI, TI and respective h/h 2.32, 3.11, 1.23 and 0.54, 1.15, 1.76 (Faria *et al*., 2015), and comparing these figures with those of baru almonds, superiority and greater possibility of cardiovascular protection for part of the almond is demonstrated.

As regards the drying process (T3), it was found that the heat has promoted a reduction ($p < 0.05$) in different fatty acids (relative to T1), however, for being the prevalent, respectively the decrease in C16:0 and C18:2 which was 1.4 and 6.6%. By analyzing all the reductions and correlate them to your degree of unsaturation, one can infer that the more affected was the polyunsaturated. A justification is the fact that this compound has a greater number of double bonds, soon has increased susceptibility to oxidation.

Conversely, this same process of drying promoted increase ($p < 0.05$) in different fatty acids, C18:0, C18:1, C22:0 and C22:2 with expansion of 18.9 respectively, 4.1, 4.9 and 7.5%. The saturated

fatty acids were the most favored by the drying process (when compared to T1 with T3), because they represented together a total increase of 134.6%. This occurs because the heating may increase the oxidation rate of these molecules. Rodríguez-Bencomo *et al*. (2015), when analyzing the pistachio oil subjected to heating (160 °C for 20 min), have found that the heat is not promoted and there is no significant increase in levels of SFA, PUFA and MUFA.

3. CONCLUSIONS

High levels of phenolic compounds, vitamin C, antioxidants (measured by the β-carotene/linoleic acids system), gallic acid, caffeic acid, rutin, sterols, total monounsaturated fatty acids and low thrombogenic, atherogenic index was found in *in natura* baru almond. During the process of drying at 65 °C for 30 min, it was observed the decline in levels of caffeic, chlorogenic acid, anthocyanins, *p*-coumaric acid, ferulic, *o*-coumaric acid, quercetin, polyunsaturated fatty acids and the free radical scavenging capacity. Under the same conditions an increase in levels of gallic acid, rutin, catechin, trans-cinnamic acid, vanillin, *m*-coumaric acid, tocopherols and monounsaturated fatty acids was observed. The temperature of 105 ° C presented the same behavior as above, however, it promoted a reduction in vitamin C content and an the increase in the presence of flavonoids. The drying temperature did not affect the levels of total phenolics, tannins, β-carotene/linoleic acid system, and sterols. The chromatographic and spectrophotometric quantification carried out in this work contributed to the increase of the scientific knowledge about the properties of baru almond submitted to different drying processes in relation to the work already done and published in the scientific community. The almond object of this study presents functional features that place it above other oilseeds. Its consumption promotes the plant biodiversity of the Cerrado Biome and contributes to a new generation of foods that can benefit the health of consumers. The bioactive properties of molecules that are intensified by processing, suggest great potential for

application of baru almond (*in natura* and/or submitted to different drying processes) in new products such as oils, cereal bars, bakery, chocolates, among others, however, their application depends on the adequacy of the industrial scale.

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ARTIGO 3 - STUDY OF THE NUTRITIONAL, SENSORY AND MICROBIOLOGICAL PROPERTIES OF FOOD PASTES MADE WITH BARU ALMONDS (*Dipteryx alata* VOG.)

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Study of the nutritional, sensory and microbiological properties of food pastes made with baru almonds (*Dipteryx alata* vog.)

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Abstract

Baru almond is an oilseed native to the Cerrado biome and stands out because of its large nutrient content. The present study sought to perform a centesimal, mineral, microbiological and sensory characterization of food pastes made with different contents of baru almonds: P1 (35%); P2 (17.5%); and P3 (0%). Hazelnuts were used as the control to counterbalance the different percentages of almond. The highest concentrations of moisture, lipids, and calcium and the highest total energy value were observed with the food pastes consisting of 35% baru almond (P1). However, a decrease in ash, protein and carbohydrate contents occurred. Higher concentrations of potassium and zinc were found in the food pastes made with 0% baru almond (P3), indicating that hazelnuts contained a greater quantity of these minerals. As for the microbiological results, no coliforms were found for any of the pastes tested. Low levels of molds and yeasts were observed at the dilutions of 10^{-1} and 10^{-2} UFC/g and none at 10^{-3} UFC/g. For the taste sensorial attributes, global impression and intention to purchase, no significant differences were observed between the formulations. However, the food pastes consisting of 17.5% baru almond and 17.5% hazelnut (P2) received the highest scores for appearance and texture. It is concluded that the food pastes made with different contents of

baru almond had satisfactory nutritional, sensory and microbiological characteristics for consumption.

Keywords: Cerrado; Oilseeds; Development of new products.

1. Introdução

Brazil is widely recognized for the biodiversity of its forests, and it has hundreds of species that provide seeds, nuts and edible kernels with unique sensory characteristics that are appropriate for the development of new products (Carvalho et al., 2012). The baru almond (*Dipteryx alata* Vog.) is an oilseed native to the Cerrado biome and stands out because of its large nutrient content and high market value and because it is part of an abundant genetic heritage from the point of view of health. Previous studies performed with this almond have demonstrated the presence of important chemical and nutritive compounds, such as proteins, fibers, lipids, unsaturated fatty acids (linoleic and oleic), minerals, antioxidant molecules, polyphenols (catechin, rutin and gallic, caffeine, chlorogenic acids, *o*-cumárico and *trans*-cinnamic) and vitamins [(C and tocopherols (alpha and gamma)] (Lemos et al., 2012; Sano et al., 2014; Lemos et al., 2016; Campidelli et al., 2019; Campidelli et al., 2020).

Among the options for food products in which oilseeds are used, hazelnut pastes stand out. They are widely consumed because of their high degree of acceptance. However, these products generally contain high concentrations of sugars and low proportions of oilseeds, which impairs their consumption by those who wish to reduce caloric intake and obtain health benefits. Furthermore, hazelnuts are included in one of the main food groups responsible for triggering food allergies (Holzhauser, Stephan & Vieths, 2002).

A viable alternative would be to substitute another oilseed, the baru almond, because its composition is superior to those of hazelnuts, and extra physiological health benefits can be obtained (Campidelli et al., 2020a; Oliveira-Alves et al., 2020). However, information that

proves the effectiveness of these products for health must be scientifically attested, despite the fact that the vast majority remaining unexplored. The present study sought to perform a centesimal, mineral, microbiological and sensory of food pastes made with different contents of baru almonds to furnish information regarding the functional potential of baru almonds in new products, as well as identifying new nutritional properties of ready-to-eat products.

2. Metodologia

A quantitative exploratory research as oriented by Pereira et al. (2018) was performed via laboratory experiments during the period from April to December of 2018.

The experiment was accomplished at the Fruit and Vegetable Post-Harvest and New Product Development Laboratories of the Federal University of Lavras, Lavras, MG, Brazil and was approved by the Ethics Committee of the Federal University of Rio Grande do Sul under the number 1716.605.

Ingredients

The following raw materials were used to manufacture food pastes: baru almonds (AB) (purchased from producers in the city of Barra do Garça, MT, Brazil), hazelnuts, erythritol, coconut oil (extra virgin), powdered skim milk, 100% cocoa powder, polydextrose, whey, emulsifier and flavoring.

Formulations

Three different paste formulations were developed through the addition of different AB contents: paste with 35% baru almonds (P1); paste with 17.5% baru almonds (P2); and control paste with 0% baru almonds (P3). Hazelnuts were used to counterbalance the different percentages of almonds (Table 1). The experimental design was two formulations plus control

with three replications.

Table 1. Formulation of pastes with different baru almond contents.

	Formulations		
Ingredients (%)	P1	P2	P3
Baru almond	35	17.5	0
Hazelnut	0	17.5	35
Erithritol	29.5	29.5	29.5
Coconut oil	14	14	14
Powdered skim milk	5.6	5.6	5.6
Cocoa 100%	10.4	10.4	10.4
Polydextrose	3	3	3
Whey	1	1	1
Emulsifyer	1	1	1
Flavoring	0.5	0.5	0.5

Prepared by the authors

In Table 1, it is possible to verify the presence of the ingredients used for the manufacture of food pastes. Erythritol was the sweetener used to replace sugar, while powdered milk and polydextrose helped in the formation of texture and contributed with the addition of dietary fiber, respectively.

Processing of pastes

Initially, AB was dried in a forced air oven (Marconi, MA0351, Piracicaba, Brazil) at 105 ºC for 30 minutes. The dried almonds were ground (experimental mill with mesh 20, Viti

Molinos, VG 2000i, Itajaí, Brazil), packed in transparent polyethylene containers and stored at 25 °C in temperature-controlled chambers (Eletrolab, model EL202).

The previously prepared AB was homogenized with the other ingredients present in its formulation (Table 1) for 5 minutes (Philips Walita model Viva RI7630 processor) to prepare the food pastes (PA). After homogenization, the PA were packed in transparent polyethylene packages and stored at -22 ºC (Eletrolab, model EL202).

Characterization of products

Proximate composition

The proximate composition was determined according to the method described by IAL (2008). The moisture content was estimated by means of direct heating in an oven with forced air at 105 °C until constant weight was obtained. The lipids were determined by the Soxhlet method through extraction with petroleum ether.

The crude protein was determined by digestion, followed by distillation of the ammonia in a Micro-Kjedahl apparatus, and using the factor 6.25 to calculate the protein concentration. The ash fraction was obtained gravimetrically, by weighing the residue obtained after heating the material at 550 °C in a muffle furnace. The total carbohydrate content was calculated by the difference obtained after subtracting the percentage of moisture, ash, lipids and proteins. The results were expressed in g.100 g^{-1}. The total energy value (VET) was determined using the conversion factors determined in RDC nº 40 (Brasil, 2001).

Microbiological analysis

The total count of molds and yeasts was performed by surface plating according to the method of the American Public Health Association (APHA, 2001). The Dicloran Rosa Bengala-1-2-Chloramphenicol culture medium was used for counting, incubating at 25 °C for

7 days, with dilutions of 10^{-1}, 10^{-2} and 10^{-3}. The results were expressed as the presence or absence of CFU/g.

The analysis of total and thermotolerant coliforms was performed according to APHA (2001) by sowing three series of tubes containing 9 mL of lauryl sulfate tryptose broth and an inverted Durhan tube with 1 mL of different sample dilutions. The tubes were incubated at 37 °C for 24 to 48 hours to check the formation of gas and turbidity of the medium, which indicated the presence of these microorganisms.

Mineral composition

The minerals were determined according to the method of Sarruge & Haag (1974) by digestion with perchloric and nitric acid at 50 °C for 10 to 15 minutes and then at 100 °C to digest all the material. The reading was performed on an atomic absorption spectrophotometer at 248.3 nm. The concentrations of phosphorus, potassium, calcium, zinc and iron were measured, and the results were expressed in $mg.100g^{-1}$ of sample.

Sensorial analysis

Ten-gram samples were served to a panel of 100 untrained tasters at a temperature of approximately 10 °C. They were offered to participants in individual booths in a random arrangement to avoid the demand effect (Minim, 2013). Participants were asked to answer questions regarding acceptance of the product with respect to the attributes of flavor, appearance, texture and overall impression based on a structured hedonic scale of nine points (1= I really disliked it, up to 9 = I really liked it).

They were also asked to respond to questions regarding the intention to purchase, using a hedonic scale of five points (1 = I would certainly not purchase, up to 5 = I would certainly purchase), and frequency of consumption of oil-based pastes, such as hazelnut

pastes, peanut pastes and others.

Statistical analysis

The data were submitted to analysis of variance (ANOVA) and a test of comparison of means (Tukey, 5% probability of error). Statistical calculations were performed using version 5.0 of the R program.

3. Resultados e Discussão

Proximate composition

The proximate composition of food pastes made with different baru almond contents can be seen in Table 2.

Table 2. Mean values for the proximate composition of food pastes made with different quantities of baru almonds [(1)]

Formulation[2]	Moisture	Lipids	Non-nitrogen extract[3]	Ash	Fiber	Protein	VET[4]
P1	2.93±0.40[a]	53.29±4.28[a]	31.3±1.32[c]	3.15±0.27[b]	2.65±0.01[a]	6.7±0.06[b]	632±0.83[a]
P2	2.5±0.49[b]	40.60±0.032[b]	43±0.93[a]	3.37±0.73[a]	2.98±0.04[a]	7.7±0.83[a]	568±1.03[b]
P3	2.47±2.62[b]	37.95±3.21[c]	47±0.73[a]	3.35±0.03[a]	1.42±0.42[b]	7.7±0.53[a]	560±1.93[b]

Notes: [1]Data are presented as mean ± standard deviation and are expressed in g.100 g^{-1}, except for VET which is expressed as kcal.100 g^{-1}; [2]Formulations: P1= paste with 35% baru almond and 0% hazelnut; P2 = paste with 17.5% baru almond and 17.5% hazelnut; P3 = control paste with 0% baru almond and 35% hazelnut. [3]Calculated from the difference of 100 minus the sum of the percentages of ash, lipids, protein and moisture; [4]Values determined using the conversion factors determined in the DRC number 40 (Brasil, 2001).

The moisture contents of the food pastes were considered to be low (less than 3%. They were below the maximum limit of 15% established by the legislation for cereal products (ANVISA, 1978).

The P1 formulation differed statistically from the others ($p<0.05$); its moisture content was higher. Nevertheless, the moisture contents of all the formulations were favorable for the conservation of the products because high concentrations of water can favor the proliferation of microorganisms and chemical reactions.

In research by Lubas et al. (2016), who developed chocolate bars containing 35% baru almonds, it was also found that the higher the percentages of this almond, the higher the moisture content present, which is similar to the results obtained in this work.

A decrease of 23.81 and 28.78% in total lipids was detected according to the respective decrease in the amount of baru almond in formulations P2 and P3. According to Campidelli et al. (2019), baru almonds contains 31.73% lipids, it being one of the oilseeds with the highest fat content.

Considering that the intake of this macronutrient is important for the nutritional balance of the human organism, it is estimated that the presence of AB can contribute good quality lipids to the paste.

The results agree with those published by Soares et al. (2018), who, when preparing cookies enriched with 10 and 20% baru almond flour, found lipid contents close to 25.15 and 26.72%. The notable increase in this value was proportional to the increase in the concentration of baru almond flour.

It was found that the concentrations of carbohydrates in formulas P2 and P3 were statistically similar ($p>0.05$) and corresponded to the highest values. This fact indicates that hazelnut can be responsible for the significant increase in this macronutrient, compared to that obtained with baru almonds.

Despite this difference, a high concentration of carbohydrates can be attributed to all the formulations so these products can be considered to be adequate sources of energy. Furthermore, the Recommended Daily Intake (RDI) for this nutrient is set at 300 grams per

day (Brasil, 2005) and, therefore, the consumption of 100 grams of P1, P2 and P3, can furnish 10.4%, 14.3% and 15.6%, respectively, of that intake.

Food pastes can contain high levels of carbohydrates, which are an immediate source of energy for the body. Despite the importance of this nutrient to the functioning of the body, it is important to note that excessive consumption has been associated with adverse effects.

The ash contents of formulas P2 and P3 were statistically equal ($p>0.05$), and they contained higher levels of minerals than P1. The hazelnut probably contains greater quantities of this nutrient than the baru almond. Despite these findings, it is estimated that all the values were high and can be beneficial for health, given that these components perform structural and biological functions essential to the functioning of the human organism.

In research by Rinaldi et al. (2016), who developed baru-based cereal bars, an average value of 1.57 g of ash/100 g was found. In the work of Soares et al. (2018), 2.42 and 2.86 g of ash.100 g^{-1} were found in cookies formulated with 10% and 20%, respectively, of baru almond flour.

The fiber contents of formulas P1 and P2 were statistically equal ($p> 0.05$), and they also contained the greatest amount of fiber, followed by formula P3. The DRI of this nutrient is 30 g per day (ANVISA, 2016), and the consumption of 100 grams of P1, P2 and P3 pastes corresponds to 8.83, 9.93 and 4.73% of the DRI, respectively.

These results demonstrate that the presence of baru almonds in food pastes can positively influence the presence of this macronutrient because fibers are important agents that influence the intestinal microbiota by modulating it positively.

These results are in line with those found by Junior et al. (2007), who found increasing values for fiber, which were 0.48, 0.83, 1.15 and 1.49%, respectively, when they developed cookies containing different quantities of almond flour (2, 4, 6 and 8%). This increase also

occurred in the cookies formulated by Soares et al. (2018), who found fiber percentages of 4.9 and 7.81% when they added 10 and 20% baru almond flour, respectively.

The protein contents of formulas P2 and P3 were greater than those found in P1, and they were statistically similar ($p>0.05$). P1, P2 and P3 food pastes represent 13.4, 15.4 and 15.4% of the DRI for this nutrient, which is 50 g.100 g^{-1} for adults, respectively (BRASIL, 2005).

According to the Brazilian legislation (Brasil, 2012), which determines that foods that are sources of protein should contain a minimum of 6 g of protein per 100 g of product. These results have recognized merit because these macronutrients contribute to the development of body structures and aid in nutrition.

The highest caloric content ($p<0.05$) was found in formula P1, followed by P2 and P3, which were statistically equal ($p>0.05$). This result is compatible with the quantities of lipids found because the greater the fat content, the greater the energy content. Furthermore, the consumption of 100 grams of P1, P2 and P3 food pastes represents 31.15, 28.4 and 26% of the Daily Reference Values (DRV), respectively (Brasil, 2003).

These pastes are considered to be foods with a high caloric content, and it is recommended that daily consumption not be excessive. Rinaldi et al. (2016) found an average value of 491.64 Kcal.100 g^{-1}, a result close to that of this study, when they prepared baru-based cereal bars.

Microbiological analysis

The results of the microbiological analysis of food pastes made with different quantities of baru almonds are described in Table 3.

Table 3 - Microbiological analyses for the presence of total and thermotolerant coliforms and the counting of molds and yeasts in food paste made with different quantities of baru almonds(1)

Formulation[2]	Total coliforms (MPN/g)[3]	Coliforms 45 ºC (MPN/g)[3]	Molds and Yeast		
			10^{-1} Dilution CFU/g	10^{-2} Dilution CFU/g	10^{-3} Dilution CFU/g
P1	Absent	Absent	17	2	0
P2	Absent	Absent	14	8	0
P3	Absent	Absent	8	2	0

Notes: [1]Data are presented as means; [2]Formulations: P1 = paste with 35% baru almond and 0% hazelnut; P2 = paste with 17.5% baru almond and 17.5% hazelnut; P3 = control paste with 0% baru almond and 35% hazelnut; [3]MPN: Most probable number (Apha, 2001).

According to RDC Resolution No. 12 of January 2001 from the National Health Surveillance Agency (ANVISA, 2001), which monitors and determines microbiological and sanitary standards for almond-based plant products, the values for coliforms at 45 °C must be lower than 10 MPN/g. In this work, all the formulations were adapted to the recommendations, no microorganism was found.

There are no established criteria in the Brazilian Food Law (ANVISA, 2001) regarding the total count of molds and yeasts in almonds. However, the low count is important information for obtaining and supplying products with adequate microbiological quality.

Low levels of molds and yeasts in the dilutions of 10^{-1} and 10^{-2} CFU/g and an absence in 10^{-3} CFU/g were observed, showing that the hygiene and quality control techniques were satisfactory during the process of preparation of the food pastes.

Therefore, the results of both analyses indicate that there were no hygienic flaws that could compromise microbiological safety during the stages of preparation, packaging and storage of the foods, demonstrating sanitary quality in all the stages of production.

Mineral composition

The concentrations of potassium, phosphorus, calcium, zinc and iron present in food pastes made with different levels of baru almonds, as well as the DRI, are shown in Table 4.

Table 4. Determination of the mineral composition (mg.100 g^{-1}) of the pastes and DRI [1,2]

Formulation[3]	Potassium (K)	Phosphorus (P)	Calcium (Ca)	Zinc (Zn)	Iron (Fe)
P1	9600±0.47^{c}	4100±0.89^{a}	3500±0.08^{a}	35.1±0.86^{c}	125.9±0.09^{a}
P2	10000±0.78^{b}	4000±0.59^{a}	2500±0.87^{b}	40.6±0.34^{b}	132.4±0.54^{a}
P3	10300±0.67^{a}	3600±0.90^{b}	2300±0.75^{b}	43.2±0.56^{a}	106.4±0.97^{b}
DRI (mg/100 g^{-1})	3510	700	1000	7	14
P value	0.0045	0.0127	0.0004	0.0000	0.0000

Notes: [1]Means followed by distinct letters in the columns differ from each other by the Tukey test ($p<0.05$); [2]DRI: Recommended Daily Intake (Brasil, 2005); [3]Formulations: P1= paste with 35% baru almond and 0% hazelnut; P2 = paste with 17.5% baru almond and 17.5% hazelnut; P3 = control paste with 0% baru almond and 35% hazelnut. Source: Prepared by the authors.

A significant difference ($p<0.05$) was observed for the minerals analyzed, demonstrating that the variation in oilseeds resulted in differences in these elements. It was found that the higher the baru (P1) almond content, the higher the concentrations of phosphorus, calcium and iron. With respect to phosphorus and iron present in formulation P2, the concentrations of these elements are significantly similar to those of P1 ($p>0.05$), indicating that the use of 17.5% baru almond and 17.5% hazelnut can provide the same result as that obtained when using 35% baru almond (P1).

As for the P3 formulation containing exclusively hazelnut, its use resulted in an increase in the levels of potassium and zinc ($p<0.05$). Regardless of the oilseed used, high values for mineral content that exceeded those recommended by the DRI were found for all

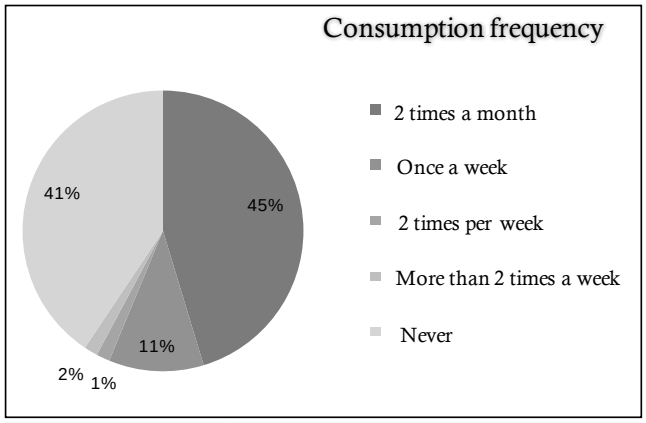
the food paste formulations, and they are classified as foods with high mineral contents (Brasil, 2005).

Sensorial analysis

The frequency of consumption of oil-based pastes was verified, and the following information was observed: 45% of the tasters consume this type of product twice a month; 41% do not consume it; 11% consume only once a week; 2% consume more than twice a week; and 1% consume twice a week. Thus, the vast majority of respondents are accustomed to consuming this type of product; on the other hand, the other majority of respondents do not consume them. The non-consumption can be justified mainly by the high cost of these foods, and it is likely that the percentage of consumers adhering to this category of foods will increase if the nutritional benefits they present are divulged.

Regarding the sensory descriptors (Figure 1), scores greater than 7.0 were obtained for all the formulations, demonstrating an adequate acceptability of the products by the tasters. However, when analyzed individually, it was clear that the P2 formulation differed significantly ($p<0.05$) from the other formulations with regard to appearance and texture. There was no difference ($p>0.05$) between formulations P1, P2 and P3 regarding flavor and global impression; that is, the percentages of these oilseeds used did not make a significant difference to the tasters ($p>0.05$), demonstrating that baru almond, little known and infrequently consumed, can sensorially resemble an oil that is frequently consumed (hazelnut).

Figure 1 - Frequency of consumption, scores for sensory acceptance and intention to purchase for food pastes made with different baru almond contents [(1).]



	Formulations[2]		
Sensory Attributes[3]	P1	P2	P3
Appearance	7.37[ab]	7.68[b]	7.24[a]
Texture	7.03[ab]	7.46[b]	7.00[a]
Flavor	7.39[a]	7.66[a]	7.59[a]
Global impression	7.28[a]	7.68[a]	7.42[a]
Purchase intention[4]	3.83[a]	4.04[a]	3.95[a]

Notes: [1]Means with different letters for the same analysis differ from each other ($p < 0.05$); [2]Formulations: P1= paste with 35% baru almond and 0% hazelnut; P2 = paste with 17.5% baru almond and 17.5% hazelnut; P3 = control paste with 0% baru almond and 35% hazelnut; [3]A 9-point scale was used; [4]A 5-point scale was used. Elaborated by the authors.

Regarding the intention to purchase, the evaluators demonstrated that "they might buy" the products if they were available for sale. Moreover, no significant differences were observed between the formulations ($p > 0.05$). In general, the food pastes pleased the consumers; however, they felt insecure regarding purchase.

The use of the baru almond, which is not commonly used in commercial products, might be one of the factors responsible for the indecision at the time of purchase. Because the pastes obtained satisfactory sensory acceptability, insecurity at the time of purchase can be minimized through marketing campaigns.

4. Final Considerations

The highest values for moisture, lipids, total energy and calcium were found in the food pastes consisting of 35% baru almonds (P1). However, the ash, protein and carbohydrate contents were lower. The food pastes made with 0% baru almonds (P3) contained higher levels of potassium and zinc, indicating that hazelnut contributes significantly to the presence of these minerals. As for the microbiological results, it was found that all the pastes developed contained no total coliforms at 45 ºC. The levels of molds and yeasts in the dilutions of 10^{-1} and 10^{-2} CFU/g were low, and none were observed at 10^{-3} CFU/g. Thus, the techniques of hygiene and quality control were satisfactory in their preparation.

For the sensory attributes of taste, global impression and intention to purchase, no significant differences were observed between the formulations; however, the food pastes consisting of 17.5% baru almonds and 17.5% hazelnut (P2) received the highest scores for appearance and texture. It was concluded that the food pastes made with different baru almond contents possessed satisfactory nutritional, sensory and microbiological characteristics for consumption, which means that the products developed are of nutritional value.

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ARTIGO 4 – FATTY ACID PROFILE, MINERAL CONTENT AND BIOACTIVE COMPOUNDS OF COCOA SPREADS SUPPLEMENTED WITH BARU ALMOND (*Dipteryx alata* VOG.)

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Fatty acid profile, mineral content and bioactive compounds of cocoa spreads supplemented with baru almond (*Dipteryx alata* Vog.)

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SUMMARY: The present study aimed to perform chromatographic and spectrophotometric characterization of bioactive compounds, antioxidants, phenolics, profile of fatty acids and minerals in spreads supplemented with different contents of baru almond. The addition of baru almond (P1 treatment) potentiated the concentrations of vitamin C, antioxidants, gallic acid, calcium, magnesium, sulfur, manganese and oleic acid. In contrast, the absence of this oil in P3 treatment provided an increase in the concentrations of vanillin, *p*-coumaric acid, ferric acid, *o*-coumaric acid, linoleic acid and saturated and polyunsaturated fatty acids. When the tannin, beta-carotene/linoleic acid, trans-cinnamic acid, monounsaturated fatty acids, hypocholesterolemic and hypercholesterolemic fatty acids content and atherogenic and thrombogenic indices were evaluated, no significant differences were detected between treatments.

KEYWORDS*: New Products; Bioactive compounds; Brazilian Cerrado; High-Performance Liquid Chromatography; Oilseeds.*

RESUMEN: El presente estudio tuvo como objetivo realizar la caracterización cromatográfica y espectrofotométrica de compuestos bioactivos, antioxidantes, fenólicos, perfil de ácidos grasos y minerales en productos para untar suplementados con diferentes contenidos de almendra baru. La adición de almendra baru (tratamiento P1) potenció las concentraciones de vitamina C, antioxidantes, ácido gálico, calcio, magnesio, azufre, manganeso y ácido oleico. En contraste, la ausencia de este aceite en el tratamiento con P3 proporcionó un aumento en las concentraciones de vainillina, ácido *p*-cumarico, ácido férrico, ácido *o*-cumarico, ácido linoleico y ácidos grasos saturados y poliinsaturados. Cuando se evaluó el contenido de tanino, ácido betacaroteno/linoleico, ácido transcinámico, ácidos grasos monoinsaturados, contenido de ácidos grasos hipocolesterolémicos e hipercolesterolémicos e índices aterogénicos y trombogénicos, no se detectaron diferencias significativas entre los tratamientos.

PALABRAS CLAVE*: Nuevos Productos; Cerrado Brasileño; Compuestos bioactivos; Cromatografía líquida de alto rendimiento; Semillas oleaginosas.*

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1. INTRODUCTION

Brazil is recognized worldwide for the plant biodiversity of its forests, boasting hundreds of species that provide edible seeds, nuts, and almonds. These foods stand out due to their sensory characteristics, making them suitable for the development of new products with economic potential (Carvalho *et al*., 2012).

The baru almond (*Dipteryx alata* Vog.) is an oleaginous native of the Cerrado biome and stands out due to its high nutrient density, high market value and because it is part of an abundant genetic heritage, but not well studied. Previous studies demonstrated the presence of important chemical/bioactive compounds in this almond, such as monounsaturated fatty acids (oleic), boron, zinc, copper, manganese, magnesium, antioxidant molecules, polyphenols (catechin, rutin and gallic acid, caffeic acid, chlorogenic acid, o-coumaric and trans-cinnamic acid), sterols and vitamins C and E (alpha and gamma tocopherols) (Lemos *et al*., 2012; Pinelli *et al.,* 2015; Lemos *et al*., 2016; Campidelli *et al*., 2019; Campidelli *et al*., 2020).

The *in vivo* effect of baru almond has previously been tested, and it has been proved that consumption can reduce adiposity, improve lipid profile, and increase antioxidant enzyme activity (Souza *et al*., 2018). These characteristics, along with its chemical properties (cited above), can minimize the incidence of different metabolic disorders, making baru almond a health-effective food.

Due to recent evidence supported by scientific trials, cocoa is also a beneficial food for consumer health. Its high content of bioactive compounds has positive effects on improving lipid function, digestion, kidney function, heart function, as well as anemia and mental fatigue. These benefits can be achieved due to its antioxidant, anti-inflammatory and antiplatelet action (Dilliinger *et al*., 2000; Herbello-Hermelo *et al*., 2018).

Amongst other options, oilseeds and cocoa products are also used in hazelnut spreads, and are extensively consumed due to their high acceptability. A disadvantage of these spreads

are that they contain high concentrations of sugar and low percentages of oilseeds, harming the consumer who wishes to obtain their health benefits. In addition, hazelnuts are included in the major food groups responsible for triggering food allergies (Holzhauser *et al*., 2002); a practical alternative would be to replace them. Baru almond is a potential substitute for hazelnut due to its chemical composition and additional physiological health benefits.

The agri-food sector keeps up with recent changes in the nutritional landscape and is interested in developing new products that are specific to meeting individual needs. Moreover, the search for information that explains how these products are beneficial is a new goal to be achieved. Nonetheless, research into the effectiveness of these new products is limited and to our detriment, there is a distribution of products below the initial quality proposed.

The present study aimed to perform chromatographic and spectrophotometric characterization of bioactive compounds, antioxidants, phenolics, fatty acid and mineral profile in spreads comprising differing content of baru almond.

2. MATERIALS AND METHODS

2.1. Reagents, materials and experimental design

All chemicals, reagents and solvents used were of analytical or high-performance liquid chromatography (HPLC) grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (>18 MΩ.cm) was obtained from a Milli-Q system (Millipore, Brussels, Belgium).

Baru almonds were obtained from the Cerrado biome, located in the city of Barra do Garças-MT, during the harvest season (between August and September 2016). The baru almond has the following centesimal composition (g/100g): 6.63 moisture, 22.96 protein, 31.73 lipid, 14.44 dietary fiber and 1.55 ashes.

The following raw materials were used for the manufacture of baru almond spreads: Baru almond (Local producer of the city of Barra do Garças, Brazil), Hazelnut (À Granel® - Lavras, Brazil), Erythritol (Tovani® - São Paulo, Brazil), Coconut Oil (Copra®- Maceió, Brazil), Skimmed Milk Powder (Molico® - Caçapava, Brazil), 100% Cocoa Powder (Garoto® - Vila Velha, Brazil), Polydextrose (Tovani® - Santana, Brazil) (Tovani® - Santana, Brazil), Emulsifying and Flavoring (Duas Rodas® - Campinas, Brazil).

Three spread formulations were developed with different baru almond content, as follows: 35% baru almond spread (P1); spreads with 17.5% baru and 17.5% hazelnut (P2); and control spread with 0% baru almond and 35% hazelnut (P3) (Table 1). The choices of the ingredients used in the processing of the spreads occurred through previous tests (for example focus group) that aimed to match the sensorial and technological characteristics. As for the experimental design, this was completely randomized with three replicates. Three independent batches were used for each treatment.

Table 1. Formulation of the spreads with different contents of baru almond

	Treatments		
Ingredients (%)	**P1**	**P2**	**P3**
Baru almond	35	17.5	0
Hazelnut	0	17.5	35
Erythritol	29.5	29.5	29.5
Coconut oil	14	14	14
Skim powdered milk	5.6	5.6	5.6
Cocoa 100%	10.4	10.4	10.4
Polydextrose	3	3	3
Whey	1	1	1
Emulsifier	1	1	1

Flavoring	0.5	0.5	0.5

2.2. Spread processing

Drying of baru almonds and hazelnuts in an air circulation oven (Marconi®, MA0351, Piracicaba, Brazil) was carried out at 105 ºC for 30 min. Thereafter, both oilseeds were ground in a home processor (Philipis Walita®, RI7632, Varginha, Brazil) for 5 min. Then, in the same processor, the remaining ingredients (Erythritol, coconut oil, skim powdered milk, cocoa 100%, polydextrose, whey, emulsifier and flavoring - Table 1) were added and homogenized with the oleaginous seeds for a further 3 min. The obtained spread was packed in transparent polyethylene packages and stored at 12 ºC in temperature controlled chambers (Eletrolab®, EL202, Sao Paulo, Brazil). Thereafter, the analytical determinations described below were performed in quadruple.

2.3 Determination of bioactive compounds

We evaluated the total content of phenolic compounds, tannins and vitamin C. The hydroalcoholic extract was prepared according to the methodology adapted from Milardovic *et al.* (2005), except for vitamin C, which followed the methodology proposed by Roe; Kuether (1943).

Total phenolics were determined by the *Folin–Ciocalteu* reagent method, using gallic acid (0.024–0.096 mg mL^{-1}) as the standard for the calibration curve. The sample (0.5 mL) and 2.0 mL of sodium carbonate (75 g L^{-1}) were added to 2.5 mL of 10% (v/v) *Folin Ciocalteu reagent.* After 30 min reaction at 37 °C, the absorbance was measured using an ultraviolet (UV)–visible spectrophotometer (Varian Cary®, 50, Campinas, Brazil) at 765 nm. The results were expressed in mg of gallic acid equivalents (GAE) per 100 g^{-1} (Roe; Kuether, 1943).

The total phenolic compounds were also evaluated using diazonium salt Fast Blue BB, with standard gallic acid (0.096-0.024 mg mM^{-1}) for the calibration curve. The extract (0.5 mL) was added

to 0.10 mL of Fast Blue BB reagent (0.1%) and 0.10 mL of NaOH (5%). After 60 min of the reaction in the dark at room temperature, the absorbance was measured using a UV-visible spectrophotometer (Varian Cary®, 50, Campinas, Brazil) at 420 nm. The results were expressed in mg gallic acid equivalents (GAE) per 100 g^{-1} (Palombini *et al*., 2016).

In order to quantify antinutritional compounds, tannin content was measured by the colorimetric method according to the Association of Official Analytical Chemists (AOAC, 1990). The method was based on the intensity of the blue color produced in reducing the *Folin-Denis* reagent for phenols, and was then measured using a UV-visible spectrophotometer (Varian Cary®, 50, Campinas, Brazil) at 760 nm the results expressed as equivalents of catechin.

Analysis of vitamin C content was achieved via the 2.4-dinitrophenylhydrazine colorimetric method and measured using a UV-visible spectrophotometer (Varian Cary®, 50, Campinas, Brazil) at 520 nm and the results expressed as equivalents of ascorbic acid (Roe; Kuether, 1943).

Antioxidant activity was evaluated by theDPPH• (2.2-diphenyl-1-picrilhidrazil radical) scavenging method, and measured using a UV-visible spectrophotometer (Varian Cary®, 50, Campinas, Brazil) at 517 nm (Milardovic *et al*., 2005). The scavenging activity of the DPPH• radical was expressed as the inhibition percentage (% I), where Ac is the control absorbance and Aam the sample absorbance, according to the equation:% I = (Ac - Aam) / Ac × 100 %. The minimum concentration of sample that inhibited 50% DPPH• radical (IC_{50}) in the reaction medium was obtained by linear regression using concentration versus percentage inhibition values.

Antioxidant activity determination by β-carotene/linoleic acid was conducted according to the methodology described by Miller (1971) and read at 470 nm using a UV-visible spectrophotometer (Varian Cary®, 50, Campinas, Brazil). The results were expressed as percentage of inhibition of oxidation.

2.4 Individual identification of phenolic compounds by high-performance liquid chromatography with diode-array detection (HPLC-DAD)

The statements for individual identification of phenolic compounds were prepared following the methodology described by Lemos *et al.* (2012). Quantification and identification of these phenols was performed in liquid chromatography (HPLC-DAD/UV-Vis) (Shimadzu Corp. ®, model Shimadzu, Kyoto, Japan). The mobile phase consisted of 2% (v/v) acetic acid in deionized water (mobile Phase) and 70:28:2 (v/v) methanol/water/acetic acid (mobile phase B), and phenolics were detected at 280 nm. Phenolic compounds were identified by comparison of retention times with standards (gallic acid, catechin, chlorogenic acid, caffeic acid, vanillin, p-coumaric acid, ferulic acid, m-coumaric acid, o-coumaric acid, trans-cinnamic acid, quercetin and rutin). The results were expressed as mg of phenolic compound in 100 g^{-1} of fresh weight.

2.5 Profile of fatty acids by CG-FID

For fatty acid profile analysis, lipids from spreads were extracted according to the procedures described by Folch *et al*., (1957). The analysis was performed by gas chromatography on a Shimatzu CG 2010 chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with a flame ionization detector, split injection at the rate of 1:50 and capillary column SPTM-2560 Supelco, 100 m × 0.25 mm × 0.20 μm (Supelco Inc., Bellefonte, PA, USA). The initial temperature of the column was 140 °C, maintained for 5 min, changing to 240 °C with increments of 4 °C, maintained by 30 min for a total of 60 min. The injector and detector were kept at the temperature of 260 °C and used helium as carrier. The fatty acids identified were compared to the retention times presented by the chromatographic pattern SupelcoTM37 FAME Mix (Supelco Inc., Bellefonte, PA, USA) and are expressed in percentage (%) of the total fatty acids. These were later were grouped as: saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The atherogenic index (AI) and thrombogenic index (TI) were determined in accordance with that specified by Ulbricht and Southgate (1991), as the Eq. (1 and 2):

$$AI: [(C12:0+(4\times C14:0)+C16:0)]/(\Sigma MUFAs+\Sigma\Omega 6+\Sigma\Omega 3) \quad (1)$$

$$TI:(C14:0+C16:0+C18:0)/[(0.5\times\Sigma MUFAs)+(0.5\times\Sigma\Omega 6)+(3\times\Sigma\Omega 3)+(\Sigma\Omega 3/\Sigma\Omega\text{-}6)] \quad (2)$$

The *ratio* of fatty acids hypocholesterolemic and hypercholesterolemic (h/H) was calculated according to the formula described by Santos-Silva (2002) and shown in Eq. (3):

h/H:(C18:1+C18:2+C20:4+C18:3+C20:5+C22:5+C22:6)/(C14:0+C16:0) (3)

2.6 Mineral composition

Mineral analysis described by the Sarruge and Haag method (Sarruge; Haag, 1974) was used in nitric-perchloric acid digestion at 50 ºC for 10–15 min and then at 100 ºC to digest all material. Then, the content levels of boron, sulfur, phosphorus, potassium, calcium, magnesium, zinc, copper, manganese and iron were quantitatively evaluated using an atomic absorption spectrophotometer (Perkim Elmer®, 3110, Wellesley, United States) at 248.3nm. The data were described in $mg.100g^{-1}$.

2.7 Statistical Analysis

The results were subjected to an analysis of variance (ANOVA), and tests for comparison of averages (Tukey's 5% probability of error). Statistical calculations were performed using the program R® version 5.0. The differences were considered significant when $p < 0.05$. The principal components analysis (PCA) was used to comprehend the similarity among the edible coatings in relation to key nutritional parameters, by the program Chemoface (Nunes *et al.,* 2012).

3. RESULTS AND DISCUSSION

3.1 Determination of bioactive compounds

The content of total phenolic compounds, tannins, vitamin C, DPPH• and beta-carotene/linoleic acid from food spreads prepared with different baru almond content are described in Table 2.

Table 2. Mean values of total phenolic compounds (measured by *Folin Ciocalteau* and Fast Blue BB), tannins, vitamin C, DPPH• and beta-carotene/linoleic acid present in food spreads made with different levels of baru almonds [1]

Analytical Determinations	Treatments [(2)]			
	P1	**P2**	**P3**	**P**
Total Phenolic Compounds (*Folin Ciocalteau*) GAE [(3)] (mg.100g^{-1})	343.60±0.32^{a}	346.66±0.43^{a}	225.89±0.54^{b}	0.0001
Total Phenolic Compounds (Fast Blue BB) GAE [(3)] (mg.100g^{-1})	46.80±0.43^{a}	45.05±0.72^{a}	33.26±0.98^{b}	0.0012
Tannins CE [(4)] (g.100g^{-1}) [ns]	622.19±1.73	645.24±0.88	517.96±1.93	0.5535
Vitamin C [(5)] (mg.100g^{-1})	24.37±0.24^{a}	19.80±0.45^{b}	18.25±1.47^{b}	0.0040
DPPH• IC50 (g of DPPH sample.g^{-1})	9.83±0.92^{c}	11.87±0.40^{b}	19.45±0.99^{a}	0.0002
Beta-carotene/linoleic acid (% protection) [ns]	87.54±1.11	85.39±0.86	84.90±1.24	0.5297

[1] Mean values ± standard deviation (in quadruplicate, n = 4). Values labeled with different letters in the same line are significantly different (Tukey's test at 95% significance; $p < 0.05$); [2] Treatments: P1 (100% baru almond and 0% hazelnuts), P2 (50% baru almond and 50% hazelnuts) and P3 (0% baru almond spread 100% hazelnuts); [3] GAE: Gallic Acid Equivalents; [4] Catechin equivalents; [5] Expressed in mg of ascorbic acid; ns: not significant ($p > 0.05$); P: p-value.

Of the bioactive compounds listed in Table 2, it was possible to observe that the P1 treatment presented a higher content of vitamin C (P1 – 24.37 mg.100g^{-1}, P2 – 19.80 mg.100g^{-1} and P3 – 18.25 mg.100g^{-1}) and a higher antioxidant activity ($p < 0.05$) as measured by DPPH• (P1 - 9.83 g of DPPH• sample.g^{-1}, P2 - 11.87 g of DPPH• sample.g^{-1} and P3 - 19.45 g of DPPH• sample.g^{-1}). As the baru almond is the main raw material in this treatment, it is worth mentioning that this oleaginous product has provided a potentiation of the bioactivity of these molecules (when compared to other treatments). When evaluating the P2 treatment, it was found that P1 did not present a statistical difference ($p < 0.05$) when total phenolic compounds were evaluated (evaluated by the two methods) (method *Folin Ciocalteau*: P1 – 343.60 mg.100g^{-1} GAE, P2 – 346.66 mg.100g^{-1} GAE and P3 – 225.89 mg.100g^{-1} GAE; method Fast Blue: P1 – 46.80 mg.100g^{-1} GAE, P2 – 45.05 mg.100g^{-1} GAE and P3 – 33.26 mg.100g^{-1} GAE), indicating that the use of a mix containing 50% of baru almond and 50% of hazelnut favors the presence of these substances. On the other hand, when the presence of tannins and

antioxidant capacity measured by beta-carotene/linoleic acid were evaluated, no significant difference was detected between treatments ($p < 0.05$).

In reference to the results obtained for total phenolic compounds (measured by the *Folin Ciocalteau* method), it has been demonstrated that these substances are high in the developed spreads. It is possible to verify that, when compared to P1, treatment with P3 gives a decrease of 34.25% of these molecules, indicating that the inclusion and exclusion of baru (P1 and P3, respectively) almonds may reduce the presence of phenolic compounds ($p < 0.05$). The obtained values of phenolic compound in other oilseeds show an average content of 32, 46, 47,137 and 239 $mg.100g^{-1}$ for pine nuts, macadamia, nuts, almonds, cashew nuts and brazil nuts, respectively (Kornsteiner *et al*., 2006). The developed spread has higher amounts of phenolic compounds than the baru almond itself, because according to Fráguas *et al.* (2014) it has 228.24 mg of phenolic compounds in 100 g. Processing, more specifically the drying process of oilseeds - which allows exposure and increases the availability of these compounds (Lemos *et al*., 2012), as well as the connection with other substances such as cocoa, which stands out for its considerable presence of polyphenols (308 $mg.100g^{-1}$) (Giacometti *et al*., 2016), may contribute to the increase of these molecules. Pinelli *et al.* (2015) found 86.16 mg/100 g of phenolic compounds in cookies made from 100% defatted baru almond flour. The removal of fat as well as the roasting process resulted in a considerable reduction in the presence of these bioactive molecules.

Similar behavior was observed when using the Fast Blue BB method, where P1 and P2 are statistically similar ($p < 0.05$) and differ from P3 ($p < 0.05$). However, this method provided a reduction in the detection of phenolic substances. This evidence is attributed to the fact that this procedure is specific and unique in the quantification of phenols, since the reagents used do not complex with other substances, like proteins, sugars and other reducing compounds such as ascorbic acid (as in the method of *Folin Ciocalteau*) (Naczk; Shahidi *et al*., 2004). Despite providing a lower result regarding the presence of phenolic compounds, this methodology is verified more accurate because it provides the exclusive dosage of these compounds.

Tannins were observed as a considerable presence in all spreads; besides, no statistical difference was detected ($p > 0.05$) indicating that the type of oleaginous seed (baru almond and hazelnut) also contributes to the presence of these molecules. According to Campidelli *et al.* (2020) and Efraim *et al.* (2011), baru almonds, hazelnuts and cocoa, which are the major ingredients in all spreads, are considerable sources of this element. Efraim *et al.* (2011) explain that cocoa seeds contain a complex series of procyanidins, formed from the condensation of individual units of catechins or epicatechins (called monomers) that are responsible for the formation of tannins in this food. Tannin compounds are considered a potent antioxidant and have antimutagenic and anticancer properties (Chu *et al*., 2016). These substances are beneficial when consumed in correct dosages and researches assert that the bioactivity of these compounds are attributed primarily to the ability to minimize the action of free radicals through the interception of active oxygen. Pinelli *et al.* (2015), when developing a cookie with 100% defatted baru almond flour, found 37.32 $mg.100g^{-1}$ of tannins. The high temperatures to which these products were subjected, could potentially be responsible for the minimization of these molecules.

Since they belong to a group of phenolic compounds, they present high reactivity and are associated in the prevention of diseases related to oxidative stress. Delfino and Canniatti-Brazaca (2010) explain that the abundant consumption of this substance must be controlled, since there is compromise in the use of minerals and proteins causing anti-nutritional effects. However, since oilseed consumption generally does not occur in natural form (mainly due to the sensorial aspect it presents in this condition), the process of drying them (which was the primary stage of processing) becomes an important step in the development of sensory aspects and reduction of antinutritional substances.

Regarding the vitamin C content, it was found that P1 presented the highest content of these substances. In addition, P2 and P3 were not statistically different ($p < 0.05$). The absence of baru almond (in P3) caused a 25% reduction of this element (when compared to P1) indicating that this oleaginous seed can contribute directly in the presence of this vitamin, because according to Campidelli *et al.*, baru almond has 39.14 $mg.100g^{-1}$ of vitamin C. Even with the reductions found, it

was found that the developed spreads had a high content of vitamin C. The consumption of 100 g of the same, allows the supply of 27.07% (P1), 22.0% (P2) and 20.27% (P3) of the Reference Daily Intake (RDI) developed by the Food and Nutrition Board at the National Academies Institute of Medicine Recommended Daily Intake (which is 90 mg^{-1} for an adult male) (Institute of Medicine, 2000). According to this same recommendation, these substances when ingested at the appropriate dosages, may provide physiological benefits to the body of a normal adult individual since ascorbic acid is a preventive antioxidant. Also, vitamin C acts as a co-factor of several enzymes in the human body, in addition to being an important natural antioxidant. Due to the inability to synthesize vitamin C, humans need to acquire this substance through diet and lack thereof may result in diseases such as scurvy and pernicious anemia (Traber; Stevens, 2011).

With regard to the means obtained for antioxidant capacity, it was considered that there was sequestering activity of DPPH• with a statistical difference in all treatments ($p < 0.05$). However, it is observed that P1 has higher values, because the lower the IC_{50}, the greater the antioxidant activity of the compound (Pelvan *et al*., 2018). , Due to treatments P2 and P3 having lower amounts of baru almond (when compared to P1), they demonstrated a respective reduction of 20.75% and 97.89% of the antioxidant capacity in said treatments and this reduction may be compatible with the antioxidant composition of hazelnuts. In addition, the interaction of this oleaginous seed with cocoa, which has a high antioxidant activity (Hernández-Hernández *et al*., 2018), has allowed the quantification of antioxidants in these spreads (even if values below P1).

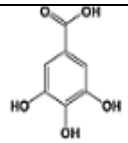
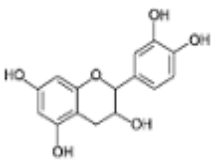
Some compounds due to being lipophilic do not react with the free DPPH• and, thus, the need arises for different methodologies to be sought to characterize such components. Therefore, total antioxidant activity was also measured by the beta-carotene/linoleic acid system and the results for this determination, according to Hassimoto *et al.* (2005), can be classified as: (i) high levels (>70%); ii) intermediate (40%-70%) and iii) low (<40%), in the inhibition of oxidation. According to this information, all spread developed demonstrated high levels and no statistical difference ($p < 0.05$) of lipophilic antioxidants, showing that both oleaginous seeds also contribute to the presence of these substances. These results are recognized as important because oxidative processes (which are

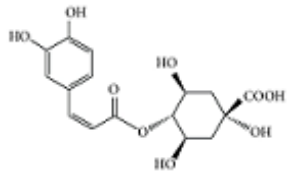
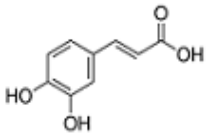
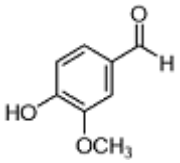
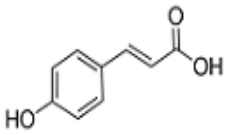
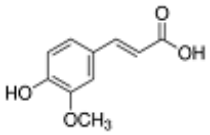
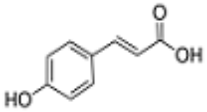
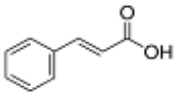
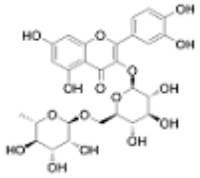
precursors of several chronic noncommunicable diseases) can be avoided by the use of antioxidant substances that have the property of preventing or reducing triggering of unwanted reactions (Dillinger *et al.*, 2000). According to Campidelli *et al.* (2020), baru almond exhibited 91.7% protection, demonstrating that much of the antioxidant capacity of pastes comes from it.

3.2 Individual identification of phenolic compounds by HPLC-DAD

A total of 10 phenolic compounds were detected in the three treatments, including flavonoids (catechin) and non-flavonoids (gallic acid, chlorogenic acid, vanillin, p-coumaric acid, o-coumaric acid, ferulic acid, trincinnamic acid and rutin). The results associated with the identification and quantification of these molecules are shown in Table 3 and the highest results were obtained for gallic acid (P1 - 31.26 mg.100g^{-1}, P2 – 24.26 mg.100g^{-1} and P3 – 10.14 mg.100g^{-1}), catechin (P1 – 32.46 mg.100g^{-1}, P2 – 31.95 mg.100g^{-1} and P3 – 14.75 mg.100g^{-1}) and chlorogenic acid (P1 – 8.80 mg.100g^{-1}, P2 – 7.61 mg.100g^{-1} and P3 – 1.31 mg.100g^{-1}).

Table 3. Identification and quantification by high-performance liquid chromatography with diode-array detection (HPLC-DAD) of phenolic compounds present in food spreads made with different contents of baru almonds

		Treatments (mg.100g^{-1}) (2)			
Phenolic Compound	Chemical Structure	P1	P2	P3	P
Gallic Acid		31.26±0.02^{a}	24.26±0.45^{b}	10.14±0.01^{c}	0.0070
Catechin		32.46±0.15^{a}	31.95±0.11^{a}	14.75±1.71^{b}	0.0001

Chlorogen Acid		8.80±0.01[a]	7.61±0.08[a]	1.31±0.16[b]	0.0001
Caffeic Acid		0.93±0.01[a]	0.60±0.02[b]	0.16±0.11[c]	0.0030
Vanillin		0.65±0.00[b]	0.57±0.03[b]	1.14±0.01[a]	0.0001
p-coumaric		-	-	0.02±0.03	0.0001
Ferrulic Acid		-	0.13±0.00[b]	0.71±0.04[a]	0.0001
o-coumaric		-	-	0.42±0.03	0.0001
Trancinnamic Acid		1.70±0.13	1.64±0.09	1.55±0.08	0.2389
Rutin		38.42±0.18[b]	45.32±0.78[a]	44.66±0.57[a]	0.0001
22Sum of phenolic compounds		114.22	112.08	74.86	

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p<0.05$); 2 [2]Treatments: P1 (100% baru almond and 0% hazelnuts), P2 (50% baru almond and 50% hazelnuts) and P3 (0% baru almond spread 100% hazelnuts); ns: not significant at the level ($p>0.05$); P: p-value.

Among the phenols identified by HPLC, rutin, catechin and gallic acid, which have antioxidant capacity, were the major phenolics present in spreads. It was found that some of the components present in the spread (gallic and caffeic acid) had their mean values increased ($p < 0.05$)

through the use of baru almonds (P1 treatment) and one of the possible explanations is associated with the fact that this is an oleaginous source of phenolic substances. Campidelli *et al.* (2020), when evaluating the profile of phenolic compounds in baru almonds, found that rutin, gallic acid and caffeic acid are major compounds in this profile. A significant increase ($p < 0.05$) in ferulic acid, vanillin, rutin and an unpublished quantification of *p*-coumaric acid in P3 treatment was also observed and this result was attributed to the use of hazelnut in this formulation. According to Pelvan *et al.* (2018) this oleaginous seed is also a source of these components. Regarding trancinnamic acid, no significant difference ($p < 0.05$) was observed between the treatments, indicating that the use of baru almonds and hazelnuts may also contribute to the inclusion of these elements in the spread.

The results obtained are not only due to the presence of baru almonds and hazelnuts, but also through the inclusion of cocoa, which in turn is also considered a source of phenolic substances (Hernández-Hernández *et al*., 2018). Authors affirm that the presence of phenolic compounds in cocoa confers antioxidant properties avoiding the formation of free radicals that act in the process of development of non-transmissible chronic degenerative diseases (Hernández-Hernández *et al*., 2018). It is emphasized that the high presence of these substances in foods is desired as they are able to perform health benefits due to the broad spectrum of medicinal properties they present and it is found that processed spreads can be considered food sources of phenolic compounds.

Żyżelewicz *et al.* (2018) investigated the profile of phenolic compounds in chocolates made with 100% cocoa liquor and also found gallic acid as the major compound (325.73 $mg.100g^{-1}$), followed by epicatechin (192.31 $mg.100^{-1}$). The superiority of these results over those obtained in this study, is due to the fact that the cocoa used was 100% pure, thus increasing its bioactivity.

3.3 Profile of fatty acids by CG-FID

The determination of the fatty acid profile present in food spreads supplemented with different levels of baru almonds is shown in Table 4.

Table 4. Identification and quantification by gas chromatography with flame-ionization detection (GC-FID) of fatty acids present in the alimentary spreads supplemented with different contents of baru almonds [1]

	Treatments [2]			
Fatty Acids (g 100 g^{-1})	**P1**	**P2**	**P3**	**P**
Hexanoate	0.03±0.00^{a}	0.03±0.00^{a}	0.02±0.00^{a}	0.10823
Octanoate	1.64±0.09^{b}	1.71±0.15^{ab}	2.39±0.24^{a}	0.02947
Undecanoate	15.50±0.56^{b}	16.55±2.06^{b}	26.58±2.50^{a}	0.01254
Laurate	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.33029
Tridecanoate	5.76±0.03^{a}	6.26±0.94^{a}	9.98±1.00^{a}	0.01152
Pentadecanoate	0.01±0.00^{b}	0.01±0.00^{b}	0.02±0.00^{a}	0.00236
Palmitate	0.13±0.01^{a}	0.09±0.01^{a}	0.09±0.01^{a}	0.08953
Methyl Behenate	2.09±0.25^{b}	4.63±0.54^{a}	5.82±0.43^{a}	0.0001
Methyl Arachidate	2.91±0.07^{b}	5.01±0.09^{a}	0.93±0.16^{c}	0.0001
Methyl Stearate	3.67±0.21^{c}	6.89±0.08^{a}	5.01±0.12^{b}	0.0001
∑ SFA [3]	31.53±0.79^{b}	41.48±3.40^{b}	51.04±4.11^{a}	0.01009
Myristoleic	0.11±0.03^{a}	0.11±0.02^{a}	0.13±0.01^{a}	0.10467
Heptadecanoate	0.09±0.01^{a}	0.07±0.01^{a}	0.10±0.01^{a}	0.13281
Heptadecenoic acid	3.35±0.08^{a}	4.05±0.51^{a}	4.70±0.42^{a}	0.09552
Octadecanoate	0.06±0.02^{a}	0.081±0.02^{a}	0.10±0.02^{a}	0.13666
Oleic acid	57.85±0.87^{a}	37.20±7.26^{b}	27.52±7.60^{b}	0.00707
Eicosenoate acid	0.14±0.00^{c}	0.76±0.51^{b}	1.75±0.46^{a}	0.00016
Erucate acid	0.05±0.00^{b}	0.05±0.00^{b}	0.10±0.00^{a}	0.00638
∑ MUFA [4]	61.63±1.10^{a}	49.14±6.18^{a}	32.83±6.88^{a}	0.53647
Linoleate acid	6.66±0.03^{b}	9.24±2.02^{b}	13.46±1.91^{a}	0.00643
Gamma-linolenic acid	0.12±0.00^{c}	0.26±0.29^{b}	1.05±0.26^{a}	0.00024
Docosadienoic acid	0.06±0.01^{c}	0.87±0.50^{b}	1.61±0.45^{a}	0.00017
∑ PUFA [5]	6.84±0.05^{b}	9.38±2.81^{b}	16.13±2.64^{a}	0,003
PUFA/SFA [6]	0.21±0.00^{b}	0.22±0.06^{b}	0.31±0.05^{a}	0.00329
MUFA/SFA [7]	1.95±0.14^{a}	1.18±0.56^{b}	0.64±0.65^{c}	0.00532
TI [8]	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.1643
AI [9]	0.05±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}	0.36538
h/H [10]	489.77±36.57^{a}	510.15±21.48^{a}	508.24±19.23^{a}	0.3455

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p<0.05$); [2] Treatments: P1 (100% baru almond and 0% hazelnuts), P2 (50% baru almond and 50% hazelnuts) and P3 (0% baru almond spread and 100% hazelnuts); [3] Total saturated fatty acids; [4] Total unsaturated fatty acids; [5] Total of polyunsaturated fatty acids; [6] Relationship between saturated and polyunsaturated fatty acids; [7] Relationship between saturated and unsaturated fatty acids; [8] Thrombogenic index; [9] Atherogenic index; [10] hypocholesterolemic/hypercholesterolemic potential; P: *p*- value.

Unsaturated fatty acids, including oleic and linoleate acids, were found to be predominant and a significant difference between treatments ($p < 0.05$) was detected (acid oleic: P1 – 57.85 %, P2 – 37.20%, P3 – 27.52 %; and acid linoleate: P1 – 6.66%, P2 – 9.24%, P3 – 13.46%). The high presence of monounsaturated fatty acids was also observed. This same profile was found in baru almond, since high concentrations of monounsaturated fatty acids were quantified, particularly oleic acid (Campidelli *et al.*, 2020).

The Dietary Guidelines for Americans (2016) states that the daily intake of MUFAs is at least 33 g^{-1} (for a 2000 kcal diet) and, in this regard, the intake of 100 g^{-1} of spread corresponding to P1, P2 and P3 can respectively provide 1.8, 1.5 and 0.9 times this recommendation. Research indicates that oilseeds are sources of these compounds and both baru almonds and hazelnuts contributed ($p < 0.05$) to this profile. Ingestion of these fats is recommended by experts as there is scientific evidence that proves they can reduce the risk of cardiovascular disease, be effective in weight loss and contribute to lowering low-density lipoprotein (LDL) levels in the blood without affecting high-density lipoprotein (HDL) levels (beneficial to health) (Lottenberg, 2009).

As for saturated fatty acids, undecanoate was the majority, and P3 was the treatment with higher averages ($p < 0.05$). Hazelnut and cocoa, which are rich in this type of fatty acid (Lottenberg, 2009), were probably responsible for the elevation of saturated fatty acids in this treatment, indicating the moderate consumption of these spreads.

For a balanced intake of fatty acids, which are essential for health, the ratio between MUFA and SFA should be at least 0.45 and in this regard, was found that the P1, P2 and P3 spread are respectively 4.33, 2.62 and 1.42 higher than this recommendation. In relation to this profile, it is estimated that the spreads may be effective in controlling the traditional risk factors for atherosclerotic cardiovascular disease (Lottenberg, 2009). The PUFA/SFA ratio should be as high as possible, preferably greater than 0.4. To this end, no treatment reached the recommendation, however, P1, P2 and P3 obtained 0.52, 0.55 and 0.77% of it, respectively. This result was expected as baru almond is not a matrix rich in polyunsaturated fatty acids (Campidelli *et al.*, 2020).

As for the atherogenic index (AI), thrombogenic index (TI) and (h/H) fatty acids, which indicate the potential for stimulation of platelet and coronary aggregation, the values obtained for P1, P2 and P3 were similar in all treatments ($p > 0.05$).Although there is no established parameter for these indices, the lower the result for AI and TI and the higher for h/H, the lower the chance of coronary changes and the healthier the food. This is due to the higher concentration of antiatherogenic fatty acids (Turan *et al*., 2007). Foods such as cheeses and meats, which are substantial sources of fatty acids, have IA, IT and h/H ratios of 2.32, 3.11, 1.23 and 0.54, 1.15, 1.76, respectively; and when comparing these products with spreads, superiority and greater possibility of cardiovascular protection for part of them is demonstrated. Żyżelewicz *et al.* (2018) found oleic (34.10%), linoleic (3.02%) and stearic (35.93%) acids as the major fatty acids in chocolates made with 100% cocoa liquor.

3.4 Mineral Composition

The determination of the mineral composition of phosphorus, calcium, magnesium, sulfur, manganese, zinc, boron, copper and iron present in food spreads supplemented with different levels of baru almonds, as well as the mineral RDI, are shown in Table 5.

Table 5. Average values of the mineral composition of food spreads supplemented with different levels of baru almonds [1]

	Treatments (mg.100g^{-1}) [2]				
Minerals	**P1**	**P2**	**P3**	**P**	**RDI**[3] **(mg.100g^{-1})**
Phosphor	4100±0.23^{ba}	4000±0.44^{a}	3600±0.93^{b}	0.0127	700
Calcium	3500±2.33^{a}	2500±1.03^{b}	2300±1.44^{b}	0.0004	1000
Magnesium	1700±1.03^{a}	1400±1.92^{b}	1200±1.96^{c}	0.0011	260
Sulfur	2000±0.45^{a}	1800±0.32^{b}	1600±0.93^{c}	0.0003	850
Manganese	52.7±0.96^{a}	35.6±0.86^{b}	20.8±0.98^{c}	0.0000	2.3
Zinc	35.1±0.95	40.6±0.65^{a}	43.2±0.74^{a}	0.0012	7
Boron	19.8±0.99^{a}	18.4±2.30^{a}	10.2±3.55^{b}	0.0067	3
Copper	15.9±2.84^{b}	29.4±3.45^{a}	29.4±0.95^{a}	0.0000	0.9
Iron	125.9±3.04^{a}	132.4±3.92^{a}	106.4±2.95^{b}	0.0000	14

[1] Mean values ± standard deviation (in quadruple, n = 4). Values followed by different letters in the same line show differences by Tukey's test at 95% significance ($p<0.05$); [2] Treatments: P1 (100%

baru almond and 0% hazelnuts), P2 (50% baru almond and 50% hazelnuts) and P3 (0% baru almond spread 100% hazelnuts); [3] Recommended Diary Ingestion (BRASIL, 2005).

As can be observed, all treatments showed a significant difference ($p < 0.05$) for analyzed minerals, showing that the variation of baru almonds and hazelnuts provided changes in these elements. It was found that the higher the baru almond content in the spread (P1), the higher the concentrations of phosphorus, calcium, magnesium, sulfur, manganese, zinc, boron and iron. Campidelli *et al.* (2019), upon analyzing the mineral composition of baru almond, found that it contained considerable amounts of boron, magnesium, copper and manganese, much higher than the daily recommendation.

With regard to the phosphorus, iron and boron present in the P2 treatment, these elements are significantly ($p < 0.05$) similar to the P1 treatment, indicating that the use of 50% baru almonds and 50% hazelnuts can provide the same result when using only baru almonds.

Regardless of the oleaginous seed used, all almonds presented high levels of minerals (Institute of Medicine, 2000) because the consumption of 100 g allows the ingestion of quantities superior to the 30% that advocates the reference RDI.

Concentrations of phosphorus, calcium, magnesium, sulfur, manganese, zinc, boron, copper and iron may, respectively, provide 5, 2, 5, 2, 15, 5, 5, 27 and 8 times this recommendation, suggesting the spread developed has a high mineral composition. It is thus established, that the consumption of these products should be stimulated since they supply the RDI of different minerals and can help in the maintenance of health. In general, oleaginous plants have high levels of minerals and almonds of baru are among the most outstanding.

3.5 Principal components analysis and hierarchical cluster analysis

Due to the relatively high number of variables studied, principal component analysis (PCA) was performed to elucidate the characteristics related to these variables, correlating them and grouping them with the treatments used (P1, P2 and P3) (Figure 1).

Figure 1. Principal Component (PC) 1 and 2 score chart for food spreads supplemented with different levels of baru almonds

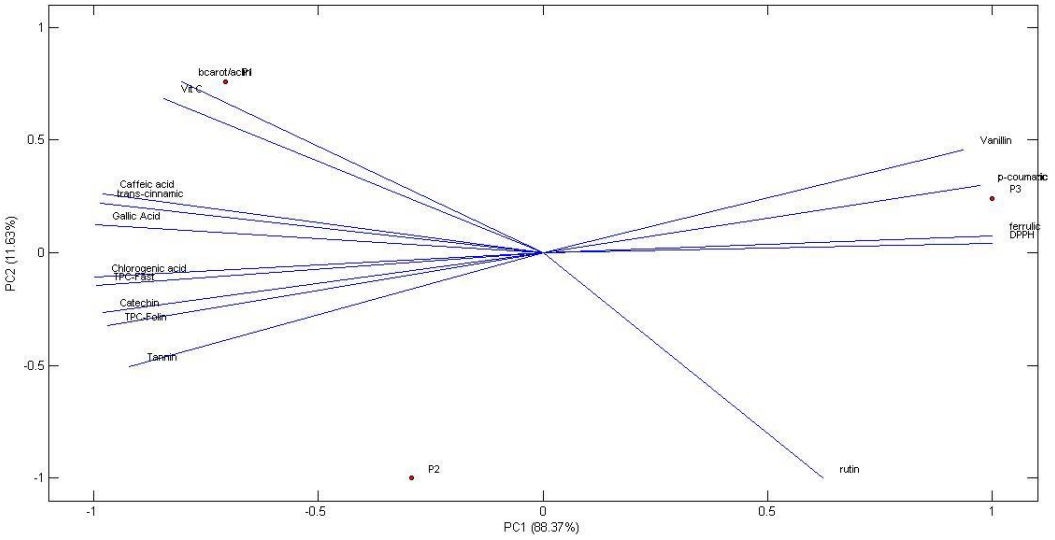


Figure 1 shows the PCA results for bioactive compounds and phenolic compounds profile. The first two main components explained 100% of the total variation between treatments for these compounds, and the first component explained 88.37% of this variation.

The spatial separation of the formulations shows that the formulation of the spreads with different contents of baru almond differed with respect to bioactive compounds and phenolic compounds. Moreover, formulation P3 presented higher values for vanillin variables, *p*-coumatic ferrulic, DPPH•, rutin. The formulation P1 presented higher values for the variables beta-carotene/linoleic acid, vitamin c, caffeic acid, trans-cinnamic, gallic acid, chlorogenic acid, total phenolic compounds (Fast method), catechin, total phenolic compounds (Folin method), tannin and formulation P2 presented intermediate values for the analyzed variables.

3. CONCLUSIONS

The addition of baru almond (P1 treatment) potentiated the concentrations of vitamin C, antioxidants, gallic acid, calcium, magnesium, sulfur, manganese and oleic acid. In contrast, the absence of this oil in P3 treatment increased concentrations of vanillin, *p*-coumaric, ferric, *o*-coumaric, acid and saturated and polyunsaturated fatty acids. When tannins, beta-carotene/linoleic acid, trans-cinnamic acid, monounsaturated fatty acids, hypocholesterolemic and hypercholesterolemic fatty acids contentand atherogenic and thrombogenic indices were evaluated, no significant ($p > 0.05$) differences were detected between treatments.

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ARTIGO 5 – BARU ALMONDS (*Dipteryx alata* VOG.) AND BARU ALMOND PASTE PROMOTE METABOLIC MODULATION AND EXERT ANTIOXIDANT EFFECTS[$]

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Baru almonds (*Dipteryx alata* Vog.) and baru almond paste promote metabolic modulation and exert antioxidant effects [$]

Running title: Metabolic modulation of baru almonds and paste

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Abstract

Chronic non-communicable diseases are associated with oxidative stress and are highly prevalent across the world, responsible for over 70% of mortality worldwide. The ingestion of bioactive compounds of natural origin has provided promising results for the prevention and treatment of these diseases. The present study sought to investigate the effects of the baru

almond, whole and as a paste, on metabolic and oxidative regulation in hyperlipidemic rats. Thirty-two rats *(Rattus norvegicus* albinus) of the Wistar strain were divided into four groups and fed various experimental diets over a period of 35 days: normolipidic diet (negative control), hyperlipidic diet (positive control), and hyperlipidic diet supplemented with baru almonds or baru almond paste. When supplemented with baru almonds or baru almond paste, hyperlipidic diets reduced the levels of CT-c, triacylglycerols, glutathione reductase, and malonaldehyde, and increased the levels of HDL-c, iron reducing power **(**FRAP), and catalase. No differences were observed between the diets with respect to the levels of liver enzymes (aspartate aminotransferase and gamma-glutamyltransferase) or the oxygen radical absorbance capacity ($ORAC_{FL}$). Supplementation with baru almond reduced oxidative stress by increasing the expression of the *Manganese Superoxide Dismutase* (*MnSod*) gene in the liver. Likewise, supplementation with baru almond paste reduced the LDL-c levels, but also increased the levels of alanine aminotransferase. These results demonstrate that supplementation with both baru almonds and baru almond paste improve the metabolic and oxidative profile of hyperlipidemic rats.

Keywords: oxidative stress; gene expression; bioactive compounds; cerrado biome.

1. Introduction

Chronic non-communicable diseases (NCDs), such as dyslipidemia, neurodegenerative diseases, obesity, and diabetes *mellitus*, are associated with oxidative stress [1, 2]. Oxidative stress is a biological condition in which there is an imbalance between the production of reactive oxygen species and antioxidant compounds. This process leads to the oxidation of biomolecules and the subsequent loss of their biological functions, predisposing cells to degeneration, senescence, and apoptosis [3]. These diseases are highly prevalent among the world's population and are responsible for 41 million fatalities each year, equivalent to 71% of deaths worldwide [4]. The main factor that modulates NCDs is the dietary habits of modern Western society, which is widely considered a public health problem. The high intake of processed foods, low levels of physical activity, high levels of stress, and, in particular, the low consumption of foods rich in bioactive compounds are examples of behavior that favor the development of these diseases [4,5]. In addition, the large intake of saturated fat (and diets rich in omega-6) also contributes to this scenario by promoting an imbalance between the production of free radicals and the effectiveness of the endogenous antioxidant system [6].

Allopathic medicine (i.e. drugs traditionally used to combat pathologies) is a traditional therapy widely used to prevent and combat these diseases. However, numerous side effects are associated with these drugs, including dizziness, changes in heart rate, headache, vomiting, and nausea, among others [7]. To avoid this situation, alternative therapies have generated promising results, in which the use of certain foods as potential adjuvant for drugs is increasing along other complementary and alternative medicines [8]. This increase is largely due to the discovery of biologically active substances in foods, which are responsible for modulating the biomarkers involved in the pathogenesis of these diseases.

Among the main foods with beneficial health properties are oilseeds. These, are known to contain significant amounts of bioactive compounds, especially monounsaturated fatty acids and vitamin E. This nutritional profile makes these foods fit the dietary pattern proposed by the Mediterranean diet, known for promoting the health and preventing the onset of diseases [9] One of the most promising almond is the baru (*Dipteryx alata* Vog.) native from Brazil. Although the information available on these foods is limited, especially *in vivo*, the baru almond is known to have a nutritional composition with potential antioxidant effects [9]. The baru almond *in natura* has the following approximate composition (in %): 6.63 (moisture), 31.73 (lipids), 1.55 (ash), 22.96 (proteins), 37.13 (carbohydrates), and 14.44 (total fibers). The almond contains oleic (48.99%) and linoleic acids (27.28%), minerals (boron, zinc, copper, manganese and magnesium), antioxidant molecules, such as polyphenols (catechin, rutin and gallic, caffeine, chlorogenic, *o*-cumaric and *trans*-cinnamic acids), and vitamins (C and E (alpha and gamma-tocopherols)) [10,11,12,13,14]. In synergism, these substances have a high potential for inhibiting oxidation and can exert positive effects in preventing diseases linked to oxidative stress [15,16,17]. Despite the many benefits presented by baru almonds, few studies yet have investigated the action of its bioactive compounds and the metabolic and molecular effects *in vivo*. Thus, the present study investigated the effect of baru almond on the metabolic and oxidative regulation of Wistar rats fed a high-fat diet [18]. Because there is a commercial demand for ready-to-eat products, it was also included a handmade food paste. Preclinical nutritional studies provide important information before proposing safe and efficacious startup doses for human studies. [19,20]. To this end, the present study investigated the effects of baru almonds and baru almond paste on the metabolic and oxidative regulation of Wistar rats fed a high-fat diet.

2. Materials and methods

2.1 Study design

This study was approved by the Ethics Committee on the Use of Animals at the Federal University of Lavras (UFLA) under protocol No. 039/17. All the procedures followed the guidelines of the National Council for the Control of Animal Experiments in Brazil (CONCEA-SBCAL) and also followed the Guidelines proposed by ARRIVE (www.nc3rs.org.uk).

Following the simple random sampling model proposed by Cochran [21], which defines the number of repetitions per plot necessary to obtain a minimum number of repetitions sufficient for statistical analysis, we used eight animals (n = 8) for each of the four treatments, totalizing thirty-two rats. The animals were adult male of Wistar rats (*Rattus norvegicus albinus*; body weights of approximately 341.10 ± 25.44 g), healthy and approximately 100-day-old provided by the Bioterism Center of the Federal University of Minas Gerais (UFMG). Rats were randomly divided into four groups (with similar initial body weight; $p>0.05$) fed over a period of 35 days with different diets: NC (normal diet - negative control), HC (high fat diet - positive control), HBA (hyperlipidic diet with added baru almonds) and HBP (hyperlipidic diet with added baru almond paste). The groups were accommodated in collective polypropylene boxes lined with autoclaved wood shavings (changed daily) and kept for thirty-five days in a room at a temperature of 22 ± 2 °C and humidity 45 ± 15% and with light/dark cycles of 12/12 hours. Throughout the experimental period, water and feeding were provided *ad libitum* and daily consumption of potable water and food was measured. These measurements being obtained from the difference between the initial and remaining quantities. No modifications were made to the protocols despite side effects and there were no animal losses during the experiment.

2.2 Food diet preparations

Baru (BA) almonds were purchased from producers in the city of Barra do Garça, state of Mato Grosso, Brazil (latitude: 15° 53’ 35” South, longitude: 52° 15’ 36” West). The BA were dried at 105°C for 30 minutes in a circulated air oven (MA0351; Marconi, Piracicaba, SP, Brazil), ground (experimental mesh mill 20, VG 2000i; Viti Molinos, Itajaí, SC, Brazil), packed in transparent polyethylene bottles, and stored at 25°C in temperature-controlled chambers (EL202; Eletrolab, São Paulo, SP, Brazil) for subsequent use in the preparation of the diets (the total storage time was 40 days).

Baru almond paste (BP) was prepared on a weekly basis. The BP was composed of 35% dry baru almonds (105°C for 30 minutes), 29.5% erythritol (Tovani®), 14% coconut oil (Copra®), 10.4% cocoa powder (Nestlé®), 5.6% skimmed-milk powder (Molico®), 3% polydextrose (Tovani®), 1% whey powder (Tovani®), 1% emulsifier (Emustab®), and 0.5% flavoring (Arcolor®). The BA was ground and homogenized for 5 minutes using a food processor (Walita®). The other ingredients were added and the mixture was homogenized for another 5 minutes to obtain the BP. The BP was then packed in transparent polyethylene packaging and stored at 12°C (EL202; Eletrolab) for subsequent use in the preparation of the diets.

The diets were formulated and adapted from AIN-93 [22]. The ingredients (Table 1) were weighed and homogenized in a stainless-steel container to obtain a mass that was manually molded into a cylindrical shape (weighing approximately 20 grams), packaged, and frozen at –22°C. To minimize possible oxidation of lipid sources, the diets were prepared on a weekly basis and offered daily *ad libitum* (at room temperature) in the collective boxes of the animals.

2.3 Euthanasia

On the morning of the last day of the experimental period and 8 hours after feeding, the rats were weighed and euthanized through cardiac puncture under anesthesia (intraperitoneal injection of 50 mg/kg of sodium thiopental). The blood was collected and distributed in polypropylene tubes and centrifuged at 10,000 rpm for 10 minutes to obtain the serum, which was stored at –22°C. A fraction of the liver was removed, kept in liquid nitrogen (–45°C to –55°C) until storage at –80°C until. The other organs, including the kidneys, spleen, pancreas, intestine, and liver remanescent, were collected and stored in formaldehyde for histopathological analysis.

2.4 Determination of nutritional and biochemical parameters

Biochemical analyses of total cholesterol (CT-c), low- and high-density lipoprotein (LDL-c and HDL-c, respectively), and triacylglycerols (TGs) were performed using the extracted blood serum and commercial colorimetric kits (Labtest Diagnostica S/A®, Lagoa Santa, MG, Brazil) [23]. The Castelli index (CI) I was calculated using the CT-c/HDL-c ratio, and the CI II was calculated using the LDL-c/HDL-c ratio [24].

2.5 Quantification of liver injury levels

Liver injury biomarkers, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT), were evaluated using the extracted blood serum by means of enzymatic and colorimetric assays provided by Labtest Diagnóstica S/A® (Lagoa Santa, MG, Brazil), as indicated by Soldin et al. [25], according to the manufacturer's instructions.

2.6 Histopathological analysis

Histopathological analyses were performed on the kidneys, liver, pancreas, spleen, and small intestine. The organs were preserved in a 4% formaldehyde solution, dehydrated with increasing concentrations of ethyl alcohol, diaphanized in xylene, and included in paraffin blocks for histological sectioning. The paraffin blocks were sectioned in 4-μm slices using a manual microtome, placed on histological slides, and dewaxed in an oven at 65°C. The sections were stained with hematoxylin and eosin (H&E) [26].

The digital images were obtained using an image capture and analysis system consisting of an Olympus BX41 microscope (Olympus Optical do Brasil Ltda, São Paulo, SP, Brazil) equipped with a camera (Color SC30 CMOS camera, Olympus Light Microscopy; Optical do Brasil Ltda, São Paulo, SP, Brazil). Measurements were performed using the Image-Pro® Express software (Targetware Informática Ltda do Brasil, Água Branca, SP, Brazil) [26]. Microscopic lesions were classified according to the nature, distribution, and intensity of the changes as to the presence of degeneration, necrosis, fibrosis, and inflammation. Scores were defined to quantify the intensities of the changes: 0 (normal), 1 (1 to 10%), 2 (11 to 30%), 3 (31 to 60), and 4 (61 to 100%).

2.7 Antioxidant defenses

2.7.1 Total proteins

The total protein concentrations in the liver samples were assessed using the Bradford colorimetric method [27].

2.7.2 Iron reducing power (FRAP)

The reducing power of the liver extracts was measured using the modified Oyaizu [28] method. Volumes of 40 μL of the sample were homogenized with 200 μL of 0.2 M sodium phosphate buffer (pH 6.6) and 200 μL of 1% (w/v) aqueous potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes. Then, 200 μL of 10% (w/v) aqueous

solution of trichloroacetic acid was added to the mixture, and the mixture was centrifuged at 5000 rpm for 10 minutes. The supernatant (0.2 mL) was mixed with 50 μL of 0.1% (w/v) aqueous ferric chloride solution. The intensity of the blue-green color was measured at 700 nm. Ascorbic acid was used as positive control. The aqueous ascorbic acid solution calibration curve was comprised of a concentration ranging from 5.0 to 0.078 mg mL^{-1}. The experiment was conducted in triplicates, with results reported (ascorbic acid equivalent antioxidant activity) as the mean values (expressed as grams of ascorbic acid equivalents (AAE)).

2.7.3 Oxygen radical absorbance capacity ($ORAC_{FL}$)

The extracts were dried at 40°C in an oven and dissolved in 75 mM phosphate buffer (pH 7.4). The $ORAC_{FL}$ assay was performed on an Infinite 200 spectrophotometer (A-5082; Tecan, Grödig, Salzburg, Austria). The analyzer was programmed to record the fluorescence of FL every minute for 150 min, with shaking before each read after the addition of AAPH [29].

2.7.4 Catalase

The measurement of catalase activity consisted of quantifying the rate of decomposition of hydrogen peroxide by the enzyme, and the change was recorded by spectrophotometry for 3 minutes at 230 nm. The result was expressed in $U.mg^{-1}$ of protein per second [30].

2.7.5 Reduced glutathione (GSH)

Liver samples (100 mg) were precipitated using 1:10 (w/v) of 12% TCA, homogenized for 20 seconds, and centrifuged at 5000 × *g* for 5 min. The concentration of thiols was evaluated immediately after obtaining the hepatic acid extracts. The analyses were performed in triplicate, and the values were expressed in $mg.g^{-1}$ of sample [31].

2.7.6 Quantification of malonaldehyde via high performance liquid chromatography (HPLC) DAD/UV-Vis

The quantification of the levels of malonaldehyde (MDA) in the liver of the animals was measured by HPLC, according to the method described by Candan and Tuzmen [32].

2.7.7 Real-time quantitative PCR assay (RT-qPCR)

Rat liver tissues (*Rattus norvergicus*) were collected and macerated directly in liquid nitrogen. Approximately 100 mg was used for extraction of total RNA with Trizol (Ambion®, Life Technology), according to the manufacturer's instructions. The integrity of the RNA samples was verified by agarose gel electrophoresis and spectrophotometry. No signs of degradation were observed, and the absorbance values were nearly 2.0 at 260/230 and 260/280 nm. cDNA was synthesized using iScript™ cDNA Synthesis Kit (BioRad) with 1 µg of RNA, according to the manufacturer's instructions. The primers were designed in an exon-exon region of the genes, with *GAPDH* and *b-Actin* as reference genes. The sequences of the oligonucleotides are provided in supplementary material. The RT-qPCR reactions were performed on a Rotor-Gene Q (Qiagen) apparatus using QuantiNova TM SYBR® Green PCR kit (Qiagen) with 7.5 µl of SYBR, 3 µl of each primer (2 µM final concentration), and 1.5 µl of cDNA for each reaction. The samples were analyzed in technical duplicates for each biological triplicate. The expression analyses followed the ddCT method [32], and the averages were normalized in relation to the treatment that yielded the lowest value for each gene.

2.7 Statistical analysis

For each variable, the experimental unit was an individual animal. The results were analyzed using one-way analysis of variance (ANOVA). If the mean values differed by the F-test, Bonferroni's post-hoc test was used. The significance index was set at $p < 0.05$. All analyses were performed using Prism 5.0 250 (GraphPad, CA, USA) statistical software.

For the statistical analysis of the hepatic steatosis methodology, the diets and steatosis levels (0, 1, 2, and 3) were used in five classes, using the prop.test function of the stats package of the statistical program R [34].

3. Results and Discussion

3.1 Determination of nutritional and biochemical parameters

Dietary consumption by the rats fed high-fat diets (HC, HBA, and HBP) was 37.97% lower than the consumption of rats fed the normolipid diet (NC) ($p < 0.05$; Fig. 1A), indicating that the addition of fat to diets may have resulted in greater satiety in the animals. The high content of lipids with a greater energy value is among the probable causes of this effect [35]. The addition of BA or BP to the high-fat diet did not cause an increase or decrease in dietary intake.

Rats fed the HC diet consumed 39.52% less water than rats fed the NC diet (Fig. 1B). The water consumption of the rats fed high-fat diets supplemented with BA or BP was similar to NC diet rats ($p > 0.05$). According to Chang et al. [36], obese individuals consume less water; therefore, the addition of BA and BP to the diet improved hydration.

The levels of LDL-c and CI II (Fig. 2A and F) were 73.94% and 80.51% lower, respectively, for rats fed the HBP diet than for those fed the HC diet. There was no significant difference ($p > 0.05$) between the LDL-c and CI II indices for the rats fed the HBA and HC diets. The addition of BA or BP to the high-fat diets contributed to a reduction in the CI I ($p < 0.05$) (Fig. 2E), HBP and NC groups showed similar CI I values ($p > 0.05$).

CIs allow to assess the risk of cardiovascular disease. Values greater than 4.4 (CI I) and 2.9 (CI II) indicate a higher risk of cardiovascular disease, adapting for rats [24]. All of the hyperlipidic diets (except HBP) yielded values higher than those recommended for CI I. All groups remained under reference value for CI I during the experimental period.

As for the decreases in LDL-c observed in rats fed the HBP diet, this result is likely to be related to the composition of the food paste, which, as a source of BA and cocoa, is rich in bioactive compounds, such as tocopherols, phenolics, and flavonoids [37,11,38, 39]. However, it is believed that the polydextrose added to this diet (Table 1; item 5) is one of the main factors responsible for this reduction, since this soluble fiber has the ability to regulate lipid metabolism, mainly reducing LDL-c levels. The viscosity of these compounds is able to create a physical barrier, which reduces lipids absorption. This results in increased hepatic conversion of cholesterol to bile acids, as well as in increased LDL-c receptors, restoring hepatic cholesterol concentrations and decreasing serum LDL-c levels. Soluble fibers also increase fermentation and the production of volatile fatty acids, which mechanism indirectly reduces the synthesis of these fractions [40, 41]. In a study by Othman et al. [41], daily doses of at least 3 g of soluble fiber reduced LDL-c in both normocholesterolemic and hypercholesterolemic subjects.

The values of triacylglycerols (Fig. 2C) for rats fed a high-fat diet (HC) were 8.37% higher ($p < 0.05$) than those of rats fed a normal diet (NC). However, when the high-fat diet contained BA or PA, these values were compatible with those obtained in the NC group ($p < 0.05$). The levels of triacylglycerols were 9.97% and 21.34% lower, respectively, then those of rats fed a HC diet ($p < 0.05$). The high concentration of monounsaturated fatty acids in BA, more specifically oleic acid [11], may have promoted the reduction of plasma triacylglycerols.

The consumption of a high-fat diet (HC) resulted in an increase ($p < 0.05$) of 20.61% in the concentration of TC compared to that of a NC diet. By contrast, the addition of BA to

the high-fat diet resulted in a significant decrease ($p < 0.05$) of 11.47% in the total cholesterol content. The addition of BP did not cause a reduction in the total cholesterol content. These results indicate that rats fed a diet with a high fat content had higher levels of TC. However, the phytosterols in BA [37] may have contributed to reducing the levels of TC, since these compounds perform structural functions analogous to CT-c, thus reducing intestinal cholesterol absorption. These molecules compete for incorporation into mixed micelles in the intestinal tract [42]. The high fiber content in BP [10] may also contribute to the decrease in the concentration of this lipoprotein, since this macronutrient reduces the gastrointestinal transit time and promotes the enteral absorption of CT-c.

The HDL-c values (Fig. 2B) of the rats fed a HC diet were 29.39% lower than those in rats fed a NC diet ($p < 0.05$). When high-fat diets were supplemented with BA and BP, it resulted in HDL-c values similar to those of the NC group ($p > 0.05$). One of the determining factors for the activation of HDL-c levels is the nutritional composition of BA, which contains 51.91% of monounsaturated fatty acids (MUFAs), corresponding to a total of 48.99% of oleic acid [11]. A plausible explanation for the protective effect of these substances is that these fatty acids are capable of remodeling lipoproteins, thereby changing their structure and reducing the concentration of cholesterol in the particle, resulting in a decreased incidence of disease [43]. HDL-c, in turn, has the biological function of extracting excess cholesterol from peripheral cells (cholesterol efflux) and transporting it to the liver for greater metabolism and excretion. This function is mainly due to its antioxidant content (α-tocopherol) [43].

The HBA and HBP diets are thought to reduce hypercholesterolemia as they were associated with improvements in the lipid profiles. Hypercholesterolemia is responsible for triggering cardiovascular diseases. According to the World Health Organization [44], is the main cause of death in the world, causing 15.2 million deaths in 2016, the equivalent to 31% of registered deaths globally.

No significant differences in the GGT or AST (Fig. 2 I and H) liver enzymes were found ($p > 0.05$) among the evaluated groups. However, the concentration of the ALT enzyme (Fig. 2G) in the rats fed the HBA diet remained statistically similar to that of rats fed a HC diet ($p > 0.05$), whereas an increase of 95.36% ($p < 0.05$) in the activity of this enzyme was observed in rats fed a HBP diet compared to a HC diet (Fig. 2G).

The increased enzymatic activity of ALT in the rats fed a HBP diet was expected since due to the combination of cocoa and baru almonds, which are both rich in tannins (i.e. antinutritional compounds) and responsible for increases in liver enzyme activity. The

combination of these compounds, together with the high dietary content of fat may have favored this result. Increased fat in the liver (which will be discussed below) is known to cause hepatocyte inflammation (non-alcoholic hepatitis) and increased ALT levels [36,37]. Despite these increases, these values were not detrimental to health because were still within recommended values for rodents (e. g. 82 U/L, 210 U/L, and 6 U/L for ALT, AST, and GGT, respectively) [45].

3.2 Histopathological analysis

The only change observed in the histopathological evaluation of the livers was the presence of steatosis despite high-fat-diet (Fig. 2). No other degenerative processes related to the diets was detected. The consumption of a NC diet did not trigger hepatic steatosis; however, 100% of the animals fed high-fat diets presented grade 2 steatosis, and between 11% and 30% of change, indicating mild degeneration ($p > 0.05$) (Fig. 3). The supplementation of the hyperlipidic diets with BA and BP did not cause an increase in the degree of steatosis ($p > 0.05$); thus, there was no damage to the rats' liver regarding this parameter.

The presence of a high fat content in diets is associated with the emergence of steatosis. Obese individuals have enlarged liver cells, which allows greater absorption of fatty acids from the blood, diet, or both to provide a larger volume for lipid storage [46]. At the same time, the liver's ability to eliminate or export this accumulated fat is reduced, increasing the accumulation of fat and leading to liver tissue damage [47].

3.3 Antioxidant defenses

Diet can favor or inhibit oxidative stress, depending on the organism's ability to activate antioxidant defenses [48]. To assess the protective effect of diets, several parameters related to antioxidant defenses were determined, including the levels of iron reducing power (FRAP), the capacity for absorbing oxygenated radicals ($ORAC_{FL}$), and the activity of the catalase and reduced glutathione enzymes (Fig. 4).

Regarding the levels of FRAP (Fig. 4A), which represents the antioxidant capacity based on the reduction of ferric ion, the iron reducing power of the HBA diet was found to be 57.24% greater than that of the HC (control) diet. There were no significant differences between the FRAP values for the rats fed received the HBA diet and those fed the HBP diet. Therefore, BA reduced the levels of oxidative stress *in vivo.* Antioxidant compounds are able

sequester free radicals and pro-oxidant metal ions, which are responsible for oxidative damage to cell membranes and DNA [48].

There was no statistical difference ($p > 0.05$) using the $ORAC_{FL}$ method (Fig. 4B). Despite this result, an average $ORAC_{FL}$ content of 12.69 mg.etrolox.g^{-1} was found for all the groups evaluated. This result is likely to be related to the presence of endogenous antioxidants. However, to evaluate a significant difference among the diets at the serum level, a longer experimental period might be necessary.

The addition of fat to the diet did not have a significant effect ($p < 0.05$) on glutathione reductase activity (Fig. 4C) as the results of the rats fed the NC and HC diets did not differ statistically. The addition of BA or BP to the hyperlipidic diet was found to increase the activity of this enzyme by 36.05%. These results demonstrate that supplementation with BA and BP reduces the levels of oxidative stress in individuals consuming high-fat diets. This takes place via the redox cycle of glutathione reductase, which is a protective system that is able to minimize any potential cellular damage caused by oxidative stress [49].

The higher the indexes for catalase activity (Fig. 4D), the greater the protection exerted by this enzyme. When evaluating the effect of the HBA and HBP diets on the rats, there was a respective decrease of 80.4% and 80.2% in catalase activity relative to rats fed the HC diet. The higher levels of enzymatic activity observed in rats fed the control diet (HC) may have occurred because the primary mechanism for combating oxidative stress occurs via the secretion of this enzyme at high levels. When stress persists and, consequently, becomes severe, this mechanism fails to mitigate the damage [50,16]. In addition, it has been reported that some enzymatic antioxidants can have their expression reduced in response to an improvement in the redox state resulting from the presence of bioactive compounds [16].

3.4 Quantification of malonaldehyde via HPLC-DAD/UV-Vis

When measuring the effect of MDA on the rats (Fig. 4E), those fed the HC diet had 30.16% more MDA in their liver than rats fed the NC diet (control). The corresponding reduction of 11.47% and 13.78% ($p < 0.05$) was observed when BA and BP were added to the high-fat diets, suggest that HBA and HBP diets decrease lipoperoxidation and, consequently, the oxidative stress.

Some bioactive substances present in BA have the ability to recover and neutralize free radicals [51,11], and are thus able to prevent or reverse the damage of reactive oxygen species to cellular macromolecules (nucleic acids, proteins, and lipids) [51]. These diets are thus able

to stabilize potential injuries caused by oxidative stress, as well as protect against lipoperoxidation generated by free radicals.

3.5 Real-time quantitative PCR assay

The expression of *IL-6, Cox-2* and *MnSod* was evaluated by quantitative PCR (RT-qPCR). In our analysis, *MnSod* was the only gene significantly expressed in the liver (Fig 4 F). Administration of the HC diet resulted in a 65.68% reduction in the expression of this gene, while a 282.8% increase in the expression of *MnSod* was observed in the rats fed the HBA diet compared to the respective control (HC diet). These results indicate that the addition of BA to the high-fat diet positively modulated antioxidant activity, as *MnSod* plays a protective role against oxidative stress [48]. Thus, the antioxidant system seemed to be stimulated via dietary supplementation with BA in cases where oxidative stress is high, such as in hyperlipidic diets.

Regarding the other genes (*IL-6* and *Cox-2*), expression levels were very low, and, therefore, were not considered. Longer experimental duration might be needed to evaluate their modulation.

4. Conclusion

The consumption of hyperlipidic diets supplemented with baru almonds or baru almond paste resulted in lower levels of triacylglycerols, total cholesterol, and MDA, and higher levels of HDL-c, FRAP, glutathione reductase, and catalase. Baru almonds increased *MnSod* expression in the liver, while the paste reduced the serum LDL-c levels. All these effects represent benefits of baru dietary supplements in high-fat diets. The only adverse effect observed was the increase in serum ALT levels in baru almond paste administration. However, it remained within the normal range for the studied species. The findings reported in this study can help guiding the development of alternative therapies for the treatment of metabolic and oxidative disorders in humans. The main mechanisms involve hypercholesterolemia and oxidative stress reduction.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Authorship

Marina Leopoldina Lamounier Campidelli, João de Deus de Souza Carneiro, Luciano José Pereira, Ellen Cristina de Souza, and Eduardo Valério de Barros Vilas Boas contributed to the design and execution of the experiment, data analysis, interpretation of results and writing of the manuscript; Suzan Kelly Vilela Bertolucci, Smail Aazza, Josilene Nascimento Seixas, Gabriel Lasmar dos Reis, Raphael Ricon de Oliveira, and Antonio Chalfun-Junior contributed to the execution of laboratory analyzes; and David Lee Nelson contributed to the translation and revision of the article. All authors read and approved the final manuscript.

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Tables

Table 1. Composition of experimental diets

	Diet[1]							
	NC		HC		HBA		HBP	
Ingredients and Suppliers	%	kcal	%	kcal	%	kcal	%	kcal
Corn starch - Maizena®	61	2440	40.04	1601.60	23.84	953.85	25.18	1028.37
Casein - Rhoster®	20	740	20	740	11.29	417.73	12.99	480.63
Sucrose - União®	5	200	5	200	5	200	5	200
Soybean oil - Sadia®	4	360	4.96	446.40	0	0	0	0
Celulose - Rhoster®	5	0	5	0	1.17	0	1.83	0
Mineral Premix ¯ Rhoster®	3.50	0	3.50	0	3.50	0	3.50	0
Vitamin Premix- Rhoster®	1	0	1	0	1	0	1	0
Methionine - Rhoster®	0.30	12	0.30	12	0.30	12	0.30	12
Choline Bitartrate -Rhoster®	0.20	8	0.20	8	0.20	8	0.20	8
Baru Nut[2,3]	0	0	0	0	30	1416.42	0	0
Kaolin - KaMin®[4]	0	0	0	0	3.68	0	0	0
Baru Paste	0	0	0	0	0	0	30	1279
Refined pork fat - Sadia®	0	0	20	1873.80	20	1873.80	20	1873.80
Total	100	3760	100	4881.80	100	4881.80	100	4881.80

[1] Diets: NC (normolipidic control diet), HC (hyperlipidic control diet), HBA (hyperlipidic diet supplemented with baru almonds) and HBP (hyperlipidic diet supplemented with baru almond paste); [2]Obtained from a producer in the city of Barra do Garça, MT, Brazil; [3]Fat content of baru almonds (%): 16.32 saturated, 51.91 monounsaturated, and 31.77 polyunsaturated; [4]Kaolin was added to increase the volume of the diets; [5] Baru almond paste composition (%): 35 baru almonds, 29.5 erythritol, 14 coconut oil, 10.4 cocoa powder, 5.6 skimmed milk powder, 3 polydextrose, 1 powdered whey, 1 emulsifier, and 0.5 of flavoring; [6]Fatty acid content of baru almond paste (%): 31.53 saturated, 61.63 monounsaturated, and 6.84 polyunsaturated.

Figures

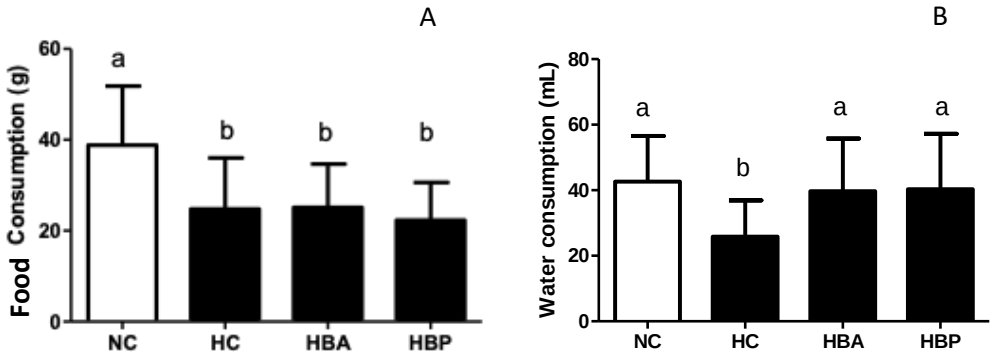

Figure 1. Diet and water consumption of rats during the experimental period. Data are presented as the mean ± standard error of the mean (SEM). Distinct lower case letters in the columns show a significant difference between the NC diets (normolipidic control diet), HC (hyperlipidic diet control), HBA (hyperlipidic diet supplemented with baru almonds) and HBP (hyperlipidic diet supplemented with baru almond paste) ($p < 0.05$).

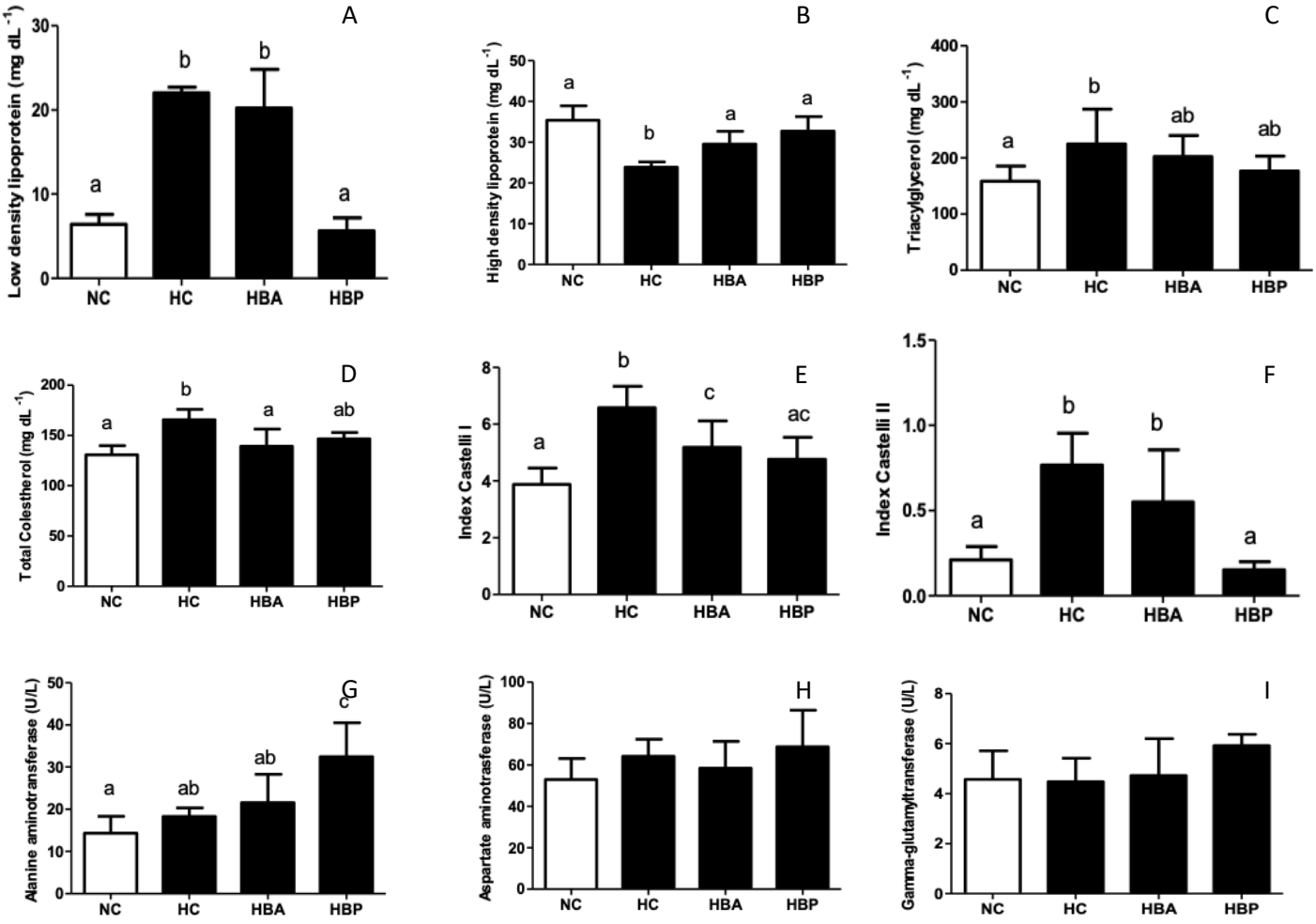

Figure 2. Biochemical parameters and liver injury indexes of rats during the experimental period. Data are presented as the mean ± SEM. Distinct lower case letters in the columns show a significant difference between the NC diets (normolipidic control diet), HC (hyperlipidic diet control), HBA (hyperlipidic diet supplemented with baru almonds) and HBP (hyperlipidic diet supplemented with baru almond paste) ($p < 0.05$).

	Prevalence of Degeneration (hepatic steatosis) - n (%)[1]				
Diet[2]	0 (Normal)	1 to 10	11 to 30	31 to 60	61 to 100
NC	7 (87.50)	1 (12.50)	0	0	0
HC	0	1(12.50)	5 (62.50)	2 (25)	0
HBA	0	2 (25)	2 (25)	4(50)	0
HBP	0	3 (37.50)	3 (37.50)	2 (25)	0

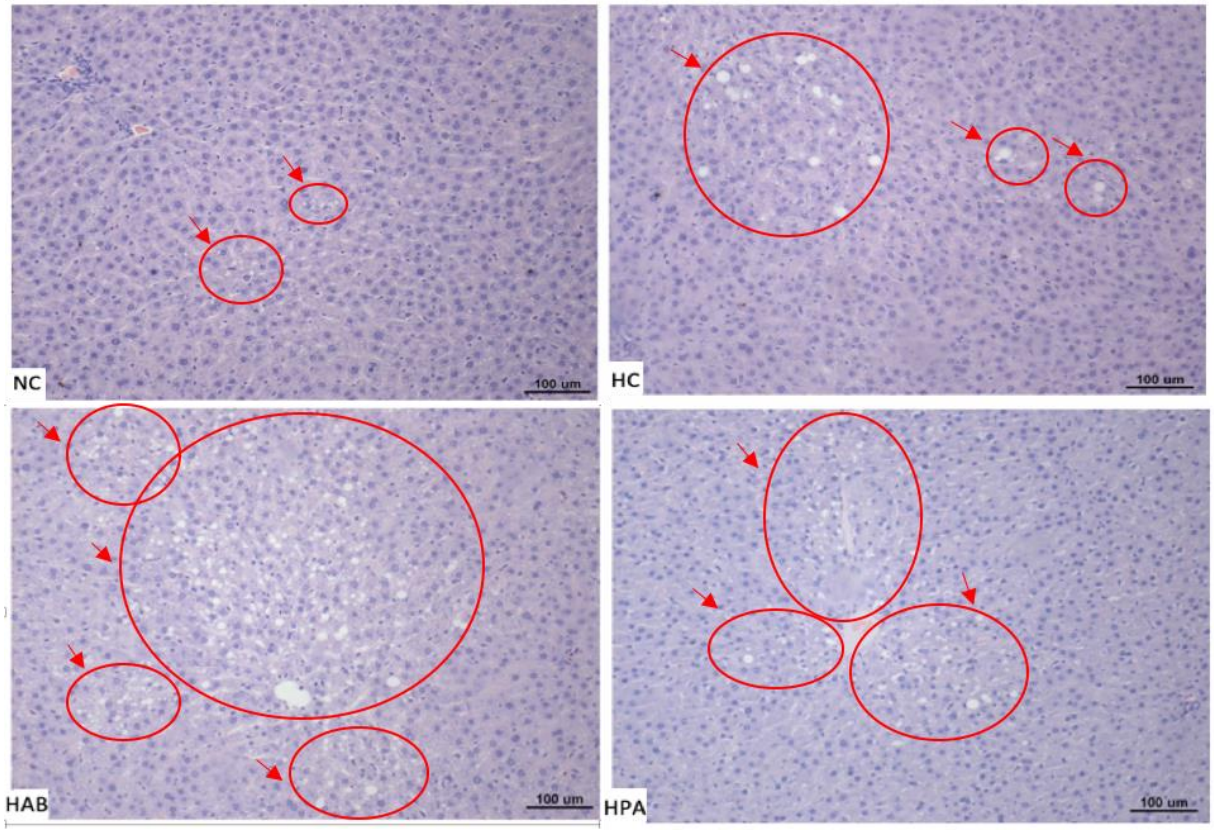

Figure 3. Histological representation (H&E, 40× increase) of degrees of hepatic steatosis of rats during the experimental period. NC (normolipidic control diet), HC (hyperlipidic control diet), HBA (hyperlipidic diet supplemented with baru almonds) and HBP (hyperlipidic diet supplemented with baru almond paste).

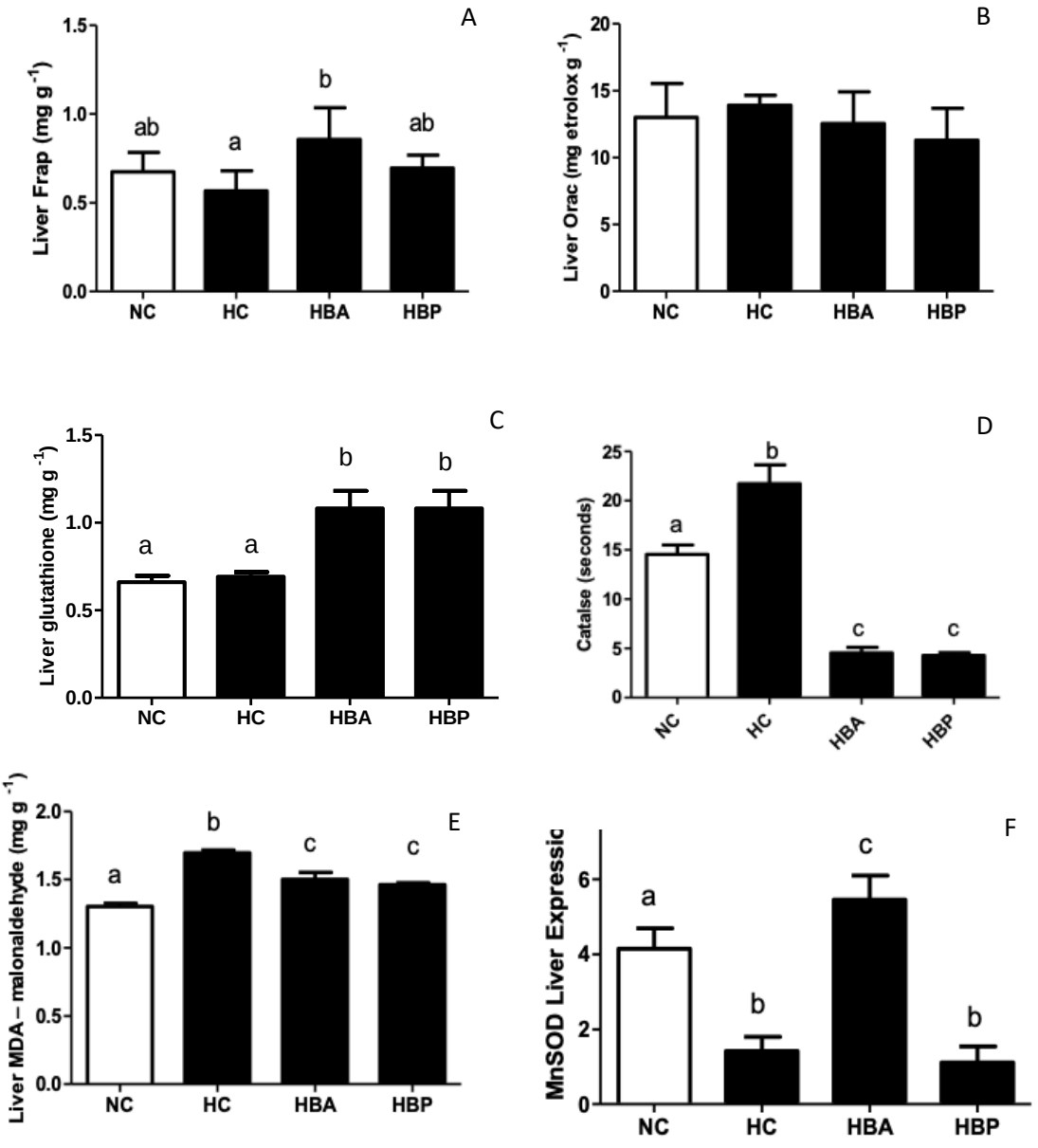

Figure 4. Analysis of antioxidants in the liver of rats during the experimental period. Data are presented as the mean ± SEM. Distinct lower case letters in the columns show a significant difference between the NC diets (normolipidic control diet), HC (hyperlipidic diet control), HBA (hyperlipidic diet supplemented with baru almonds) and HBP (hyperlipidic diet supplemented with baru almond paste) ($p < 0.05$).

Supplementary material

Sequence of the oligonucleotides (forward and reverse) utilized in q-PCR

Genes	Initiators 1 (Forward)	Initiators 2 (Reverse)
β-Actina[1]	AGCCTTCCTTCCTGGGTATG	CGGATGTCAACGTCACACTT
GAPDH[1,2]	CCATCTTCCAGGAGCGAGA	GGCGGAGATGATGACCCTTT
MnSod[3]	TCCCTGACCTGCCTTACGA	TGTAACATCTCCCTTGGCCAG
IL-6[4]	TGGAAATGAGAAAAGAGTTGTGC	TCCAGAAGACCAGAGCAGAT
Cox-2[5]	CTCAGCCATGCAGCAAATCC	GGGTGGGCTTCAGCAGTAAT

[1]Reference genes used to calculate the CT delta; [2]*Glyceraldehyde-3-Phosphate Dehydrogenase*; [3]*Manganese Superoxide Dismutase*; [4]*Interleukin-6*; [5]*Cyclooxygenase*; [6]*Tumor Necrosis Factor-alpha*; [7]*Zinc Superoxide Dismutase*