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AVALIAÇÃO DA ATIVIDADE ANTIOXIDANTE, MUTAGÊNICA E ANTIMUTAGÊNICA DE POLPAS DE FRUTAS

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Tese apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade de Caxias do Sul, visando a obtenção de grau de Doutor em Biotecnologia

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Prof. Dr. João A. P. Henriques

Caxias do Sul

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Dedicar... O que realmente isso quer dizer?

Doar? Partilhar? Homenagear?

Isso e muito mais, pois quem dedica ama...

Àqueles que mais amo no mundo.

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CAT	**Catalase**
DPPH$^\bullet$	**1,1-difenil-2-picrilhidrazil**
FRAP	***Ferric reducing-antioxidant power***
IBRAF	**Intituto Brasileiro de Frutas**
LDL	***Low Density Lipoproteins***
OMS	**Organização Mundial da Saúde**
PIXE	***Particle induced X-ray emission***
SOD	**Superóxido dismutase**
TEAC	***Trolox equivalent antioxidant capacity***
TRAP	***Total radical-trapping antioxidant parameter***

RESUMO

Vários estudos têm demonstrado que o consumo de frutas está associado a redução nos índices de doenças neurodegenerativas, cardiovasculares e câncer. Embora diversas frutas *in natura* e compostos isolados de frutas já tenham sido estudados, existem poucos dados acerca da composição e atividade biológica de frutas congeladas, as quais tem sido amplamente utilizadas pela população no preparo de sucos e, também, como matéria-prima pelas indústrias de alimentos. Em vista disso, este trabalho teve como objetivo avaliar a composição, a atividade antioxidante, mutagênica e antimutagênica de 23 polpas de frutas congeladas. Foram avaliados os teores de carboidratos, lipídios, proteínas, vitamina C, polifenóis totais, carotenóides e minerais de todas as frutas. A atividade antioxidante foi determinada através da capacidade de varredura do radical livre 1,1-difenil-2-picrilhidrazil (DPPH$^\bullet$) e atividades superóxido dismutase e catalase-*like*. Para a polpa de açaí, foram realizados, também, ensaios em homogeneizados de cérebro de ratos Wistar. Para avaliação da atividade mutagênica e antimutagênica, utilizou-se a linhagem XV185-14C de *S. cerevisiae,* que permite a detecção de dois tipos de mutações, revertentes *locus* específica e mudança na leitura do quadro genético (*frameshift*). Conforme o esperado, as polpas apresentaram baixos teores de lipídios e proteínas (ausência ou inferior a 1 mg%). O coco foi a única fruta com valores insignificantes de carboidratos em sua composição. Mesmo congeladas, as frutas mostraram um conteúdo significativo de polifenóis, carotenóides, ácido ascórbico, macro e microminerais. A maioria das polpas avaliadas (74%) apresentou capacidade antioxidante *in vitro* (DPPH$^\bullet$) igual ou superior à da vitamina C. Este efeito mostrou correlação positiva com o conteúdo de carotenóides ($r = 0{,}366$; $p \leq 0{,}01$). Todas as amostras apresentaram atividade catalase-*like* e 56% das frutas, atividade superóxido dismutase-*like*. O açaí mostrou-se capaz de reverter os danos oxidativos induzidos pelo peróxido de hidrogênio em córtex, cerebelo e hipocampo de ratos Wistar. Quando ensaiadas em altas concentrações (5, 10, 15 % p/v) as polpas de açaí, caju, kiwi e morango mostraram atividade mutagênica, a qual apresentou correlação positiva com carotenóides ($r = 0{,}793$, $p \leq 0{,}05$), polifenóis ($r = 0{,}793$, $p \leq 0{,}05$) e ácido ascórbico ($r = 0{,}793$, $p \leq 0{,}05$). Doze frutas apresentaram importante atividade antimutagênica, a qual mostrou correlação positiva com a atividade catalase-*like* ($r = 0{,}400$, $p \leq 0{,}01$). Embora outros estudos sejam necessários, os dados obtidos neste trabalho mostram que as frutas, mesmo congeladas, apresentam importante atividade antioxidante e antimutagênica, podendo minimizar os danos oxidativos associados a diversas doenças.

ABSTRACT

Several studies have pointed out that consumption of fruits is related to the reduced risk of diseases, such as coronary heart and neurodegenerative diseases, as well as certain types of cancer. Although several fruits *in natura* and compounds isolated from them have been already studied, there exist little data on frozen fruits composition and their biological activity. Frozen fruits are widely employed to prepare juices, and also as a raw material for the food industry. Therefore, this study aims to evaluate the composition and the antioxidant, mutagenic and antimutagenic activities of 23 frozen fruit pulps. Carbohydrates, lipids, proteins, vitamin C, total polyphenols, carotenoids and mineral contents were assessed in all fruits. Antioxidant activity was determined by the chemical measurement of 1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging activity and superoxide dismutase and catalase-like activities. Tissue homogenates from Wistar rats' brain only assayed in açai pulp. Mutagenic and antimutagenic activities, strain XV185-14C of *S. cerevisiae*, that allows the detection of specific locus and frameshift mutations. As expected, all pulps showed low lipids and proteins contents (absence or lower than 1 mg%). Coconut was the only fruit which presented insignificant carbohydrate values in its composition. Even frozen, the fruits contained significant polyphenols, carotenoids, vitamin C, macro and micro minerals and trace elements. Most of the assayed pulps (74%) showed in vitro antioxidant capacity equal or superior to vitamin C. This effect was positively correlated with carotenoid content ($r = 0.366$; $p \leq 0.01$). All samples showed catalase-like activity and 56% of the fruits showed superoxide dismutase activity. Acai fruit was able to revert hydrogen peroxide-induced oxidative damages in Wistar rats' cortex, cerebellum and hippocampus. When tested in high concentrations (5, 10, 15 %w/v), acaí, cashew apple, kiwi and strawberry pulps showed mutagenic activity, which was positively correlated with carotenoids ($r = 0.793$, $p \leq 0.05$), polyphenols ($r = 0.793$, $p \leq 0.05$) and vitamin C ($r = 0.793$, $p \leq 0.05$). Twelve fruits demonstrated important antimutagenic activity, which was positively correlated with catalase-like activity ($r = 0.400$, $p \leq 0.01$). Our findings show that frozen fruits have important roles in antioxidant and antimutagenic activities, reducing oxidative damages associated to several diseases.

APRESENTAÇÃO

Nos últimos anos, especialistas na área de nutrição têm recomendado o aumento no consumo de frutas e verduras, a fim de minimizar a incidência de doenças gastro-intestinais, neurodegenerativas e câncer. Além de carboidratos, fibras e minerais, as frutas são ricas em compostos com reconhecida atividade antioxidante, como vitaminas, carotenóides e polifenóis. No entanto, a dificuldade de obtenção de frutas frescas em diferentes regiões e épocas do ano pode limitar sua utilização. Uma das soluções encontradas pela indústria foi o processamento/congelamento das frutas, permitindo, desta forma, a disponibilização, ao consumidor, de uma grande variedade de frutas em todas as estações do ano e regiões geográficas.

Embora existam vários estudos com frutas *in natura*, principalmente quanto aos seus efeitos benéficos a saúde do homem, não são conhecidos, até o momento, dados acerca da composição e atividade biológica de polpas congeladas. Em vista disso, esse trabalho teve como objetivo avaliar a atividade antioxidante, mutagênica e antimutagênica de polpas de frutas congeladas, bem como determinar o conteúdo de polifenóis, carotenóides, vitamina C e valor nutricional. Os resultados obtidos estão apresentados em quatro capítulos. No primeiro deles, são mostrados os resultados sobre a composição (polifenóis, carotenóides e ácido ascórbico) e a atividade biológica das 23 frutas estudadas, incluindo as mais utilizadas pela população, bem como algumas frutas nativas da Amazônia. O teor de 14 minerais das 23 polpas de frutas está apresentado no segundo capítulo. Considerando que o açaí é bastante consumido, principalmente no norte e nordeste do Brasil, esta fruta foi escolhida para ser estudada quanto a sua atividade antioxidante em homogeneizados de cérebro de ratos, sendo esse o tema do artigo que compõe o terceiro capítulo. O quarto capítulo é composto de uma revisão sobre os compostos majoritários e atividade biológica de frutas *in natura* e congeladas.

Estes resultados, em conjunto, compõem o primeiro banco de dados conhecido sobre composição química e atividade biológica de frutas congeladas e pode contribuir, significativamente, para o cálculo da ingestão diária recomendada de nutrientes, minerais e compostos bioativos.

1. INTRODUCÃO

1.1 As frutas: constituintes e efeitos benéficos

Existe um número considerável de evidências epidemiológicas que mostram a associação entre dietas ricas em frutas e a diminuição dos índices de doenças cardiovasculares, neurodegenerativas (para revisão, ver Sing *et al.*, 2008), câncer (para revisão, ver Johnson, 2007) e diabetes (para revisão, ver Jenkins, 2003). Estes efeitos têm sido atribuídos, principalmente, a presença de polifenóis (D'Archivio, 2007), carotenóides (Hoelzl *et al.*, 2005) e vitaminas nas frutas, os quais apresentam reconhecida atividade antioxidante (para revisão, ver Sing *et al.*, 2008).

Segundo o Instituto Brasileiro de Frutas (IBRAF) o consumo de frutas frescas no Brasil, em 2007, foi em torno de 24 kg per capita, cerca de um quarto do recomendado pela Organização Mundial da Saúde (OMS). As frutas mais consumidas pela população brasileira são banana, maçã, abacaxi, limão, melão, mamão, manga e uva (IBRAF, 2007). No entanto, um dos fatores que tem limitado o consumo de frutas é a sua disponibilidade nas diferentes regiões geográficas e épocas do ano. Parte dessa limitação vem sendo solucionada através do congelamento de polpas de frutas, manejo industrial que tem tido grande aceitação, tanto para uso doméstico, como pela indústria de alimentos.

Segundo o Ministério da Agricultura, polpa/suco de fruta congelada é o produto não fermentado, não concentrado, não diluído, resultante do esmagamento de frutas polposas, através de um processo tecnológico adequado, específico para cada fruta. As polpas devem ser preparadas com frutas sãs, limpas, isentas de matéria terrosa, de parasitas e detritos de animais ou vegetais. Não são permitidos fragmentos das partes não comestíveis da fruta, nem substâncias estranhas à sua composição normal, como sujidades, parasitas e larvas (Ministério da Agricultura, 2000). De forma geral, a obtenção de polpas e sucos de frutas congelados segue o fluxograma descrito na Figura 1. Neste trabalho foram utilizados sucos de limão, laranja, uva e tangerina e polpas de açaí, acerola, maçã, caju, coco, cupuaçu, kiwi, manga, melão, mamão, maracujá, abacaxi, framboesa, goiaba, graviola, morango e pitanga.

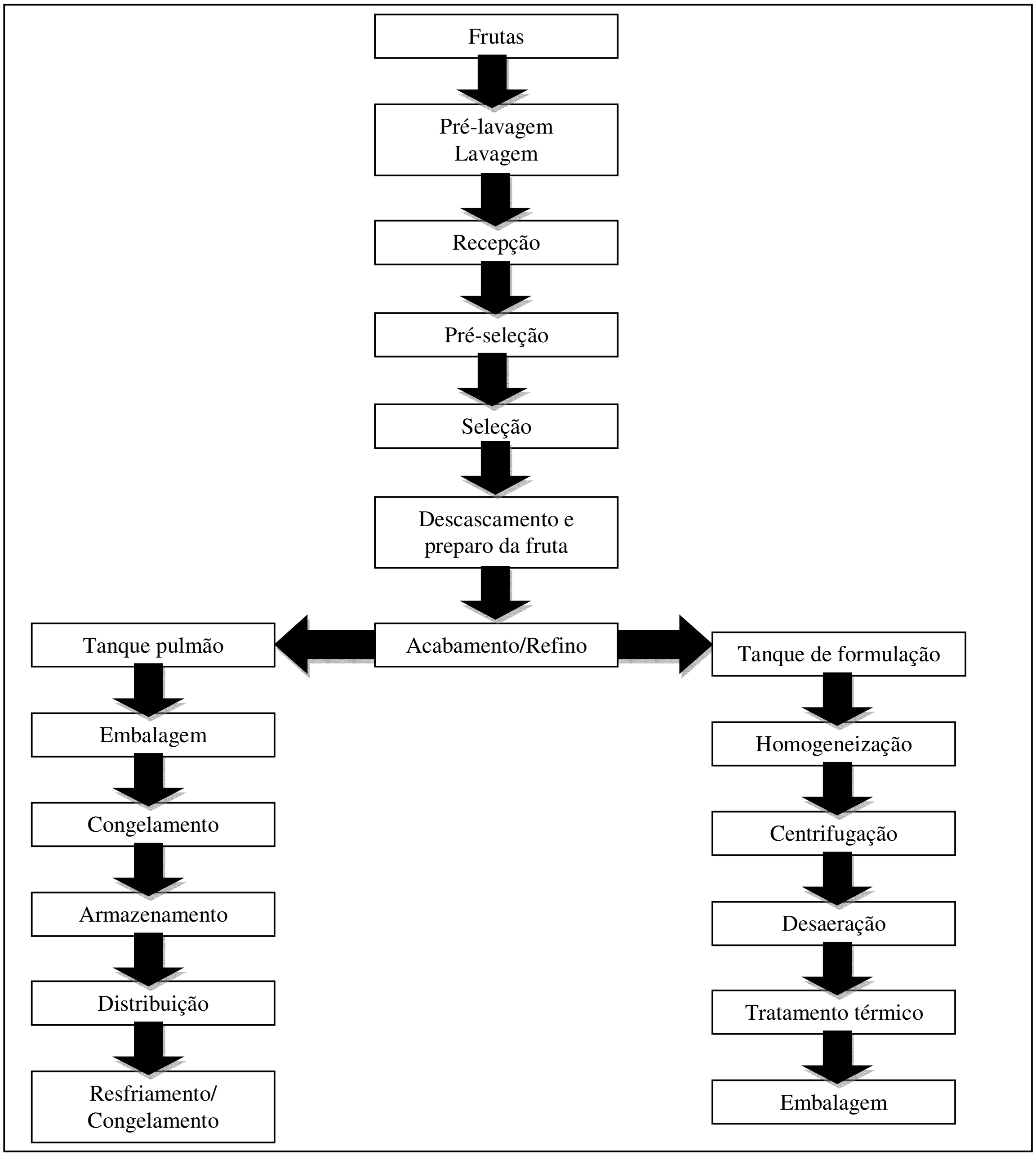

Figura 1. Fluxograma do processamento de frutas nas indústrias de polpas de frutas congeladas (lado esquerdo) e sucos de frutas (lado direito). (Adaptado de acordo com as informações fornecidas pela empresa produtora – Mais Fruta – Antonio Prado, RS)

Em 2007, o Brasil produziu 43,7 milhões de toneladas de frutas, das quais apenas 47% foram consumidas como frutas *in natura*. O restante da produção foi utilizado nas agroindústrias para fabricação de suco de laranja e de polpas congeladas (IBRAF, 2007). Atualmente, polpas congeladas, podem ser encontradas, facilmente, em qualquer supermercado e a um custo acessível. Essas frutas têm sido utilizadas não só para preparação de sucos, mas, também, como matéria-prima na produção de doces, preparo com leite, sorvetes, geléias, bolos, etc.

As frutas são alimentos considerados de alto valor nutricional, apresentando em sua composição, carboidratos, lipídios, proteínas, fibras, vitaminas, minerais, carotenóides e compostos fenólicos (Harbone *et al.*, 2000; Silalahi, 2002). De modo geral, as frutas possuem quantidades insignificantes de lipídios e, por serem de origem vegetal, não têm colesterol. O teor de proteínas também é baixo, sendo que quantidades maiores são encontradas na goiaba, coco e maracujá (Matsuura *et al.*, 2004). As calorias das frutas provêm, principalmente, dos carboidratos, que podem estar presentes em maior ou menor grau, sendo a banana uma das frutas mais ricas nesse nutriente (Matsuura *et al.*, 2004). A presença de fibras nas frutas contribui para o bom funcionamento do sistema digestório (Li *et al.*, 1994) e tem sido associada à redução de alguns tipos de câncer (Jones *et al.*, 1990).

As frutas contêm, ainda, quantidades importantes de vitaminas, principalmente as vitaminas A e C (Gey, 1998). A vitamina A é o termo genérico para descrever todos os compostos com atividade biológica de retinol. Essa vitamina possui papel essencial na visão, crescimento e desenvolvimento, no desenvolvimento e manutenção do tecido epitelial, nas funções imunológicas e de reprodução (Hoover *et al.*, 2008). A carência dessa vitamina na dieta ou por dificuldades de absorção, pode causar deficiências visuais, desenvolvimento embrionário prejudicado, espermatogênese alterada, aborto espontâneo, anemia e ressecamento das mucosas (Hoover *et al.*, 2008). Além dos aspectos nutricionais, a vitamina

A é um importante antioxidante, mas que pode agir também como pró-oxidante, dependendo da quantidade de oxigênio presente no sistema (Burton & Ingold, 1984; Palozza *et al.*, 1997, Klamt *et al.*, 2003).

Os carotenóides, precursores da vitamina A, são compostos naturais que formam um grupo de pigmentos coloridos, normalmente amarelos, vermelhos ou alaranjados, encontrados em frutas, tecidos animais (lagostas) e determinadas bactérias. Mais de 600 carotenóides estão bem descritos e cerca de 50 deles são precursores de vitamina A (Halliwell & Gutteridge, 2007). Os carotenóides apresentam efeito antitumoral, pois, entre outros fatores, aumentam a síntese de proteínas que favorecem a comunicação entre as células, evitando, assim a proliferação do tumor. Também exercem efeitos benéficos ao sistema imunológico, inibem a lipoperoxidação e são varredores de radicais livres (Halliwell & Gutteridge, 2007). Sua ingestão está associada à diminuição dos níveis de *Low Density Lipoproteins* - LDL (Rao, 2002) e doenças coronárias (Castenmiller *et al.*, 1999).

A vitamina C (ácido L-ascórbico) é encontrada em quantidades significativas em frutas (Toit *et al.*, 2001), sendo sintetizada a partir de glicose (Wheeler *et al.*, 1998). Os humanos e outros primatas são incapazes de sintetizar a vitamina C, obtendo-a através da dieta. Essa vitamina funciona como co-fator enzimático e participa de várias reações de óxido-redução do organismo, sendo importante para a cicatrização de feridas e queimaduras, na proteção do tecido conjuntivo (Halliwell & Gutteridge, 2007), e é um importante estimulador do sistema imunológico (Combs, 1998). Sua deficiência ou má absorção causa o escorbuto. Além disso, a vitamina C é um importante antioxidante, protegendo várias biomoléculas contra o dano causado por espécies reativas (Halliwell & Gutteridge, 2007). Além de sua ação direta contra radicais livres, afeta indiretamente o balanço entre oxidantes e antioxidantes, já que é o principal doador de elétrons ao radical da vitamina E, α-tocoferil, promovendo a regeneração do α-tocoferol (Liu *et al.*, 1998).

Dietas ricas em ácido L-ascórbico foram associadas com o decréscimo da incidência de câncer (Van Poppel & Van Der Berg, 1997; Ocké *et al.*, 1997; Yong *et al.*, 1997), diminuição dos níveis de colesterol, pressão arterial (Fotherby *et al.*, 2000), de doenças cardíacas (Flagg *et al.*, 1995) e da progressão da aterosclerose no homem (Halliwell & Gutteridge, 2007). A vitamina C pode, ainda, prevenir a indução de mutações no DNA (Lutsenko *et al.*, 2002). Por outro lado, alguns estudos demonstraram que essa vitamina pode agir como pró-oxidante pela reação de Fenton ou reações de óxido-redução semelhantes (Wang *et al.*, 1996; Ferguson, 2001). Em altas concentrações pode apresentar atividade mutagênica (Franke *et al.*, 2004), e na presença de íons de cobre, pode gerar radical hidroxila (De Flora, 1998; De Flora *et al.*, 2001), altamente danoso ao DNA (Halliwell & Gutteridge, 2007).

Os minerais desempenham uma função vital no desenvolvimento e boa saúde do corpo humano e as frutas são consideradas as principais fontes de minerais necessários na dieta humana (Seifried *et al.*, 2007). Estes compostos regulam o metabolismo de diversas enzimas, o equilíbrio ácido-base, a pressão osmótica, a atividade muscular e nervosa, facilitam a transferência de compostos essenciais através das membranas e, em alguns casos, fazem parte dos elementos constituintes dos tecidos do organismo (Shils *et al.*, 1994).

A maior parte dos minerais é considerada essencial e são, tradicionalmente, divididos em macrominerais (elementos de volume) e microminerais (elemento traço). Mais recentemente, o termo ultra-traço tem sido utilizado para descrever elementos que são consumidos em quantidades medidas em microgramas a cada dia. Os macrominerais são necessários em quantidade maiores que 100 mg/dia, enquanto os microminerais são necessários em quantidades menores que 100 mg/dia (Mahan & Scott-Stump, 2004).

Os minerais representam ao redor de 4 a 5% do peso corpóreo. Cerca de 50% deste peso é devido ao cálcio e 25% ao fósforo (fosfatos), encontrados, principalmente em ossos e dentes. Os outros macrominerais essenciais (Mg, Na, Cl e S) e os microminerais (Fe, Zn, Se, Mn, F, Mo, Cu, Cr, Co e Br) constituem os 25% restantes. Os elementos ultra-traço, tais como iodo, arsênico, alumínio, estanho, níquel, vanádio e silício fornecem uma quantidade insignificante de peso (Mahan & Scott-Stump, 2004).

O cálcio atua na contração muscular, no controle de enzimas, como por exemplo a proteína quinase C, nos processos de transcrição, ativação de genes e apoptose (Mahan & Scott-Stump, 2004). A baixa ingestão de cálcio está associada a maior incidência de câncer de cólon e hipertensão (Mahan & Scott-Stump, 2004). Além disso, o cálcio atua na prevenção da osteoporose (Flynn, 2003).

Na forma de fosfatos, o fósforo participa de várias funções essenciais, tais como, a formação do DNA, RNA, ATP, AMP cíclico, tamponamento celular e formação de ossos e dentes (Mahan & Scott-Stump, 2004). Os eletrólitos sódio e cloro participam no balanço e distribuição de água, equilíbrio osmótico e formação do potencial de ação celular (Mahan & Scott-Stump, 2004). O Mg é um co-fator para a DNA-polimerase e um protetor efetivo contra carcinogênese (Rojas *et al.*, 1999), enquanto o enxofre participa de efeitos antitrombóticos (Reinhold *et al.*, 1975).

Os microminerias mais abundantes em frutas são o Mn, Cu, Zn e Fe. O manganês é um importante co-fator enzimático e sua carência pode causar perda de peso, fragilidade óssea, dermatite, degeneração do ovário ou testículos e náuseas (Mahan & Scott-Stump, 2004). O Fe apresenta-se em duas formas estáveis e interconversíveis, Fe^{+2} e Fe^{+3}. Normalmente está ligado a proteínas e participa de reações redox e transporte de oxigênio. Quando livre pode ser potencialmente tóxico e gerar espécies reativas de oxigênio pela reação de Fenton, formando os radicais superóxido e hidroxil. A deficiência de Fe está relacionada com anemia e a hipotransferrina (De Freitas & Meneghini, 2001).

Traços de Cu são essenciais ao organismo, pois este elemento participa como co-fator de enzimas como a citocromo C oxidase, superóxido dismutase Cu/Zn, tirosinases, lisil oxidases, entre outras. Estudos *in vitro* mostraram que o Cu, a exemplo do Fe, pode induzir a formação de espécies reativas de oxigênio, causando por sua vez danos a lipídios, proteínas e DNA, possivelmente pela reação de Fenton (Linder, 2001).

A maior parte do zinco no organismo está ligada a metaloenzimas e, aproximadamente, 80% encontra-se nos eritrócitos. O Zn está envolvido na manutenção da estabilidade do DNA, estabilização de membranas estruturais e na proteção celular, prevenindo a peroxidação lipídica (Dreosti, 2001). Zinco e magnésio têm grande importância na estabilidade genômica, sendo co-fatores de processos como o reparo por excisão de bases (BER), por excisão de nucleotídeos (NER) e nos múltiplos estágios de formação de tumores (Hartwig, 2001).

Apesar dos microelementos e elementos ultra-traço serem imprescindíveis à manutenção da homeostase celular, o papel de grande parte deles não está elucidado (Mahan & Scott-Stump, 2004).

Mais recentemente, as frutas têm sido estudadas em função da presença de compostos fenólicos (Singh *et al.*, 2008), com reconhecida atividade antioxidante (para revisão, ver Ferguson, 2001). Os polifenóis são compostos que apresentam em sua estrutura um ou mais anéis aromáticos com grupos hidroxilas (Ferguson, 2001). São capazes de quelar metais e varrer radicais livres através da formação de radicais fenoxil (Mello & Santos, 1999). Os flavonóides, principais polifenóis encontrados em frutas, apresentam uma estrutura ao qual pode estar ligado uma aglicona ou glicosídeo. Os substituintes presentes nos carbonos conferem maior ou menor atividade antioxidante para os diferentes flavonóides, assim como podem, também, favorecer uma possível ação pró-oxidante (Trueba & Sánchez, 2001).

Os flavonóides têm sido empregados na prevenção de enfermidades cardiovasculares e circulatórias (Ness & Powles, 1997; Stoclet *et al.*, 2004), cancerígenas (Wang & Mazza, 2002; Katsube *et al.*, 2003), no diabetes e no mal de Alzheimer (Hertog *et al.*, 1997; Ishige *et al.*, 2001). Possuem, ainda, atividade antibacteriana (Taguri *et al.*, 2004) e podem induzir a apoptose (Sánchez-Moreno, 2002; Yeh & Yen, 2005; Heo & Lee, 2005).

As frutas *in natura* têm sido bastante estudadas e apresentam vários efeitos benéficos à saúde humana (Tabela 1), principalmente relacionados à atividade antioxidante. Além destes, há vários estudos sobre compostos isolados de frutas, como flavonóides (para revisão, ver Ferguson, 2001; Ferguson & Philpott, 2008), vitamina C (para revisão, ver De Tulio, 2004) e carotenóides (para revisão, ver Riccioni, 2008). No entanto, considerando-se a forma de ingestão das frutas pela população, torna-se importante estudar, integralmente, toda a parte comestível da fruta, e não apenas extratos ou compostos isolados.

Até o momento, há poucas informações sobre polpas congeladas. Existem dois estudos sobre a atividade antioxidante *in vitro* de frutas congeladas, um deles sobre açaí (Schauss *et al.*, 2004) e outro sobre extratos metanólicos de polpas congeladas de acerola, açaí, amora, caju, goiaba e graviola (Hassimoto, 2005). É sabido, no entanto, que durante o processamento das frutas, inclusive congelamento, pode ocorrer perda de vitamina C (Brunini *et al.*, 2002; Yamashita *et al.*, 2002; Franke *et al.*, 2004; Beekwilder *et al.*, 2005) e carotenóides (Melo *et al.*, 2000; Agostini-Costa *et al.,* 2003), o que torna importante o estudo de frutas congeladas, as quais têm sido cada vez mais utilizadas pela população em substituição às frutas *in natura*.

Tabela 1. Principais atividades biológicas de frutas *in natura* descritas na literatura.

Atividade biológica	Fruta	Referências
Anticarcinogênica	Coco	Nalini *et al.*, 1997
	Limão	National Toxicology Program, 1990
	Manga	Rodriguez *et al.*, 2006
	Uva	Lala *et al.*, 2006; Stagos *et al.*, 2005
Anticonvulsivante	Maracujá	Nassiri-Asl *et al.*, 2007
Antifúngica	Limão	Ben-Yehoshua *et al.*, 2008
Antigenotóxica	Laranja	Franke *et al.*, 2006
Antiinflamatória	Açaí	Rodrigues *et al.*, 2006
	Amora	Kim & Park, 2006
	Manga	Knödler *et al.,*2007
Antimutagênica	Caju	Melo Cavalcante *et al.*, 2003; Trevisan *et al.*, 2006; Petta *et al.*, 2004; Nalini *et al.,* 1997
	Coco	Grover & Bala, 1993
	Goiaba	Deters *et al.*, 2005; Tang & Edenharder, 1997
	Kiwi	Miyazawa *et al.*, 1999
	Laranja	Higashimoto *et al.*, 1998; Franke *et al.*, 2004
	Limão	Bala & Grover, 1989

Antioxidante	Abacaxi	Herraiz & Galisteo, 2003.
	Açaí	Rocha *et al.*, 2007 ; Rodrigues *et al.*, 2006 ; Schauss *et al.*, 2006; Lichtenthaler *et al.,*2005
	Acerola	Hanamura *et al.*, 2005
	Amora	Kim & Park, 2006
	Caju	Green *et al.*, 2007; Konan *et al.*, 2006
	Cupuaçu	Yang *et al.*, 2003
	Framboesa	Viljanen *et al.*, 2004, Wada & Ou, 2002; Mullen *et al.*, 2002; Wang & Jiao, 2000; Kahle *et al.*, 1999
	Goiaba	Jime'nez-Escrig *et al.*, 2001
	Laranja	Jayaprakasha *et al.*, 2007 ; Deyhim *et al.*, 2006 ; Hosseinimehr & Karami, 2005 ; Franke *et al.*, 2004.
	Maçã	Leu *et al.*, 2006
	Manga	Mahattanatawee *et al.,*2006; Rodriguez *et al.,*2006; Percival *et al.,*2006
	Mamão	Lohiya *et al.*, 2008 ; Gambera *et al.*, 2007; Mehdipour *et al.*, 2006 ; Imao *et al.*, 1998 ; Emeruwa, 1982
	Maracujá	Nassiri-Asl *et al.*, 2007
	Melão	Lester, 2008; Vouldoukis *et al.,*2004; Lester *et al.*, 2004
	Morango	Kiselova *et al.,*2006; Rababah *et al.*, 2005; Kahkonen *et al.*, 2001
	Uva	Kedage *et al.*, 2007; El-Ashmawy *et al.*, 2007; Devi *et al.*, 2006 ; Janisch *et al.*, 2006; Stagos *et al.*, 2005; Shafiee *et al.*, 2003; Castillo *et al.*, 2000
Bactericida	Goiaba	Pelegrini *et al.*, 2008; Abdelrahim *et al.*, 2002

	Manga	Sairam *et al.,*2003
	Mamão	Nayak *et al.*, 2007 ; Osato *et al.*, 1993 ; Emeruwa, 1982
	Uva	Thimothe *et al.*, 2007
Contraceptiva	Abacaxi	Garg *et al.*, 1970
Mutagênica	Caju	Melo Cavalcante *et al.*, 2003; Trevisan *et al.*, 2006
	Coco	Sandhya & Rajamohan, 2006; Petta *et al.*, 2004; Narasimhamurthy *et al.*, 1999
	Kiwi	Deters *et al.*, 2005
Vasodilatadora	Açaí	Rocha *et al.*, 2007
	Framboesa	Wada & Ou, 2002
	Uva	Madeira *et al.*, 2005; Soares *et al.*, 2004

1.2 Avaliação da atividade antioxidante, mutagênica e antimutagênica

Diferentes metodologias têm sido desenvolvidas para obter uma medição, seja qualitativa ou quantitativa, da capacidade antioxidante de diversos compostos, tanto através de testes *in vitro*, como pelo tratamento de tecidos ou, ainda, utilizando culturas celulares (testes *in vivo*). Dentre os testes *in vitro* existentes, a capacidade de varredura do radical DPPH$^\bullet$ (1,1-difenil-2-picrilhidrazil) vem sendo cada vez mais utilizada (Rice-Evan*s et al.*, 1995). O DPPH$^\bullet$ é um radical livre estável que pode ser reduzido por um antioxidante, resultando em perda de coloração, a qual é determinada a 517 nm (Yamaguchi *et al.*, 1998; Espín *et al.*, 2000; Fukumoto & Mazza, 2000). Testes que avaliam a atividade *like* de algumas enzimas também vêm sendo utilizados (Hanamura *et al.*, 2005; Schauss *et al.*, 2006; Leu *et al.*, 2006), como por exemplo, a medida da capacidade de varredura de radicais superóxido (SOD-*like*) e do peróxido de hidrogênio (CAT-*like*). A enzima superóxido dismutase (SOD) dismuta os ânions superóxido em O_2 e H_2O_2, sendo, este último, substrato para a enzima catalase (CAT), que forma água e oxigênio (Harris, 1992). Além disso, a avaliação de substâncias com possível atividade antioxidante em tecidos de rato têm tido aceitação no meio científico (Dal-Pizzol *et al.*, 2001). A utilização de diferentes tecidos animais (fígado, hipocampo, cerebelo, córtex cerebral) como modelo experimental permite a avaliação de parâmetros de estresse oxidativo, tais como nível de peroxidação lipídica e protéica, atividades de enzimas antioxidantes, entre outros (Kappel *et al.*, 2008; Caregnato *et al.*, 2008).

Considerando que a atividade antioxidante de um composto é influenciada pelo modelo de estudo (Halliwell *&* Gutteridge, 2007), costuma-se empregar mais de um tipo de teste. Ensaios *in vivo*, utilizando microrganismos, têm se mostrado muito adequados na triagem rotineira de vários produtos, sendo testes rápidos, sensíveis, econômicos e reprodutíveis (Da Silva & Henriques, 1987; Rabello-Gay *et al.*, 1991). A descrição do ciclo de vida de *Saccharomyces cerevisiae* e o conhecimento das características genéticas básicas, além da facilidade de manipulação, e principalmente, do fato deste microrganismo ser eucarioto, proporcionaram a grande difusão deste sistema biológico como metodologia experimental para estudos da atividade antioxidante (Soares *et al.,* 2003; Picada *et al.*, 2003; Lopes *et al.,* 2004, Raspor *et al.,* 2005; Spada & Salvador, 2005) e mutagênica de inúmeros compostos (Henriques *et al.*, 2001). Os dados obtidos nesse tipo de teste apresentam uma correlação de, aproximadamente, 70% em relação ao observado no homem (Da Silva & Henriques, 1987; Rabello-Gay *et al.*, 1991).

O estudo da atividade antioxidante baseia-se na avaliação do crescimento celular após tratamentos realizados na presença e ausência do composto a ser testado adicionado de um agente reconhecidamente formador de espécies reativas, como por exemplo, o peróxido de hidrogênio (Soares *et al.*, 2003; Spada & Salvador, 2005; Saffi *et al.*, 2006).

Para o estudo da atividade mutagênica, tem sido utilizada uma linhagem de *S. cerevisiae* denominada de XV-145-14c isolada por Von Borstel. Esta linhagem permite a detecção de dois tipos de mutações *locus* específicas: reversão do alelo ocre *lys1-1* (alteração para o códon UAA de término de cadeia) ou do alelo *missense his1*-7 (códon alterado codifica um aminoácido diferente), e rever são por deslocamento do quadro de leitura do DNA (*frameshift*) verificadas no *locus hom3-10.* As células revertentes podem ser detectadas pela utilização de meios seletivos nos quais o fator de crescimento inicialmente requerido não está presente, ou está em quantidades muito pequenas, permitindo somente um crescimento inicial (Parry & Parry, 1984; Zimmermann, 1984; Boeira *et al.,*2002; Pungartnik *et al.*, 2005).

Os testes de antimutagênese seguem o mesmo princípio do ensaio de mutagênese, com a ressalva de haver um co, pré, ou pós-tratamento com um agente reconhecidamente mutagênico (Henriques *et al.*, 2001; Melo Cavalcante *et al.*, 2003) acrescido da amostra a ser avaliada.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar a atividade antioxidante, mutagênica e antimutagênica de 23 polpas/sucos de frutas congeladas e determinar os principais compostos e minerais presentes nestas frutas.

2.2 Objetivos específicos

- Quantificar a atividade antioxidante *in vitro* (capacidade de varredura do radical DPPH$^\bullet$ e atividade superóxido dismutase e catalase-*like*) das polpas de frutas;
- Avaliar possíveis efeitos mutagênicos/antimutagênicos das polpas de frutas em células eucarióticas da levedura *S. cerevisiae;*
- Determinar o conteúdo de carboidratos, lipídios, proteínas, carotenóides, polifenóis totais e ácido ascórbico nas diferentes frutas;
- Quantificar os teores de minerais (PIXE) presentes nas polpas de frutas estudadas;
- Avaliar a capacidade antioxidante do açaí em homogeneizados de tecido nervoso de ratos Wistar;
- Correlacionar os dados obtidos nos ensaios de atividade biológica com o conteúdo de carotenóides totais, polifenóis totais, ácido ascórbico e minerais encontrados nas diferentes amostras de frutas.

3. RESULTADOS E DISCUSSÃO

3.1 CAPÍTULO 1

Antioxidant, mutagenic, and antimutagenic activity of frozen fruits

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3.2 CAPÍTULO 2

Macro and micromineral: are frozen fruit a good source?

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Macro and microminerals: are frozen fruits a good source?

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Abstract

Fruits are rich in minerals, which are essential for a wide variety of metabolic and physiologic processes in the human body. The intake of frozen fruits has spread widely in the last years, not only in the preparation of juices, but also, as raw material for yogurts, candies, cookies, cakes, ice creams, and children's food. However, until now, there is no data about the mineral profile of frozen fruits. This is the first database to quantify the levels of 14 minerals (macro and micro) in 23 samples of frozen fruits, including the most used around the world and some native fruits from the Amazonian rainforest in Brazil. Although geographical differences should be considered, these data can be used to achieve Dietary Reference Intakes for minerals in most parts of the world.

Keywords: frozen fruits, minerals, diet.

1.Introduction

Many of the current diets are rich in fat, salt, and sugar and poor in complex carbohydrates, vitamins, and minerals and they are responsible for an increase in diet-related diseases such as obesity, diabetes, cardiovascular problems, hypertension, osteoporosis, and cancer. It is believed that the ingestion of fruits and vegetables helps in the prevention of these diseases. Fruits are an important component of diet, responsible not only for adding variety of color and texture to meals, but also for providing important nutrients. Fruits are low-fat and low-calorie foods, with relatively small amounts of protein and carbohydrates, but they are rich in fibers and add significant amounts of micronutrients to the human diet (Zhi, Moore *et al.*, 2003).

Among the micronutrients found in fruits, minerals represent a class of inorganic substances present in all kinds of fruits. The human body needs about twenty different minerals in order to function properly (Williams, 2006). These elements can be classified into macro and micro minerals. Macro minerals are needed in amounts higher than 100mg/day and include Ca, P, Mg, S, Na, Cl and K. Micro minerals (Fe, Zn, I, Se, Mn, Cr, Cu, Mo, F, B, Co, Si, Al, Ar, Sn, Li and Ni), found in minimal quantities in human tissues, are needed in amounts lower than 100mg/day. In this group, the minerals that are needed in amounts lower than 1mg/day can be called, also, ultra trace elements (Mahan e Escott-Stump, 2005). Fruits are the most important source of both macro and micro minerals (Pellerano, Mazza *et al.*, 2008), which are indispensable for the maintenance of life, growth, and reproduction (Alsafwah, Laguardia *et al.*, 2007).

Nowadays, a high intake of fruits is recommended in order to prevent many kinds of diseases (Lampe, 1999). However, it is difficult to find fruits *in natura* – which are perishable – during all year round and/or in places far from the harvesting field. Frozen fruit intake has been widely spread in many countries. They are easy to commercialize and are very important as a source of raw material. They are used in yogurts, candies, cookies, cakes, ice creams, fresh drinks and children's food (Hassimotto, Genovese *et al.*, 2005). In a recent work (Spada, De Souza *et al.*, 2008), it was demonstrated that fruits, even frozen, are rich in carotenoids, ascorbic acid and phenolic compounds and present an important antioxidant activity. However, to our knowledge, there is no data about mineral levels in frozen fruits.

Therefore, the aim of this study was to determine the mineral levels in 23 samples of frozen fruit through PIXE (particle induced X-ray emission) assay. The results can be important to help the population to achive the recommended dietary allowance (RDA) threshold for minerals.

2. Material and methods

2.1 Frozen fruits

Frozen pulps of acerola (*Malpighia glabra L.*), apple (*Malus domestica B.*), acai (*Euterpe oleracea* L.), black mulberry (*Morus nigra* M.), cashew apple (*Anacardium occidentale* L.), coconut (*Cocos nucifera* L.), cupuacu (*Theobroma grandiflorum* W.), kiwi fruit (*Actinidia chinensis* P.), mango (*Mangifera indica* L.), melon (*Cucumis melo* L.), papaya (*Carica papaya* L.), passion fruit (*Passiflora alata* C.), peach (*Prunus persica* L.), pineapple (*Ananas sativus* L.), raspberry (*Rubus idaeus* L.), red guava (*Psidium guajava* L.), soursop (*Annona muricata* L.), strawberry (*Fragaria vesca* L.), Surinam cherry (*Eugenia uniflora* L.), and frozen juices of red grape (*Vitis vinifera* L.), lemon (*Citrus limon* B.), orange (*Citrus aurantium* L.), and tangerine (*Citrus reticulata* L.) were obtained from the industry Mais Fruta (Antonio Prado, RS, Brazil). Pulps and juices were produced with fresh and clean fruits; free of filthy substances, parasites, and plant or animal debris. Only edible portions of the fruits were pressed in order to prepare pulps and juices. In red grape, lemon, orange, and tangerine juices, flesh was separated from fluid obtained from pressing. Pulps and juices were divided into aliquots of 100 g and kept frozen at -20 ºC. No organophosphorus or carbamate pesticides were detected in the samples, through assay described by Bastos, Cunha & de Lima (1991) and de Lima, Cunha Bastos & Cunha Bastos (1996).

2.2 Particle induced X-ray emission analysis

Quantification of mineral compounds present in frozen fruits was carried out using PIXE. This technique has a truly multielemental capability; that is, all elements with atomic number higher than 11 can be simultaneously detected in a single measurement on the same target (Johansson, Campbell *et al.*, 1995). The sensitivity is very good and varies smoothly as a function of atomic number. It is important to note that PIXE sensitivity depends on the sample being analyzed. Typically, sensitivity is of the order of a few parts per million. The analysis is relatively fast and the measuring time is a few minutes. Since this technique is non-destructive, it preserves the original samples, allowing extra measurements if required. Sample preparation in its solid form (for a variety of samples) does not require either sophisticated handling or chemical treatment, thus reducing drastically any chance of contamination. Nowadays, PIXE is widely used to characterize a variety of materials, including biological, geological and environmental samples (Kern, Bonatto *et al.*, 2005; Franke, Pra *et al.*, 2006). For PIXE analysis, the fruit samples were dried and transformed in tablets, as described by Franke et al. (2006). Measurements were carried out at the Ion Implantation Laboratory of the Physics Institute of the Federal University of Rio Grande do Sul. A 3 mV Tandetron accelerator provided a 2 MeV proton beam with an average current of 2 nA for the experiments. Details of the experimental set-up are described in Dias et al. (2002). The characteristic X-rays induced by the proton beam were detected with a lithium doped silicon detector with an energy resolution of 155 eV at 5.9 keV, which was positioned at an angle of 45 ^{o}C with respect to the beam direction. The data were analyzed using the GUPIX code (Maxwell, Campbell *et al.*, 1989 1989; Campbell, Hopman *et al.*, 2000 2000). The standardization procedure was carried out using a bovine standard from NIST (SRM–

1577b). Quantitative PIXE analysis of a sample in a thick target approximation (pellets) requires knowledge of its matrix composition. Therefore, another ion beam technique, Rutherford Backscattering Spectroscopy (Chu, Mayer *et al.*, 1978), was employed to obtain this information for the liver and bovine standard used to calibrate the PIXE analysis. The matrix composition consisted of approximately C (70%), O (15%) and H (15%). All assays were performed in triplicate.

2.3 Statistical analysis

Data were subjected to analysis of variance and means were compared using Tukey's post-hoc test using the SPSS program, version 12.0 (SPSS, Chicago, IL).

3. Results and discussion

This is the first work to evaluate the mineral content of 23 frozen fruits, including the most used around the world and some native fruits from the Amazonian rainforest in Brazil. Frozen fruits are used not only in the preparation of juices, but also as raw material for yogurts, candies, cookies, cakes, ice creams, and children's food (Spada, De Souza *et al.*, 2008).

The minerals content found in fruits is presented as macro (Table 1) and micro minerals (Table 2). The Recommended Dietary Allowances (RDA), which is defined as the average daily intake level that is sufficient to meet the nutrient requirement of nearly all – 97 to 98% - healthy individuals in a particular life-stage and gender group (ref), was also showed. When there is insufficient scientific evidence to establish a RDA, it was used the Adequate Intake (AI), which is the recommended average daily nutrient intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group, or groups, of apparently healthy people who are assumed to be maintaining an adequate nutritional state (IOM, 2004.). All the studied fruits presented Mg, Cl, P, K and Ca. Sulfur was found in all fruits, except cupuacu and passion fruit, two native fruits of Brazil. Only coconut, lemon and papaya presented Na.

The micro mineral Fe was found in all fruits, Mn in 65.2% of them and Cu and Zn in 30.4% of the analyzed fruits. A low level of Cr was found in melon, orange and papaya. The ultra trace elements, Si and Al, were found in 91.30% and 39.13% of fruits, respectively.

Macro minerals are essential for a wide variety of metabolic and physiologic processes in the human body. Calcium, magnesium, potassium, sodium and chlorine, for example, are important for many enzymes activities, for the composition of the skeletal system and for ATP formation (Williams, 2006). It was observed that frozen fruits (100 g) are able to provide around 2.1% (for men) and 2.8% (for women) of the RDA for Mg and around 0.5% (for both sexes) of the Ca and P RDAs. Cl, K and Na were found in low amounts, reaching only around 0.2% of the AI.

Micro minerals, such as Fe, Mn, Cu and Zn are cofactors of many enzymes and are part of the active site of some oxidases and oxygenases (Halliwell e Gutteride, 2007). Iron is a component of hemoglobin, myoglobin, cytochromes, and various enzymes in the muscle cells (Donabedian, 2006). About 3.5% (for men) and 1.5% (for women) of the RDA for Fe can be provided by frozen fruits (100g). Acai, apple and tangerine are rich in Fe, and 100 g of these frozen fruits can contribute with approximately 7% of the RDA for men. Red grape and coconut (100 g) can reach 22.2% of the Mn RDA. All fruits provide more than 100% of the Cu RDA for men and women. Melon, orange and papaya (100 g) are able to provide more than 100% of the Cr AI.

Our results showed the mineral profile of 23 frozen pulp fruits, which can help to provide the flexibility needed to achieve the optimal dietary mineral content for a healthy human diet. However, it should be taken into consideration that the mineral profile can be influenced by the growing conditions, such as soil and geographical factors (Ercisli e Ohran, 2007). Also, the bioavailability of minerals appears to be dependent on cultivar, environment and harvest year (Bohn, Meyer *et al.*, 2008). Some compounds, such as ascorbic acid, can help Fe absorption, but, on the other side, iron uptake could be inhibited by strong chelators such phytic acid, some polyphenols and the divalent cations Ca, Zn, Co and Mg. The relationship among all the fruit constituents and between them and the other elements present in the diet should be better studied.

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References

Alsafwah, S., Laguardia, S. P., Arroyo, M., Dockery, B. K., Bhattacharya, S. K., Ahokas, R. A., & Newman, K. P. (2007). Congestive heart failure is a systemic illness: a role for minerals and micronutrients. *Clin Med Res*, *5*(4), 238-243.

Bastos, V. L. F. C., Cunha, J. C. B., Lima, J. S. (1991) Brain acetylcholinesterase as in vitro detector of organophosphorus and carbamate insecticides in water. *Water Research*, 25:835-840.

Bohn, L., Meyer, A. S., & Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *J Zhejiang Univ Sci B*, *9*(3), 165-191.

Campbell, J. L., Hopman, T. L., & Maxwell, J. A. (2000). The Guelph PIXE software package III: alternative proton database. *Nucl Instrum Methods Phys Res B.*, *170*, 193-204.

Chu, W. K., Mayer, J. W., & Nicolet, M. A. (1978). *Backscattering spectrometry.* New York.

de Lima, J. S., Cunha Bastos, J., Cunha Bastos, V. L. F. (1996) Methyl parathion activation by partially purified rat brain fraction. *Toxicology Letters*, 87:53-60.

Donabedian, H. (2006). Nutritional therapy and infectious diseases: a two-edged sword. *Nutr J*, *5*, 21.

Ercisli, S., & Ohran, E. (2007). Chemical composition of withe (*Morus alba)*, red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. *Food Chemistry*, *103*, 1380-1384.

Franke, S. I., Pra, D., Giulian, R., Dias, J. F., Yoneama, M. L., da Silva, J., Erdtmann, B., & Henriques, J. A. (2006). Influence of orange juice in the levels and in the genotoxicity of iron and copper. *Food Chem Toxicol*, *44*(3), 425-435.

Halliwell, B., & Gutteride, J. M. C. (2007). *Free Radicals in Biology and Medicine*. New York.

Hassimotto, N. M., Genovese, M. I., & Lajolo, F. M. (2005). Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J Agric Food Chem*, *53*(8), 2928-2935.

IOM (2004) Institute of Medicine. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Available online. http://www.nap.edu/catalog/10925.html

Johansson, S. A., Campbell, J. L., & Malmqvist, K. G. (1995). *Particleinduced x-ray emission spectrometry (PIXE)*. New York.

Kern, A. L., Bonatto, D., Dias, J. F., Yoneama, M. L., Brendel, M., & Henriques, J. A. P. (2005). The importance of yeast Alr proteins in cadmium detoxification as indicated by particle-induced X-ray emission and phenotypic analyses. *X-ray spectrometry*, *34*(4), 355-358.

Lampe, J. W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am J Clin Nutr*, *70*(3 Suppl), 475S-490S.

Mahan, K. L., & Escott-Stump, S. (2005). *Krause's Food, Nutrition & Diet Therapy*. Philadelphia: Saunders.

Maxwell, J. A., Campbell, J. L., & Teesdale, W. J. (1989). The Guelph PIXE software package. *43*, 218-230.

Pellerano, R. G., Mazza, S. S., Marigliano, R. A., & Marchevsky, E. J. (2008). Multielement analysis of argentinean lemon juices by instrumental neutronic activation analysis and their classification according to geographical origin. *J Agric Food Chem*, *56*(13), 5222-5225.

Spada, P. D., de Souza, G. G., Bortolini, G. V., Henriques, J. A., & Salvador, M. (2008). Antioxidant, mutagenic, and antimutagenic activity of frozen fruits. *J Med Food*, *11*(1), 144-151.

Williams, M. (2006). Dietary supplements and sports performance: metabolites, constituents, and extracts. *J Int Soc Sports Nutr*, *3*, 1-5.

Zhi, J., Moore, R., & Kanitra, L. (2003). The effect of short-term (21-day) orlistat treatment on the physiologic balance of six selected macrominerals and microminerals in obese adolescents. *J Am Coll Nutr*, *22*(5), 357-362.

Table 1. Levels of macro minerals (mg%) in frozen fruits

Samples	Mg	Cl	P	Ca	K	S	Na
Acai	8.47 ± 0.17^{a}*	1.96 ± 0.12^{a}	3.05 ± 0.07^{a}	3.50 ± 0.13^{a}	1.03 ± 0.40^{a*}	1.91 ± 0.01^{a}	nd
Acerola	8.64 ± 0.22^{a}	2.38 ± 0.19^{a}	3.25 ± 0.16^{a}	5.82 ± 0.05^{b}	1.22 ± 0.50^{b}	2.17 ± 0.09^{ab}	nd
Apple	7.74 ± 0.24^{a}	2.05 ± 0.02^{a}	2.61 ± 0.14^{a}	3.60 ± 0.50^{a}	0.97 ± 0.10^{a}	1.73 ± 0.03^{a}	nd
Black mulberry	8.21 ± 0.05^{a}	2.26 ± 0.04^{a}	3.01 ± 0.42^{a}	4.83 ± 0.02^{c}	1.35 ± 0.30^{b}	1.99 ± 0.07^{a}	nd
Cashew apple	8.22 ± 0.01^{a}	2.99 ± 0.34^{b}	2.53 ± 0.47^{c}	3.49 ± 0.05^{a}	1.43 ± 0.10^{b}	1.46 ± 0.55^{a}	nd
Coconut	9.67 ± 0.24^{a}	2.60 ± 0.01^{a}	4.43 ± 0.09^{b}	7.60 ± 0.59^{d}	2.41 ± 0.16^{b}	3.59 ± 0.21^{c}	26.95 ± 1.05^{a}
Cupuacu	8.48 ± 0.02^{a}	0.23 ± 0.07^{d}	3.13 ± 0.12^{a}	6.59 ± 0.07^{d}	1.55 ± 0.07^{b}	nd	nd
Kiwi fruit	8.91 ± 0.19^{a}	2.66 ± 0.05^{b}	3.34 ± 0.01^{a}	7.03 ± 0.03^{d}	2.07 ± 0.07^{b}	2.48 ± 0.09^{b}	nd
Lemon	8.98 ± 0.15^{a}	2.73 ± 0.02^{b}	3.47 ± 0.02^{a}	7.02 ± 0.03^{d}	2.21 ± 0.53^{b}	2.23 ± 0.03^{ab}	23.15 ± 1.44^{ab}
Mango	7.62 ± 0.05^{a}	1.89 ± 0.02^{a}	2.69 ± 0.07^{c}	3.43 ± 0.01^{a}	1.23 ± 0.16^{c}	1.79 ± 0.02^{a}	nd
Melon	11.15 ± 0.28^{b}	3.55 ± 0.84^{c}	4.17 ± 0.27^{b}	10.33 ± 0.36^{e}	2.22 ± 0.21^{b}	2.96 ± 0.86^{b}	nd
Orange	8.08 ± 0.02^{a}	2.16 ± 0.02^{a}	2.85 ± 0.02^{c}	4.45 ± 0.02^{c}	1.49 ± 0.02^{c}	1.87 ± 0.07^{a}	nd
Papaya	8.44 ± 0.49^{a}	1.82 ± 0.13^{a}	2.96 ± 0.02^{c}	5.32 ± 0.04^{c}	1.55 ± 0.26^{b}	2.01 ± 0.07^{a}	21.80 ± 1.02^{b}
Passion fruit	8.44 ± 0.23^{a}	2.36 ± 0.02^{a}	1.26 ± 0.07^{d}	5.42 ± 0.09^{c}	1.21 ± 0.16^{c}	nd	nd
Peach	11.32 ± 0.34^{b}	3.68 ± 0.06^{c}	3.91 ± 0.26^{a}	5.58 ± 0.06^{c}	1.53 ± 0.21^{c}	2.57 ± 0.05^{b}	nd
Pineapple	8.40 ± 0.33^{a}	2.30 ± 0.17^{a}	3.12 ± 0.09^{a}	4.57 ± 0.24^{c}	1.34 ± 0.52^{c}	2.01 ± 0.06^{a}	nd
Raspberry	8.4 ± 0.09^{a}	2.20 ± 0.02^{a}	3.10 ± 0.01^{a}	4.48 ± 0.25^{c}	1.52 ± 0.18^{c}	1.97 ± 0.02^{a}	nd
Red grape	8.14 ± 0.23^{a}	1.94 ± 0.01^{a}	2.81 ± 0.08^{c}	3.25 ± 0.26^{a}	0.90 ± 0.30^{a}	1.77 ± 0.06^{a}	nd
Red guava	8.61 ± 0.14^{a}	2.55 ± 0.02^{a}	3.16 ± 0.05^{a}	6.09 ± 0.09^{d}	14.4 ± 0.50^{b}	2.24 ± 0.06^{ab}	nd
Soursop	8.48 ± 0.61^{a}	2.36 ± 0.31^{a}	3.21 ± 0.10^{a}	5.72 ± 0.15^{c}	1.09 ± 0.20^{b}	2.19 ± 0.05^{ab}	nd
Strawberry	9.15 ± 0.08^{a}	2.69 ± 0.03^{b}	3.40 ± 0.04^{a}	6.57 ± 0.15^{d}	1.61 ± 0.42^{b}	2.21 ± 0.03^{ab}	nd
Surinam cherry	8.57 ± 0.14^{a}	2.12 ± 0.15^{a}	3.05 ± 0.09^{a}	4.43 ± 0.06^{c}	1.38 ± 0.07^{b}	1.87 ± 0.15^{a}	nd
Tangerine	10.66 ± 0.14^{b}	3.48 ± 0.17^{c}	2.55 ± 0.07^{c}	5.40 ± 0.80^{c}	1.49 ± 0.50^{b}	2.67 ± 0.83^{b}	nd
Men/day	*420mg*	**2000mg**	*700mg*	*1200mg*	**4700mg**	not determinable	**1300mg**
Women/day	*320mg*	**2000mg**	*700mg*	*1200mg*	**4700mg**	not determinable	**1300mg**

Data are mean ± SD values of three independent experiments.

*Different letters indicate a significant difference according to analysis of variance and Tukey's *post hoc* test (*p≤0* .05) for each mineral evaluated. nd: non detected.

This table presents *Recommended Dietary Allowances (RDAs)* in italics and **Adequate Intakes (AIs) in bold type.**

Table 2. Levels of micro minerals (mg%) in frozen fruits

Samples	Fe	Mn	Cu	Zn	Cr	Si	Al
Acai	0.51 ± 0.28^{a*}	0.27 ± 0.07^{a}	nd	0.20 ± 0.01^{a}	nd	1.22 ± 0.03^{a}	nd
Acerola	0.26 ± 0.85^{b}	nd ±	nd	nd	nd	1.35 ± 0.07^{a}	nd
Apple	0.54 ± 1.32^{a}	0.29 ± 0.04^{ab}	1.80 ± 0.14^{a}	0.28 ± 0.05^{ab}	nd	1.12 ± 0.04^{a}	4.16 ± 0.03^{a}
Black mulberry	0.28 ± 0.57^{b}	0.31 ± 0.01^{b}	nd	nd	nd	1.28 ± 0.02^{a}	nd
Cashew apple	0.28 ± 0.64^{b}	nd ±	nd	nd	nd	1.54 ± 0.53^{a}	nd
Coconut	0.24 ± 0.42^{b}	0.40 ± 0.07^{b}	nd	nd	nd	1.60 ± 0.07^{a}	nd
Cupuacu	0.20 ± 0.19^{c}	0.20 ± 0.01^{a}	2.25 ± 0.78^{ab}	nd	nd	1.30 ± 0.06^{a}	nd
Kiwi fruit	0.21 ± 0.42^{b}	0.19 ± 0.02^{a}	nd	nd	nd	nd	nd
Lemon	0.27 ± 0.85^{b}	0.21 ± 0.06^{a}	2.30 ± 0.42^{b}	0.26 ± 0.04^{ab}	nd	1.37 ± 0.07^{a}	5.35 ± 0.05^{b}
Mango	0.18 ± 0.76^{c}	0.17 ± 0.06^{a}	1.85 ± 0.35^{a}	0.21 ± 0.03^{a}	nd	1.11 ± 0.01^{a}	4.17 ± 0.10^{a}
Melon	0.28 ± 0.07^{b}	0.31 ± 0.04^{b}	nd	nd	0.15 ± 0.01^{a}	1.66 ± 0.60^{a}	nd
Orange	0.28 ± 0.92^{b}	0.18 ± 0.01^{a}	nd	0.30 ± 0.08^{ab}	0.13 ± 0.02^{a}	1.22 ± 0.02^{a}	4.47 ± 0.01
Papaya	0.16 ± 0.28^{c}	nd ±	2.05 ± 0.07^{c}	0.22 ± 0.01^{a}	0.18 ± 0.04^{b}	1.31 ± 0.07^{a}	4.72 ± 0.30^{a}
Passion fruit	0.16 ± 0.07^{c}	0.20 ± 0.04^{a}	1.55 ± 0.05^{a}	0.30 ± 0.01^{b}	nd	1.26 ± 0.04^{a}	4.73 ± 0.07^{a}
Peach	0.34 ± 0.27^{b}	nd ±	nd	nd	nd	1.74 ± 0.07^{a}	nd
Pineapple	0.30 ± 0.56^{b}	0.30 ± 0.06^{b}	nd	nd	nd	1.25 ± 0.06^{a}	nd
Raspberry	0.25 ± 0.42^{b}	0.21 ± 0.05^{a}	nd	nd	nd	1.29 ± 0.07^{a}	nd
Red grape	0.18 ± 0.07^{c}	nd ±	3.10 ± 0.85^{c}	nd	nd	1.18 ± 0.01^{a}	4.12 ± 0.35^{a}
Red guava	0.18 ± 0.02^{c}	0.14 ± 0.02^{a}	nd	nd	nd	nd	nd
Soursop	0.18 ± 0.21^{c}	nd ±	nd	nd	nd	1.29 ± 0.06^{a}	4.83 ± 0.25^{a}
Strawberry	0.35 ± 0.78^{b}	0.28 ± 0.03^{a}	nd	nd	nd	1.39 ± 0.03^{a}	5.21 ± 0.35^{b}
Surinam cherry	0.14 ± 0.28^{c}	nd ±	nd	nd	nd	1.32 ± 0.04^{a}	nd
Tangerine	0.61 ± 0.21^{a}	nd ±	nd	nd	nd	3.82 ± 0.42^{b}	nd
Men/day	*8mg*	*2.3mg*	*900µg*	*11mg*	**30µg**	not determinable	not determinable
Women/day	*18mg*	*1.8mg*	*900µg*	*8mg*	**20µg**	not determinable	not determinable

Data are mean ± SD values of three independent experiments.

[*]Different letters indicate a significant difference according to analysis of variance and Tukey's *post hoc* test (*p≤0* .05) for each mineral evaluated. nd: non detected.

This table presents *Recommended Dietary Allowances (RDAs)* in italics and **Adequate Intakes (AIs) in bold type.**

3.3 CAPÍTULO 3

Frozen fruit pulp of Euterpe oleraceae Mart. (Acai) prevents hydrogen peroxide induced damage in the cerebral cortex, cerebellum and hippocampus of rats

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Frozen fruit pulp of Euterpe oleraceae Mart. (Acai) prevents hydrogen peroxide induced damage in the cerebral cortex, cerebellum and hippocampus of rats

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Abstract

Oxidative stress is implicated in several human illnesses, including neurological disorders such as Parkinson's and Alzheimer's diseases. Acai is largely consumed in Brazil and contains high levels of antioxidant compounds. This work aims to study the antioxidant activity of acai frozen fruit pulp in the cerebral cortex, hippocampus and cerebellum of rats treated with the oxidizing agent hydrogen peroxide (H_2O_2). Pre-treatment of tissue with acai decreased H_2O_2-induced damage of both lipids and proteins in all tissues tested. This fruit was also able to reduce the activities of the antioxidant enzymes superoxide dismutase and catalase to basal levels. We observed a negative correlation between the polyphenol content of acai and the levels of lipid ($r = -0.689$; $p \leq 0.05$) and protein damage ($r = -0.569$; $p \leq 0.05$), suggesting the participation of polyphenols in the observed antioxidant activity. These data suggest that acai could prevent the development of age-related neurodegenerative diseases.

Keywords: *Euterpe oleraceae*, acai, brain tissues, superoxide dismutase, catalase, oxidative stress.

INTRODUCTION

Several studies have shown that the consumption of fruits and vegetables is associated with a reduced risk of many diseases, including neurodegenerative diseases such as Parkinson's and Alzheimer's diseases.[1] Fruits and vegetables synthesize a vast array of secondary chemical compounds that, although not involved in primary metabolism, are important for a multitude of beneficial effects that have been reported for fruits.[2]

The palm fruit of *Euterpe oleraceae* M., commonly known as acai, is consumed in a variety of beverages and food preparations, mainly in Brazil[3]. This fruit supplies several antioxidant compounds such as polyphenols, carotenoids and ascorbic acid.[4-6] Acai has, in fact, already been demonstrated to possess antioxidant activity *in vitro*,[6,7] and possible roles for acai as a food additive are under investigation.[8-11]

The brain is especially susceptible to oxidative stress.[12] While only making up about 2 % of the total body mass, the brain consumes 20 % of the total oxygen used by the body. It is enriched with readily peroxidizable polyunsaturated fatty acids and does not possess any antioxidant defenses. The brain also has high levels of iron, which are the key catalysts for lipid peroxidation. Additionally, many neurotransmitters are themselves autoxidized to generate reactive species.[13-14] Lipid peroxidation in brain tissues is associated with a progressive loss of membrane permeability and cellular damage, which leads to an increased susceptibility to various diseases.[15] In order to protect the brain against oxidative damage, there exist intricate and interrelated processes, which include superoxide dismutase (SOD) and catalase (CAT) enzymes. SOD catalyzes the dismutation of superoxide anion ($O_2^{-\bullet}$) to oxygen and hydrogen peroxide, while CAT converts hydrogen peroxide to water and molecular oxygen.[16] SOD and CAT enzymes have an important role in maintaining physiological redox equilibrium, avoiding or decreasing the oxidative stress.[16]

The aim of the present study was to investigate the biological effects of frozen pulp of acai in reducing the oxidative stress induced by hydrogen peroxide in the cerebellum, cerebral cortex and hippocampus from Wistar rats.

MATERIALS AND METHODS

Acai Frozen Fruit Pulp

Acai (*Euterpe oleracea*) was obtained from the company Mais Fruta (Antonio Prado, Brazil). The pulp was produced with fresh and clean fruits, free of dirt, parasites and plant or animal debris. Only the edible portion of acai was used. After pressing, the pulp was frozen at –20 °C. Immediately prior to the assays frozen pulp was mixed with distilled water in a blender to achieve a final concentration of 40% (wt/v). Nutritional composition and the main secondary compounds of acai are shown in Table 1. No organophosphorus or carbamate pesticides were detected in the sample.

Animal and Tissue Preparation

Ten-day-old Wistar rats were obtained from our own breeding colony. They were maintained at approximately 25°C, on a 12-h light/12-h dark cycle, with free access to food and water. Assays were performed as described by Leipnitz *et al.*[17]. Briefly, animals were killed by decapitation without anesthesia, and the brain was rapidly excised on a Petri dish placed on ice. The cerebral cortex, cerebellum and hippocampus were dissected, weighed and kept chilled until homogenization, which was performed using a ground glass type Potter-Elvejhem homogenizer in 1.5% KCl. The homogenates were centrifuged at 800 g for 10 min at 4 °C, the pellet was discarded and the supernatants were used immediately. Aliquots were treated with acai pulp (40% wt/v) for 30 min, and 1mM H_2O_2 was subsequently added to the mixture. Samples were incubated for 1 h at 30 ºC with agitation. All experiments were conducted in accordance with the Guiding Principles of the Use of Animals in Toxicology, adopted by the Society of Toxicology in July 1989.

Biochemical assays

Superoxide dismutase activity was determined spectrophotometrically by measuring the inhibition of self-catalytic adrenochrome formation at 480 nm (model UV-1700 spectrofotometer, Shimadzu, Kyoto Japan) in a reaction medium containing 1 mmol/L adrenaline (pH 2.0) and 50 mmol/L glycine (pH 10.2). This reaction was performed at 30ºC for 3 min.[18] One unit of SOD activity is defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50% per gram of protein. The CAT-like activity assay was performed according to the method described in Aebi[19] by determining the hydrogen peroxide decomposition rate at 240 nm. A total of 1 unit of catalase decomposed 1 μmol of H_2O_2/mg of protein in 1 min at pH 7.4. Protein concentration was determined by the Bradford method[20] using serum bovine albumin as standard.

Lipid peroxidation was monitored by the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, which has been widely adopted as a sensitive method for measurement of lipid peroxidation, as previously described[21]. The oxidative damage to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH), as previously described.[22] All chemicals were purchased from Sigma (Sigma Chemical Co., São Paulo).

Statistical Analyses

Values were determined as parametric or nonparametric by using the Kolmogorov-Smirnoff test. Data were subjected to analysis of variance, and means were compared using Tukey's test. Relationships between variables were assessed with Pearson's product-moment correlation coefficient. SPSS version 12.0 (SPSS, Chicago, IL) was used in all statistical analysis.

RESULTS

Tissue treatments with hydrogen peroxide induced an increase in lipid (TBARS) and protein (protein carbonyl groups) damage in the cerebellum, cerebral cortex and hippocampus of rats relative to the untreated control (Figure 1 A and B). H_2O_2 also induced an increase in both SOD and CAT activities in all the assayed tissues (Figure 2 A and B). Acai treatment was neither able to change enzyme activities (Figure 2), nor to induce oxidative damage in lipids or proteins (Figure 1). However, when tissues were pre-treated with acai and then with H_2O_2, a significant decrease in lipid and protein damage and in SOD and CAT activities was observed (Figure 2). The reduction of lipid damage was around 48 % in the cerebral cortex, 64 % in the hippocampus and 72 % in the cerebellum. Reduction of protein damage was 55 % in the cerebral cortex, 36 % in the hippocampus and 42 % in the cerebellum.

DISCUSSION

Brain cells are continuously threatened by the damage caused by reactive species produced during normal oxygen metabolism or induced by exogenous sources.[23] The mechanisms by which reactive species interfere with cellular function are not fully understood, but one of the most important events seems to be oxidative damage. The loss of motor function due to cell death and region-specific loss of neurons in the mammalian brain by oxidative stress is more prominent in the cerebral cortex, hippocampus, and cerebellum. The cerebral cortex and hippocampus are regions associated with cognition and feed-back control of stress, while the cerebellum is concerned with motor function.[24] *In vitro* studies of the responses of the cerebral cortex to vitamin E have revealed its neuroprotective role in the brain of mice.[25] Green tea catechins and polyphenols have also been reported for their antioxidant properties and protective effects against oxidative stress in rat brain.[26]

Acai, a very popular Brazilian fruit, is known to possess antinociceptive, anti-inflammatory[27] and inhibitory effects on nitric oxide production by activated macrophage cell line RAW 264.7. This fruit is rich in antioxidants compounds,[6,28] which are known to reduce the risks of diseases related to oxidative stress.

Brain tissue pre-treatments with acai were able to prevent oxidative damage induced by hydrogen peroxide. This oxidant causes cell damage through the production of hydroxyl radicals via the Haber-Weiss/Fenton reaction.[16] Unlike the free radicals $O_2^{-\bullet}$ and $OH^{\bullet}$, hydrogen peroxide is very diffusible within and between cells *in vivo*, causing damage in membranes, proteins, lipids, and the cellular nucleus.[16] Negative correlations between total phenolic content of acai and TBARS ($r = -0.689$; $p \leq 0.05$) and carbonyl assay ($r = -0.569$; $p \leq 0.05$) levels were observed, suggesting that the polyphenols present in this fruit could be responsible, at least in part, for the antioxidant activity of this fruit, as already described for the *in vitro* antioxidant activity of acai seeds and methanol and ethanol acai seed extracts.[5]

Hydrogen peroxide and/or the oxidative stress produced by it are able to induce the antioxidant activity of the SOD and CAT enzymes,[29] as observed in this work (Figure 2 A and B). Acai treatments were able to return SOD and CAT antioxidant activities to basal levels (Figure 2 A and B). In fact, a negative correlation was observed between SOD and CAT activities and total phenolic content of acai ($r = -0.701$; $r = -0.698$; $p \leq 0.05$, respectively). Among the polyphenols found in acai are epicatechin, catechin, cyanidin 3-glucoside, ferulic acid, *p*-hydroxy benzoic acid, pelargonidin 3-glucoside, gallic acid, protocatechuic acid and free ellagic acid.[4,28] Some polyphenols, such as catechin,[1] have been reported to be superoxide radical and H_2O_2 scavengers. In fact, acai frozen pulp has already been shown to possess SOD and CAT-like activities[6] and the ability to scavenge superoxide radicals,[7] which could be responsible, at least in part, for its beneficial effects against oxidative stress.

Structural damage and loss of brain function is associated with aging and age-related diseases in the central nervous system. Oxidative stress is considered a risk factor and contributes to increased lipid and protein damage.[30] The data obtained in this study showed that the frozen pulp of acai possesses an important antioxidant activity in the brain tissue of rats. Other polyphenol-rich fruits, such as berries[31] and grapes,[32] have already been found to be beneficial to brain function. Thus, it is possible to assert that nutritional interventions containing polyphenols could retard or prevent the development of age-related neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases.

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LITERATURE CITED

1. Silalahi J: Anticancer and health protective properties of citrus fruit components. *Asia Pac J Clin Nutr* 2002;11:79–84.

2. Joseph JA, Shukitt-Hale B, Casadesus G: Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* 2005;81:313S-316S.

3. Strudwick J, Sobel GL; Uses of Euterpe oleraceae Mart. In the Amazon estuary. *Braz Adv Econ Bot* 1988;6:225-253.

4. Del Pozo-Insfran D, Brenes CH, Talcott ST: Phytochemical composition and pigment stability of Açai (*Euterpe oleracea* Mart.). *J Agri. Food Chem* 2004;54:1539-1545.

5. Rodrigues RB, Lichtenthäler R, Zimmermann BF, Papagiannopoulos M, Fabricius H, Marx F, Maia JG, Almeida O: Total oxidant scavenging capacity of

Euterpe oleracea Mart. (açaí) seeds and identification of their polyphenolic compounds. *J Agric Food Chem* 2006;54:4162-4167.

6. Spada PDS, Souza GGN de, Bortolini GV, Henriques JAP, Salvador M: Antioxidant, mutagenic, and antimutagenic activity of frozen fruits. J. Med. Food 2008;11:144-151.

7. Schauss AG, Wu X, Prior RL, Ou B, Huang D, Owens J, Agarval A, Jensen GS, Hart AN, Shanbro E: Antioxidant capacity and other bioactivities of the freeze-dried amazonian palm berry *Euterpe oleraceae* Mart. (acai). *J Agric Food Chem* 2006;54:8604-8610.

8. Arruda DC, Felippi R, Mantovani ISB, Santos GB, Gabriel FT, de Sa AC, Fernandez SBO, Ribeirodo-Valle RM, Menezes FS: Antioxidant activity and levels of phenolics in *Euterpe oleracea* Mart extract (Acai). *Rev Bras Plan Med* 2004;6:5-10.

9. Lichtenthäler R, Rodrigues RB, Maia JGS, Papagiannopoulos M, Fabricius H, Marx F: Total oxidant scavenging capacities of *Euterpe oleraceae* Mart. (Acai) fruit. *Int J Food Sci Nutr* 2005;56:53-64.

10. Hassimotto NMA, Genovese MI, Lajolo FM: Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J Agric Food Chem* 2005;53:2928-2935.

11. Coïsson JD, Travaglia F, Piana G, Capasso M, Arlorio M: *Euterpe oleraceae* juice as functional pigment for yogurt. *Food Res Int* 2005;38:847-853.

12. Halliwell B, Gutteridge JMC: Oxygen radicals and the nervous system. *Trend. Neurosci* 1985;8:22-26.

13. Olanow CW: An introduction to the free radical hypothesis in Parkinson's disease. *Ann Neurol* 1992;32:S2-9.

14. Savory J, Rao JK, Huang Y, Letada PR, Herman MM: Age-related hippocampal changes in Bcl-2:Bax ratio, oxidative stress, redox-active iron and apoptosis associated

with aluminum-induced neurodegeneration: increased susceptibility with aging. *Neurotoxicology* 1999;20:805-817.

15. Balu M, Sangeetha P, Haripriya D, Panneerselvam C: Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. *Neurosci Lett* 2005;383: 295–300.

16. Halliwell B, Gutteridge JMC: *Free Radicals in Biology and Medicine*, 3rd ed. Clarendon Press, Oxford, 2007.

17. Leipnitz G, Schumacher C, Dalcin KB, Scussiato K, Solano A, Funchal C, Dutra-Filho CS, Wyse ATS, Wannmacher CDW, Latini A, Wajner M: In vitro evidence for an antioxidant role of 3-hydroxykynurenine and 3-hydroxyanthranilic acid in the brain. *Neurochem Int* 2007;50:83-94.

18. Bannister JV, Calabrese L: Assays for SOD. *Methods Biochem Anal* 1987;32:279–312.

19. Aebi H: Catalase in vitro. *Methods Enzymol* 1984;105:121–126.

20. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem1976*;7:248-254.

21. Wills ED: Mechanism of lipid peroxide formation in animal tissues. *Biochem J* 1966;99:667–676.

22. Levine RL: Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990*;*186:464– 478.

23. Grace PA: Ischaemia-reperfusion injury. *Br J Surg* 1994; 81:637-647.

24. de Groot H: Reactive oxygen species in tissue injury. *Hepatogastroenterology* 1994;41:328-332.

25. Merrill DA, Chiba AA, Tuszynski MH: Conservation of neuronal number and size in the entorhinal cortex of behaviorally characterized aged rats. *J Comp Neurol* 2001;438:445-456.

26. Kan S, Devi SA, Kawashima S. Effect of vitamin E on the accumulation of fluorescent material in cultured cerebral cortical cells of mice. Ex*p Gerontol* 1991**;**26:365-374.

27. Suzuki M, Tabuchi M, Ikeda M, Umegaki K, Tomita T: Protective effects of green tea catechins on cerebral ischemic damage. *Med Sci Monit* 2004;10:20-25.

28. Del Pozo-Insfran D, Percival SS, Talcott ST: Açai (*Euterpe oleracea* Mart.) polyphenolics in their glycoside and aglycone forms induce apoptosis of HL-60 leukemia cells. *J Agric Food Chem* 2006**;**54:1222-1229.

29. Spada PKWDS, Salvador M: Antioxidant activity of the flavonoid hesperidin in chemical and bilogical systems. *J Agric Food Chem* 2005**;**53:4757-4761.

30. Schoneich C: Reactive oxygen species and biological aging: a mechanistic approach. *Exp Gerontol* 1999;34:19–34.

31. Devi A, Jolitha AB, Ishii N: Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. *Med Sci Monit* 2006;12:124-129.

32. Dani C, Pasquali MAB, Oliveira MR, Umezu FM, Salvador M, Henriques JAP, Moreira JCF: Protective effects of purple grape juice on carbon tetrachloride-induced oxidative stress in brains of adult wistar rats. J. Med. Food 2008;11:55–61.

Table 1. Main secondary compounds and nutritional composition of acai (per 100g of frozen pulp).

	Total Phenolic content (mg of catechin)	Total Carotenoid content (mg)	Vitamin C (mg)	Proteins	Lipids	Carbohydrates	Caloric values (kJ)
Acai	1.19 ± 0.20	1.02 ± 0.55	15.70 ± 0.97	0.55 ± 0.03	0.67 ± 0.04	7.63 ± 0.02	163.25 ± 0.03

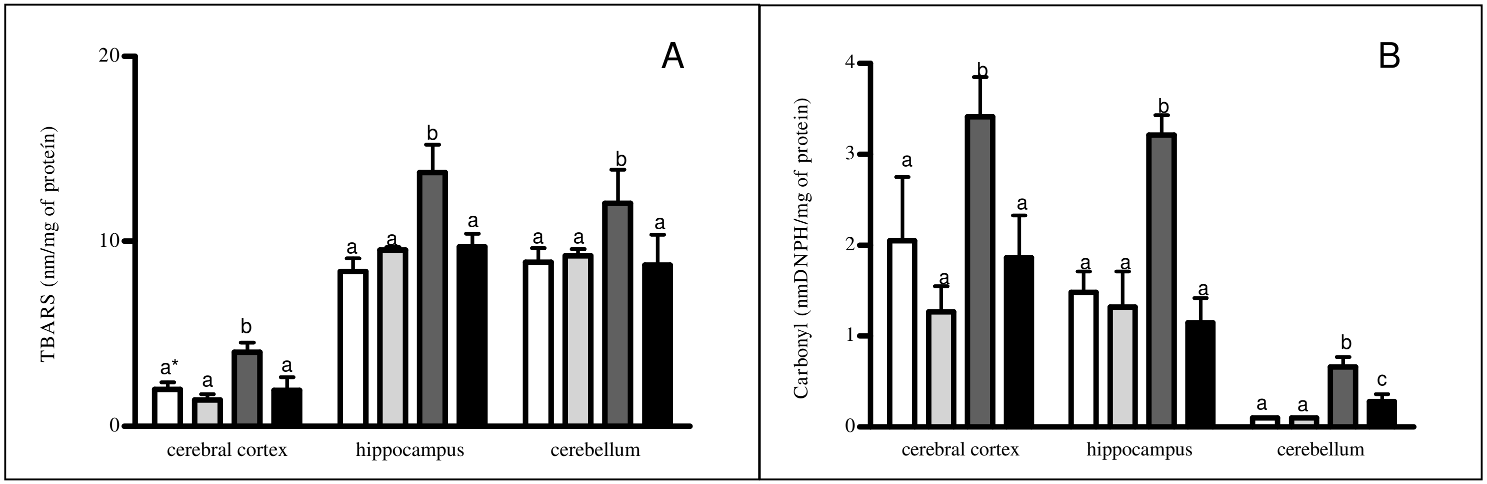

Figure 1. Lipid damage (A) and protein damage (B) in the cerebral cortex, hippocampus and cerebellum of rats.

Untreated tissues (☐); acai 40% wt/v (░); H_2O_2 1 mM (▓); acai 40% wt/v plus H_2O_2 1 mM (■).

Data are mean ± SD values of five independent experiments. *Different letters indicate a significant difference according to analysis of variance and Tukey's *post hoc* test ($p \leq 0.05$) for each tissue evaluated.

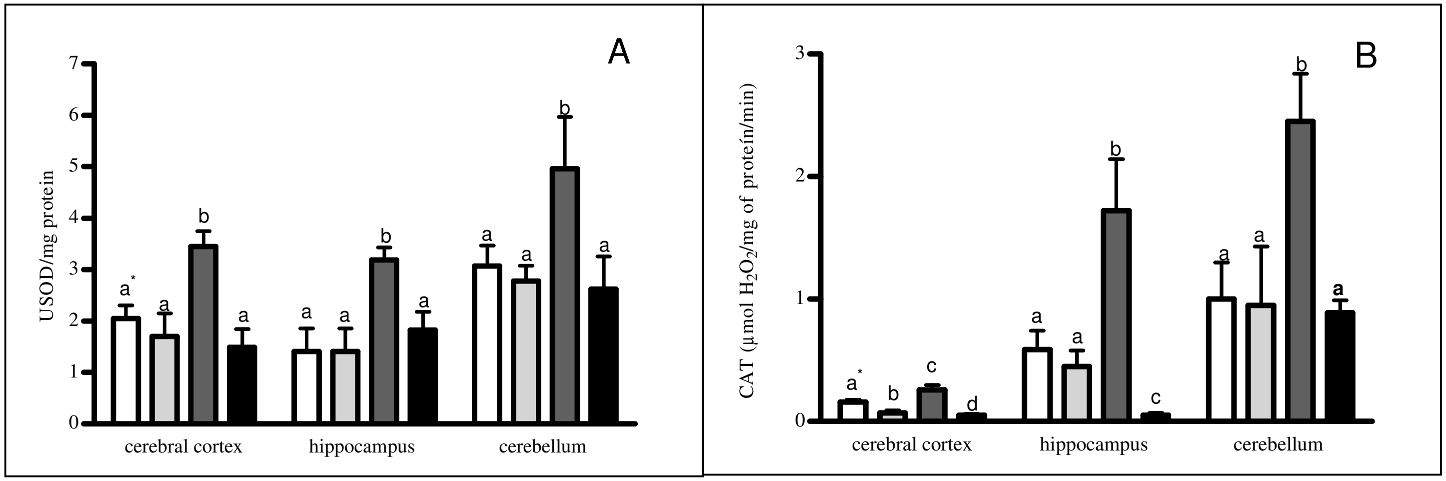

Figure 2. Superoxide dismutase (A) and catalase (B) activities in the cerebral cortex, hippocampus and cerebellum of rats.

Untreated tissues (□); acai 40% wt/v (░); H_2O_2 1 mM (▓); acai 40% wt/v plus H_2O_2 1 mM (■).

Data are mean ± SD values of five independent experiments. *Different letters indicate a significant difference according to analysis of variance and Tukey’s *post hoc* test ($p \leq 0.05$) for each tissue evaluated.

3.4 CAPÍTULO 4

Biological activities and main compounds of fruits

Revisão da literatura para publicação no livro *Recent Progress in Medicinal Plants – Search for Natural Drugs* – Editora *Studium Press* (ISSN: 09761849-5-8)

BIOLOGICAL ACTIVITIES AND MAIN COMPOUNDS OF FRUITS

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Abstract

Many studies have shown that the consumption of fruits and vegetables is associated with a reduced risk of many diseases, including cancer, atherosclerosis, and neurovegetative diseases, which are related to elevated levels of oxidative stress. Antioxidant compounds can decrease oxidative stress, minimizing the incidence of these diseases. Fruits supply several antioxidant compounds, as for example vitamin C, carotenes, and/or polyphenols. On the other hand, some compounds present in fruits have themselves been identified as being mutagenic. This chapter reviews the major compounds and their corresponding biological activities of 23 fruits commonly consumed in the world.

Keywords: fruits, main compounds, biological activities.

In the last years, there has been a growing interest in nutraceuticals and functional foods. Plants, including food plants (fruits and vegetables), synthesize a vast array of secondary chemical compounds that, although not involved in primary metabolism, are important for a variety of ecologic functions that enhance the plant's ability to survive. Interestingly, these compounds may be responsible for the multitude of beneficial effects that have been reported for fruits with an array of health-related bioactivities (Joseph *et al*., 2005). Many studies (Joseph *et al*., 1999; Joseph *et al*., 1998; Prior *et al*., 1998; Cao *et al*., 1996; Wang *et al*., 1996) have suggested that the most important benefits of such compounds may be derived from their antioxidant, antimutagenic, anticarcinogenic, and anti-inflammatory properties.

Fruits present a large spectrum of constituents. Besides carbohydrates, lipids, and proteins (for review see Spada *et al*., 2008), carotenoids, vitamins and polyphenols are the most widely and best-studied compounds of fruits (Table 1).

Many fruits present high levels of carotenoids, for example acerola, mango, papaya and Surinam cherry (Table 1). About fifty to sixty different carotenoids are typically present in the human diet, and the most abundant forms found in plasma are β-carotene (precursor of vitamin A), lycopene, lutein, β-cryptoxanthin and zeaxanthin (Halsted, 2003). The biological effects of carotenoids are related to their antioxidant properties (Faulks and Southon, 2001), which can prevent the appearance of serious diseases such as cancer, pulmonary disorders, cataract (Tapiero, 2004) and atherogenesis (Faulks and Southon, 2001; Voutilainen *et al*., 2006).

Vitamins, mainly C and E, can also be found in fruits (Table 1). Vitamin C or ascorbic acid is ubiquitous in fruits. This compound is an important antioxidant (Fenech and Ferguson, 2001), antimutagenic (Kojima *et al*., 1992; Guha and Khuda-Bukhsh, 2002) and a regulator of DNA-repair enzymes (Cooke *et al*., 1998; Lunec *et al*., 2002). It is also involved in wound healing, tyrosine metabolism, conversion of folic acid to folinic acid, carbohydrate metabolism, synthesis of lipids and protein, iron metabolism, and resistance to infections (Suntornsuk *et al.*, 2002, Saffi *et al.*, 2006).

Vitamin E can be found in cashew apple, mango, red grapes, and peaches (Table 1). This vitamin is able to donate its hydrogen to free radicals, thereby forming a stable species (Rimbach *et al.*, 2002). Vitamin E radical can be regenerated by ascorbate, resulting in the formation of an ascorbyl radical (Rimbach *et al.*, 2002). There is epidemiologic and clinical evidence that high intake of vitamin E may be associated with a decreased risk of coronary heart disease (Diaz *et al.*, 1997; Kohlmeier *et al.*, 1997). Chronic oral administration of vitamin E prevented the loss of mitochondrial function and reduced ROS-induced damage in aging mice (Navarro *et al.*, 2005). These beneficial effects were paralleled by an increased lifespan, better neurological performance and higher exploratory activity (Panetta *et al.*, 2004).

Phenols (hydroxybenzenes) and especially polyphenols (containing two or more phenol groups) are ubiquitous in plant foods and, apart from known vitamins and minerals, may be one of the most widely marketed groups of dietary supplements. This class of plant metabolites contains more than 8000 known compounds, ranging from simple phenols such as phenol itself through to materials of complex and variable composition such as tannins (Bravo, 1998). Phenolic compounds in fruits (Table 1) include flavonoids (mainly quercetin, hesperidin, anthocyanins, catechins, and kaempherol), phenolic acids (salicylic acid), hydroxycinnamic acids (coumaric and caffeic), and stilbenes (resveratrol).

Much of the literature on polyphenolic compounds concerned about the deleterious effects associated with the ability of certain phenols to bind and precipitate macromolecules including protein and carbohydrates, thereby reducing the digestibility of foods (Singleton and Rossi, 1983). More recently, interest has been rekindled in the recognition that many polyphenols, although non-nutrients, show antibacterial effects

(Avorn, 1994), ability to reduce blood pressure (Lampe, 1999), antioxidant, anti-inflammatory, antimutagenic and/or anticarcinogenic effects, at least in *in vitro* systems (Saiko *et al*., 2008; Rodrigues *et al*., 2006; Sairam *et al.,*2003; Miyazawa *et al.,*1999; Bravo, 1998). A prospective study of 800 elderly men showed that the ingestion of flavonoids, mainly in tea, onions, and apples, was associated with significant reduction in mortality from coronary heart disease (Hertog *et al*., 1993). In addition, polyphenols can also inhibit platelet aggregation and vascular relaxation through the production of nitric oxide (Dubick *et al*., 2001).

Almost all the fruits present in this review show antioxidant activity (Table 2), which can be associated with the presence of carotenoids, vitamins, and mainly, polyphenols. The mechanisms of the antioxidant action of polyphenols are complex and they are still been studied. In a general way, they can avoid reactive species formation either by inhibition of enzymes or by chelation of trace elements involved in free radical production, scavenging reactive species, and up-regulating or protecting antioxidant defense (Halliwell and Gutteridge, 1999). Some compounds can also act in a similar way to the enzymatic defenses, since they are able to neutralize reactive species such as superoxide anion and hydrogen peroxide (Silalahi, 2002).

Many fruits (Table 2) can also present antimutagenic activity. There are a number of different mechanisms, which have been implicated in the antimutagenic effects of polyphenols. Some of these are non-specific as for example, polyphenols can exert an antioxidant action (Hartman and Shankel, 1990; Hoensch and Kirch, 2005; Anisimov *et al*., 2006; Valcheva-Kuzmanova and Belcheva, 2006, Srinivasan, 2007) or inhibit the uptake of mutagens such as benzo[a]pyrene (Hatch *et al*., 2000). Different polyphenols may act to upregulate the activity of glutathione S-transferase and/or may directly interfere with DNA adduct formation (Ferguson, 2001).

Although many polyphenols can present antimutagenic effects, some of them can act as a weak mutagenic agent (Ferguson, 2001). The exact reason why a polyphenol can be a mutagenic or an antimutagenic compound is not known, but structure-activity relationships among the flavonoids suggested that bacterial mutagenicity required a double bond between positions 2 and 3 and a hydroxyl group at position 3 (Nagao *et al.*, 1981). It is also known that a number of polyphenols, including quercetin, can bind to DNA (Alvi *et al.*, 1986) and this direct interaction may be an important mechanism of bacterial mutagenicity. Interestingly, some fruits (cashew apple, coconut and kiwi fruit) can present both mutagenic and antimutagenic activities (Table 2). It is known that high concentrations of ascorbic acid (Franke *et al.*, 2005) and some kinds of polyphenols can induce mutagenic effects (De Flora, 1998; De Flora *et al.*, 2001) depending on factors such as pH and the presence of Cu(II) and Fe(III) in the media (Wang *et al.*, 1996; Ferguson, 2001; De Flora, 1998).

Intensive research conducted over the last few years has shown that polyphenols, carotenoids, and vitamins derived from fruits interfere with tumor progression by acting directly on tumor cells as well as by modifying the tumor's microenvironment (stroma) and creating physiological conditions that are hostile to tumor growth (Béliveau and Gingras, 2007). Anticarcinogenic activity can also be related to the antioxidant effect (Béliveau and Gingras, 2007). Some fruits, like coconut, kiwi fruit, lemon, mango, and red grape can present anticarcinogenic activities *in vivo* assays (Table 2).

Various plant polyphenols have profound effects on the function of immune and inflammatory cells (Middleton Jr. *et al.*, 1992). Polyphenols present in green tea (mainly epigallocatechin gallate) can inhibit the inducible nitric oxide (NO) synthase and block NO-associated DNA damage (Bartsch *et al.*, 1996). Acai, black mulberry, mango, and raspberry have shown important anti-inflammatory effects (Table 2).

Plants have developed sophisticated active defense systems against pathogens, among them the production of antibiotic compounds. Centuries of folk wisdom have identified certain fruits or vegetables as having antibacterial potential (Lampe, 1999). Cashew apple, red grape, red guava, lemon, mango and papaya present antibacterial and/or antifungal actions (Table 2) acting against *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneumonia* (Sairam *et al*., 2003; Osato *et al*., 1993). The microbial activity of fruits is related to the presence of different types of polyphenols, mainly procyanidins (Taguri *et al*., 2004).

Briefly, this review compiles data from some studies about biological activity and the main secondary compounds of fruits, reinforcing the idea that a diet rich in fruits could be used to prevent many kinds of pathologies, providing a genuine beneficial effect on human populations.

Acknowledgements

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Table 1. Compounds with potential biological activity in different fruits

Fruit	Compounds with potential biological activities	References
Acai (*Euterpe oleracea* L.)	Vitamin C[1], carotenoids[1], polyphenols (cyanidin[2,4,5], procyanidin[2,3], peonidin[2], pelargonidin[2], catechin[2], epicatechin[2,3], resveratrol[2], protocatechuic acid[3])	[1]Spada *et al.*, 2008; [2]Rocha *et al.*, 2007; [3]Rodrigues *et al.*, 2006; [4]Lichtenthaler *et al.*, 2005; [5]Del Pozo-Insfran *et al.*, 2004.
Acerola (*Malpighia glabra* L.)	Vitamin C[1], carotenoids[1], polyphenols[1,2]	[1]Spada *et al.*, 2008; [2]Mezadri *et al.,*2006.
Apple (*Malus domestica* B.)	Vitamin C[1,5], polyphenols (procyanidins[2,3], anthocyanins[3], catechin[3,4], epicatechin[4], quercetin[5])	[1]Spada *et al.*, 2008; [2]Kahle *et al.*, 2005; [3]Vrhovsek *et al.*, 2004; [4]Sanoner *et al.*, 1999; [5]Ballot *et al.*, 1987.
Black mulberry (*Morus nigra* M.)	Vitamin C[1], carotenoids[1], polyphenols (coumaric acid[2], salicylic acid[2], caffeic acids[2])	[1]Spada *et al.*, 2008; [2]Zadernowski *et al.*, 2005.
Cashew apple (*Anacardium occidentale* L.)	Vitamin C[1], vitamin E[2], carotenoids[1], polyphenols[3] (quercetin, procyanidin, anacardic acid)	[1]Spada *et al.*, 2008; [2]Ryan *et al.,*2006; [3]Melo Cavalcante *et al.*, 2003.
Coconut (*Cocos nucifera* L.)	Vitamin C [1,3], polyphenols (catechin[4], phenolic acids[2])	[1]Spada *et al.*, 2008; [2]Dey *et al.*, 2005; [3]Mantena *et al.*, 2003; [4]Kirszberg *et al.*, 2003.
Cupuacu (*Theobroma grandiflorum* W.)	Vitamin C[1], polyphenols (catechin[2], epicatechin[2], quercetin[2], kaempferol[2])	[1]Spada *et al.*, 2008; Yang *et al.*, 2003.
Kiwi fruit (*Actinidia chinensis* P.)	Vitamin C[1,3], carotenoids[1], polyphenols[1,2]	[1]Spada *et al.*, 2008; [2]Chang and Case, 2005; [3]Kvesitatdze *et al.*, 2001.
Lemon (*Citrus limon* B.)	Vitamin C[1,2], polyphenols (eriocitrin[2,3], hesperidin[2,3], diosmetin[2,3], diosmin[2,3], diosmetin[2,3,4], quercetin[4], apigenin[4], hesperetin[4], homoeriodictyol[4])	[1]Spada *et al.*, 2008; [2]González-Molina *et al.*, 2008; [3]Miyake *et al.*, 2007; [4]Gil-Izquierdo *et al.*, 2004.
Mango *Mangifera indica* L.	Vitamin C[1,2,10], vitamin E[3], carotenoids[1,3,4,5,8]; polyphenols (quercetin[4,8], rhamnetin[5,8], gallotannins[6], flavonols[8], kaempferol[8], xanthone[8], isomangiferin[8], galloyl derivatives[8], mangiferin[8,9], catechin[9], epicatechin[9], tannic acid[7], caffeic acid[7], gallic acid[9], benzoic acid[9])	[1]Spada *et al.*, 2008; [2]Ribeiro *et al.*, 2007; [3]Ornelas-Paz Jde *et al.*, 2007; [4]Berardini *et al.*, 2005; [5]Chen *et al.*, 2004; [6]Berardini *et al.*, 2004; [7]Singh *et al.,* 2004; [8]Schieber *et al.*, 2003; [9]Nunez Selles *et al.*, 2002; [10]Ballot *et al.*, 1987.
Melon (*Cucumis melo* L.)	Vitamin C[1,3,4], carotenoids[1,2], polyphenols[1]	[1]Spada *et al.*, 2008; [2]Portnoy *et al.*, 2008; [3]Lester, 2008; [4]Gil *et al.*, 2006.
Orange (*Citrus aurantium* L.)	Vitamin C[1], carotenoids[1], polyphenols[2] (neoeriocitrin, narirutin, naringin, hesperidin, neohesperidin, naringenin, hesperetin)	[1]Spada *et al.*, 2008; [2]Pellati *et al.*, 2004.

Papaya (*Carica papaya* L.)	Vitamin C[1,4], carotenoids[1-3], polyphenols[1]	[1]Spada *et al.*, 2008; [2]Mutsuga *et al.*, 2001; [3]Cano *et al.*, 1996; [4]Osato *et al.*, 1993.
Passion Fruit (*Passiflora alata* L.)	Vitamin C[1], carotenoids[1,2]	[1]Spada *et al.*, 2008; [2]Mourvaki *et al.*, 2005.
Peach (*Prunus persica* L.)	Vitamin C[1,3,4,5], vitamin E[3], carotenoids[1], polyphenols (catechin[2], epicatechin[2], quercetin[2], eriodictyol[2], naringenin[2], kaempferol[2], isorhamnetin[2], protocatechuic acid[2], vanillic acid[2], coumaric acid[2])	[1]Spada *et al.*, 2008; [2]Wijeratne *et al.*, 2006; [3]Carbonaro *et al.*, 2002; [4]Gil *et al.*, 2002; [5]Ballot *et al.*, 1987.
Pineapple (*Ananas ssp*)	Vitamin C[1], carotenoids[1,3], polyphenols[1,2]	[1]Spada *et al.*, 2008; [2]Wen *et al.*, 1999; [3]Ballot *et al.*, 1987.
Raspberry (*Rubus idaeus* L.)	Vitamin C[1], carotenoids[1], polyphenols (anthocyanins[2-4], procyanidins[3], ellagitannins[3,4], kaempferol[4], quercetin[4], ellagic acid[4])	[1]Spada *et al.*, 2008; [2]Fang Chen *et al.*, 2007; [3]Beekwilder *et al.*, 2005; [4]Mullen *et al.*, 2002.
Red grape (*Vitis vinifera* L.)	Vitamin C[1], vitamin E([2)3], tocotrienol[2)3], carotenoids[1], polyphenols (malvidin[2], quercetin[4,5,6], catechin [4,5,6], epicatechin[4,5,6], resveratrol[2,4,5,6], delphinidin[2,5], cyanidin [2,5], petunidin[4], peonidin[2,5], malvidin[5], procyanidin[5], epicatechin gallate[5], *trans*-polydatin[5], isorhamnetin[5], kaempferol[5], gallic acid[5], protocatechuic acid[5], caftaric acid[5], *p*-hydroxybenzoic acid[5], caffeic acid[5], *p*-coumaric acid[5], ferulic acid[5], ellagic acid[6])	[1]Spada *et al.*, 2008; [2]Dani *et al.*, 2008; [3]Horvatha *et al.*, 2006, [4]Chafer *et al.*, 2005; [5]Kammerer *et al.*, 2004; [6]Yilmaz and Toledo, 2004.
Red guava (*Psidium guajava* L.)	Vitamin C[1,5], carotenoids[1,4], polyphenols (guajadial[2], quercetin[3], myricetin[3], kaempferol[3], apigenin[3])	[1]Spada *et al.*, 2008; [2]Carasek *et al.*, 2006; [3]Miean *et al.*, 2001; [4]Mercadante *et al.*, 1999; [5]Ballot *et al.*, 1987.
Soursop (*Annona muricata* L.)	Polyphenols[1,2]	[1]Spada *et al.*, 2008; [2]Augusto *et al.*, 2000.
Strawberry (*Fragaria vesca* L.)	Vitamin C[1,3], carotenoids[1,2], polyphenols[1,2]	[1]Spada *et al.*, 2008; [2]Kiselova *et al.*, 2006; [3]Ballot *et al.*, 1987.
Surinam cherry (*Eugenia uniflora* O.)	Polyphenols[1,2]	[1]Spada *et al.*, 2008; [2]Almeida *et al.*, 1995.
Tangerine (*Citrus reticulata* L.)	Vitamin C[1], carotenoids[1,2,4], polyphenols[1,3]	[1]Spada *et al.*, 2008; [2]Ricón *et al.*, 2005; [3]Gil-Izquierdo *et al.*, 2004; [4]Nogata *et al.*, 2003.

Table 2. Biological activities of fruits

Fruits	Biological activities	References
Acai	Antioxidant activity[1-5] Vasodilatory activity[2] Anti-inflammatory effect[3] Mutagenic activity[1]	[1]Spada *et al.*, 2008; [2]Rocha *et al.*, 2007; [3]Rodrigues *et al.*, 2006; [4]Schauss *et al.*, 2006; [5]Lichtenthaler *et al.,* 2005.
Acerola	Antioxidant activity[1,2]	[1]Spada *et al.*, 2008, [2]Hanamura *et al.*, 2005.
Apple	Antioxidant activity[1,2]	[1]Spada *et al.*, 2008, [2]Leu *et al.*, 2006
Black mulberry	Antioxidant activity[1,2] Anti-inflammatory effect[2]	[1]Spada *et al.,* 2008, [2]Kim and Park, 2006.
Cashew apple	Antioxidant activity [1-3] Mutagenic/comutagenic activities [1,4,5] Antibacterial activity[2] Antimutagenic activity [4,5]	[1]Spada *et al.*, 2008, [2]Green *et al.*, 2007; [3]Konan *et al.*, 2006; [4]Melo Cavalcante *et al.*, 2003; [5]Trevisan *et al.*, 2006
Coconut	Antioxidant activity[1,5,6] Mutagenic activity[2-4] Antimutagenic activity[3,5] Anticarcinogenic activity[5]	[1]Spada *et al.*, 2008, [2]Sandhya and Rajamohan, 2006; [3]Petta *et al.*, 2004; [4]Narasimhamurthy *et al.*, 1999; [5]Nalini *et al.*, 1997; [6]Bell and Kamens, 1990.
Cupuacu	Antioxidant activity[1,2] Antimutagenicity[1]	[1]Spada *et al.*, 2008; [2]Yang *et al.*, 2003.
Kiwi fruit	Comutagenic activity; low antimutagenic and mutagenic effects[1-3] Anticarcinogenic activity[4]	[1]Spada *et al.*, 2008; [2]Deters *et al.*, 2005; [3]Tang and Edenharder, 1997; [4]Edenharder *et al.*, 1994.
Lemon	Antifungal activity[1] Antimutagenic activity[2-4] Anticarcinogenic activity[5]	[1]Ben-Yehoshua *et al.*, 2008; [2]Spada *et al.*, 2008; [3]Higashimoto *et al.*, 1998; [4]Bala and Grover, 1989; [5]National Toxicology Program, 1990.
Mango	Antioxidant activity[1,3-5] Anti-inflammatory activity[2] Anticarcinogenic activity[4] Antimutagenic activity[1,6] Antidiarrhoeal activity[7] Antibacterial activity[7]	[1]Spada *et al.*, 2008; [2]Knödler *et al.,* 2007; [3]Mahattanatawee *et al.,* 2006; [4]Rodriguez *et al.,* 2006; [5]Percival *et al.,* 2006; [6]Pardo-Andreu *et al.,* 2006; [7]Sairam *et al.,* 2003
Melon	Antioxidant activity[1-4]	[1]Spada *et al.*, 2008; [2]Lester, 2008; [3]Vouldoukis *et al.,* 2004; [4]Lester *et al.*, 2004.
Orange	Antioxidant activity[1,3,5,6] Adrenergic activity[2] Antigenotoxic activity[4] Antimutagenic activity[1,7]	[1]Spada *et al.*, 2008; [2]Nelson *et al.*, 2007; [3]Jayaprakasha *et al.*, 2007 ; [4]Franke *et al.*, 2006; [5]Deyhim *et al.*, 2006 ; [6]Hosseinimehr and Karami, 2005 ; [7]Miyazawa *et al.,*1999;
Papaya	Antioxidant activity[1,2,4,5,8,14] Antibacterial activity[3,11,14] Inhibitory effect on sperm motility[6] Antifertility activity[7,9,10,12,15] Androgenic activity[13]	[1]Spada *et al.*, 2008; [2]Lohiya *et al.*, 2008; [3]Nayak *et al.*, 2007 ; [4]Gambera *et al.*, 2007 ; [5]Mehdipour *et al.*, 2006 ; [6]Rahmat *et al.*, 2004 ; [7]Lohiya *et al.*, 1999 ; [8]Imao *et al.*, 1998; [9]Lohiya *et al.*, 1994 ; [10]Chinoy *et al.*, 1994, [11]Osato *et al.*, 1993 ; [12]Lohiya and Goyal., 1992; [13]Chinoy and Ranga Geetha, 1984 ; [14]Emeruwa, 1982 ; [15]Gopalakrishnan and Rajasekharasetty, 1978.
Passion Fruit	Antioxidant activity[1,2]	[1]Spada *et al.*, 2008; [2]Nassiri-Asl *et al.*, 2007.

	Anticonvulsant effect[2]	
Peach	Antioxidant and antimutagenic activities[1]	[1]Spada *et al.*, 2008
Pineapple	Antioxidant activity[1-3] Antifertility activity[4]	[1]Spada *et al.*, 2008; [2]Herraiz and Galisteo, 2003; [3]Sun *et al.*, 2002 ; [4]Garg *et al.*, 1970
Raspberry	Antioxidant activity[1,2-6] Antimutagenic activity[1] Vasodilatory activity[3]	[1]Spada *et al.*, 2008; [2]Viljanen *et al.*, 2004, [3]Wada and Ou, 2002; [4]Mullen *et al.*, 2002; [5]Wang and Jiao, 2000; [6]Kalt *et al.*, 1999.
Red grape	Antioxidant activity[1,2,4,6,8,9,13,14] Antimutagenic activity[1] Antibacterial activity[3] Anticarcinogenic activity[5,9] Antiarrhythmic and cytoprotective effects[7] Protective effects against ischemia-reperfusion[10,15] Radioprotective effects[14] Antiexudative and capillaritonic effects[16] Vasodilatory activity[11,12]	[1]Spada *et al.*, 2008; [2]Kedage *et al.*, 2007; [3]Thimothe *et al.*, 2007; [4]El-Ashmawy *et al.*, 2007; [5]Lala *et al.*, 2006 ; [6]Devi *et al.*, 2006 ; [7]Al-Makdessi *et al.*, 2006 ; [8]Janisch *et al.*, 2006; [9]Stagos *et al.*, 2005; [10]Nakagawa *et al.*, 2005; [11]Madeira *et al.*, 2005; [12]Soares *et al.*, 2004, [13]Shafiee *et al.*, 2003; [14]Castillo *et al.*, 2000; [15]Maffei Facinó *et al.*, 1996; [16]Zafirov *et al.*, 1990.
Red guava	Antioxidant activity[1,5] Antibacterial activity[2,4] Antimutagenic effect[1,6] Hypoglycemic effects[3,7]	[1]Spada *et al.*, 2008; [2]Pelegrini *et al.*, 2008; [3]Rai *et al.*, 2007; [4]Abdelrahim *et al.*, 2002; [5]Jime'nez-Escrig *et al.*, 2001; [6]Grover and Bala, 1993; [7]Cheng and Yang, 1983.
Strawberry	Antioxidant activity[1-4] Mutagenic activity[1]	[1]Spada *et al.*, 2008; [2]Kiselova *et al.,* 2006; [3]Rababah *et al.*, 2005; [4]Kahkonen *et al.*, 2001.
Soursop	Antioxidant and antimutagenic activities[1]	[1]Spada *et al.*, 2008.
Tangerine	Antioxidant and antimutagenic activities[1]	[1]Spada *et al.*, 2008.

References

Abdelrahim, S. I., Almagboul, A. Z., Omer, M. E. and Elegami, A. (2002). Antimicrobial activity of Psidium guajava L. *Fitoterapia.* **73(7-8)**:713-715.

Almeida, C. E., Karnikowski, M. G., Foleto, R. and Baldisserotto, B. (1995). Analysis of antidiarrhoeic effect of plants used in popular medicine. *Rev Saude Publica.* **29(6)**:428-433.

Alvi, N. K., Rizvi, R. Y. and Hadi, S. M. (1986). Interaction of quercetina with DNA. *Biosci. Rep.* **6:**861-868.

Anisimov, V. N., Popovich, I. G., Zabezhinski, M. A., Anisimov, S. V., Vesnushkin, G. M. nd Vinogradova, I. A. (2006). Melatonin as antioxidant, geroprotector and anticarcinogen. *Biochim Biophys Acta.* **1757(5-6):**573-589.

Augusto, F., Valente, A. L., dos Santos Tada, E. and Rivellino, S. R. (2000). Screening of Brazilian fruit aromas using solid-phase microextraction-gas chromatography-mass spectrometry. *J Chromatogr A*. 2000. **873(1)**:117-127.

Avorn, J., Monane, M., Gurwitz, G. R., Choodnovsky, I. and Lipsitz, L. (1994). Reduction of bacteriuria and pyruria after ingestion of cranberry juice. *JAMA*. **271:**751–154.

Bala, S. and Grover, I. S. (1989). Antimutagenicity of some citrus fruits in Salmonella typhimurium. *Mutat Res*. **222(3)**:141-148.

Ballot, D., Baynes, R. D., Bothwell, T. H., Gillooly, M., MacFarlane, B. J., MacPhail, A. P., Lyons, G., Derman, D. P., Bezwoda, W. R. and Torrance, J. D. (1987). The effects of fruit juices and fruits on the absorption of iron from a rice meal. *Br J Nutr.* **57(3):**331-343.

Bartsch, H. and Frank, N. (1996). Blocking the endogenous formation of *N*-nitroso compounds and related carcinogens. *IARC Scientific Publications*. **139:**189-201.

Beekwilder, J., Jonker, H., Meesters, P., Hall, R. D., Van der Meer, I. M. and Ric de Vos, C. H. (2005). Antioxidants in raspberry: on-line analysis links antioxidant activity to a diversity of individual metabolites. *J Agric Food Chem.* **53(9):**3313-3320.

Béliveau, R. and Gingras, D. (2007). Role of nutrition in preventing cancer. *Can Fam Physician*. **53:**1905-1911.

Bell, D. A. and Kamens, R. M. (1990). Evaluation of the mutagenicity of combustion particles from several common biomass fuels in the Ames/Salmonella microsome test. *Mutat Res*. **245(3)**:177-183.

Ben-Yehoshua, S., Rodov, V., Nafussi, B., Feng, X., Yen, J., Koltai, T. and Nelkenbaum, U. (2008). Involvement of limonene hydroperoxides formed after oil gland injury in the induction of defense response against *Penicillium digitatum* in lemon fruit. *J Agric Food Chem*. **56(6):**1889-1895.

Berardini, N, Carle, R. and Schieber, A. (2004). Characterization of gallotannins and benzophenone derivatives from mango (Mangifera indica L. cv. 'Tommy Atkins') peels, pulp and kernels by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom.* **18(19):**2208-2216.

Berardini, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R. and Schieber, A. (2005). Screening of mango (Mangifera indica L.) cultivars for their contents of flavonol O- and xanthone C-glycosides, anthocyanins, and pectin. *J Agric Food Chem.* **53(5):**1563-1570.

Bravo, L. (1998) Polyphenols: chemistry, dietary sources, metabolism and nutritional significance, *Nutr. Rev*. **56**:317–333.

Cano, M. P., Ancos, B., Lobo, G. and Monreal, M. (1996). Effects of freezing and canning of papaya slices on their carotenoid composition. *Z Lebensm Unters Forsch.* **202(4):**279-284.

Cao, G., Sofic, E. and Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *J Agric Food Chem.* **44:**3426-3431.

Carasek, E. and Pawliszyn, J. (2006). Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber. *J. Agric. Food Chem.* **54**:8688-8696.

Carbonaro, M., Mattera, M., Nicoli, S., Bergamo, P. and Cappelloni, M. (2002). Modulation of antioxidant compounds in organic vs conventional fruit (Peach, *Prunus persica* L., and Pear, *Pyrus communis* L.). *J. Agric. Food Chem.* **50**:5458-5462.

Castillo, J., Benavente-García, O., Lorente, J., Alcaraz, M., Redondo, A., Ortuño, A. and Del Rio, J. A. (2000). Antioxidant activity and radioprotective effects against chromosomal damage induced in vivo by X-rays of flavan-3-ols (Procyanidins) from grape seeds (Vitis vinifera): comparative study versus other phenolic and organic compounds. *J Agric Food Chem.* **48(5):**1738-1745.

Chafer, A., Pascual-Martí, M. C., Salvador, A. and Berna, A. (2005). Supercritical fluid extraction and HPLC determination of relevant polyphenolic compounds in grape skin. *J Sep Sci.* **28(16)**:2050-2056.

Chang, J. and Case, R. (2005) Cytotoxic phenolic constituents from the root of Actinidia chinensis.*Planta Med.* **71(10)**:955-999

Chen, J. P., Tai, C. Y. and Chen, B. H. (2004). Improved liquid chromatographic method for determination of carotenoids in Taiwanese mango (Mangifera indica L.). *J Chromatogra.* **1054(1-2):**261-268.

Cheng, J. T. and Yang, R. S. (1983). Hypoglycemic effect of guava juice in mice and human subjects. *Am J Chin Med.* **11(1-4):**74-76.

Chinoy, N. J., D'Souza, J. M. and Padman, P. (1994). Effects of crude aqueous extract of Carica papaya seeds in male albino mice. *Reprod Toxicol.* **8(1)**:75-79.

Chinoy, N. J. and Ranga Geetha, M. (1984). Effects of *Carica papaya* seed extracts on the physiology of the vas deferens of albino rats. *Acta Eur Fertil.* **15(1):**59-65.

Cooke, M. S., Evans, M. D., Podmore, I. D., Herbert, K. E., Mistry, N., Mistry, P., Hickenbothanm, P. T., Hussieni, A. and Griffiths, H. R., Lunec, J. (1998). Novel repair action of vitamin C upon in vivo oxidative DNA damage. *FEBS Left.* **439:**363-367.

Dani, C., Oliboni, L. S., Pasquali, M. A. B., Oliveira, M. R., Umezu, F. M., Salvador, M., Moreira and J. C. F., Henriques, J. A. P. (2008) Intake of purple grape juice as a hepatoprotective agent in wistar rats. J. Med. Food .**11 (1):** 127–132.

De Flora, S. (1998) Mechanisms of inhibition of mutagenesis and carcinogenesis. *Mutat Res.***402**:151–158.

De Flora, S., Izzottti, A., D'Agostini, F., Balansky, R. M., Noonan, D. and Albini, A. (2001) Multiple points of intervention in the prevention of cancer and other mutation-related diseases. *Mutat Res.* **480-481**:9–22.

Del Pozo-Insfran, D., Brenes, C. H. and Talcott, S. T. (2004). Phytochemical composition and pigment stability of Acai (Euterpe oleracea Mart.). *J Agric Food Chem.* **52(6)**:1539-1545.

Deters, A. M., Schroder, K. R. and Hensel, A. (2005). Kiwi fruit (Actinidia chinensis L.) polysaccharides exert stimulating effects on cell proliferation via enhanced growth factor receptors, energy production, and collagen synthesis of human keratinocytes, fibroblasts, and skin equivalents. *J Cell Physiol.* **202(3)**:717-722.

Devi, S. A., Jolitha, A. B. and Ishii, N. (2006). Grape Seed Proanthocyanidin Extract (GSPE) and antioxidant defense in the brain of adult rats. *Med Sci Monit.* **12(4):** 124-129.

Dey, G., Chakraborty M. and Mitra, A. (2005). Profiling C6-C3 and C6-C1 phenolic metabolites in Cocos nucifera. *J Plant Physiol.* **162(4)**:375-381.

Deyhim, F., Lopez, E., Gonzalez, J., Garcia, M. and Patil, B. S. (2006). Citrus juice modulates antioxidant enzymes and lipid profiles in orchidectomized rats. J. Med. Food. **9(3)**:422-426.

Diaz, M. N., Frei, B., Vita, J. A. and Keaney, J. F. (1997). Antioxidants and atherosclerotic heart disease. *N Engl J Med.* **337:**408-416.

Dubick, M.and Omaye, S. T. (2001) Modification of atherogenesis and heart disease by grape wine and tea polyphenols. In: Wildman REC, ed. Handbook of nutraceuticals and functional foods. Boca Raton, FL: CRC Press. 235–260.

Edenharder, R., Kurz, P., John, K., Burgard, S. and Seeger, K. (1994). In vitro effect of vegetable and fruit juices on the mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline. *Food Chem Toxicol.* **32(5)**:443-459.

El-Ashmawy, I. M., Saleh, A. and Salama, O. M. (2007). Effects of marjoram volatile oil and grape seed extract on ethanol toxicity in male rats. *Basic Clin Pharmacol Toxicol.* **101(5):**320-327.

Emeruwa, A. C. (1982). Antibacterial substance from Carica papaya fruit extract. *J Nat Prod.* **45(2):**123-127.

Fang, C., Yangzhao, S., Guanghua, Z., Xiaojun, L., Xiaosong, H., Jihong, W. and Zhengfu, W. (2007). Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography–mass spectrometry. *Ultrasonics Sonochemistry*. **14**:767–778.

Faulks, R. M. and Southon, S. Carotenoids, metabolism and disease. In: Wildman REC, ed. Handbook of nutraceuticals and functional foods. Boca Raton, FL: *CRC Press*, 2001:143–156.

Fenech, M. and Ferguson, L. R. (2001). Vitamins/minerals and genomic stability in humans. *Mutat. Res.* **475:**1-6.

Ferguson, L. R. (2001). Role of plant polyphenols in genomic stability. *Mutat Res.* **475(1-2)**:89-111.

Franke, S. I. R., Prá, D., Silva, J., Erdtmann, B. and Henriques, J. A. P. (2005) Possible repair action of vitamin C on DNA damage induced by methyl methanesulfonate, cyclophosphamide, FeSO4 and CuSO4 in mouse blood cells in vivo. *Mutat Res* **583**:75–84.

Franke, S. I., Pra, D., Giulian, R., Dias, J. F., Yoneama, M. L., Silva, J., Erdtmann, B. and Henriques, J. A. P. (2006). Influence of orange juice in the levels and in the genotoxicity of iron and copper. *Food Chem Toxicol.* **44(3):**425-435.

Gambera, L., Campanella, G., Piomboni, P., Serafini, F., Morgante, G. and De Leo, V. (2007). Association of antioxidants and natural immune activators in the treatment of astheno-teratospermia and abacterial leukocytosis. *Minerva*

Ginecol. **59(5):**473-479.

Garg, S. K., Saksena, S. K. and Chaudhury, R. R. (1970). Antifertility screening of plants. VI. Effect of five indigenous plants on early pregnancy in albino rats. *Indian J Med Res.* **58(9):**1285-1289.

Gil, M. I., Aguayo, E. and Kader, A. A. (2006). Quality Changes and Nutrient Retention in Fresh-Cut versus whole fruits during Storage. *J. Agric. Food Chem.* **54**:4284-4296.

Gil, M. I., Tomaä, F. A., S-Barberaä, N., Hess-Pierce, B. and Kader, A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin c contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem.* **50**:4976-4982.

Gil-Izquierdo, A., Riquelme, M. T., Porras, I. and Ferreres, F. (2004). Effect of the rootstock and interstock grafted in lemon tree (Citrus limon (L.) Burm.) on the flavonoid content of lemon juice. *J Agric Food Chem.* **52(2)**:324-331.

González-Molina, E., Moreno, D. A. and García-Viguera, C. (2008). Genotype and Harvest Time Influence the Phytochemical Quality of Fino Lemon Juice (*Citrus limon* (L.) Burm. F.) for Industrial Use. *J. Agric. Food Chem.* **56(5)**:1669-1675.

Gopalakrishnan, M. and Rajasekharasetty, M. R. (1978). Effect of papaya (Carica papaya linn) on pregnancy and estrous cycle in albino rats of Wistar strain. *Indian J Physiol Pharmacol.* **22(1):**66-70.

Green, I. R., Tocoli, F. E., Lee, S. H., Nihei, K. I. and Kubo, I. (2007). Design and evaluation of anacardic acid derivatives as anticavity agents. *Eur J Med Chem.* Epub ahead of print.

Grover, I. S. and Bala, S. (1993). Studies on antimutagenic effects of guava (*Psidium guajava*) in Salmonella typhimurium. *Mutat Res.* **300(1)**:1-3.

Guha, B. and Khuda-Bukshs, A. R. (2002). Efficacy of vitamin-C (l-ascorbic acid) in reducing genotoxicity in fish (Oerochromis mossambicus) induced by ethyl methane sulphonate. *Chemosphere*. **47:**49-56.

Halliwell, B. and Gutteridge, J. M. C. (1999). Free Radicals in Biology and Medicine, 3rd ed. Clarendon Press, Oxford. 936p.

Halsted. C. H. (2003) Dietary supplements and functional foods: 2 sides of a coin? *Am J Clin Nutr.* **77**(suppl):1001S–1007S

Hanamura, T., Hagiwara, T. and Hirokazu, K. (2005). Strutural and Functional Characterization of polyphenols isolated from Acerola fruit. *Bios. Biotechnol. Biochem.* **69(2)**:280-286.

Hartman, P. E. and Shankel, D. M. (1990). Antimutagens and anticarcinogens: a survey of putative interceptor molecules, *Environ. Mol. Mutagen*. **16:**136-142.

Hatch, F. T., Lightstone, F. C. and Colvin, M. E. (2000). Quantitative structure-activity relationship of flavonoids for inhibition of heterocyclic amine mutagenicity. *Environ. Mol. Mutagen.* **35:**279-299.

Herraiz, T. and Galisteo, J. (2003). Tetrahydro-beta-carboline alkaloids occur in fruits and fruit juices: activity as antioxidants and radical scavengers. *J Agric Food Chem.* **51(24)**:7156-7161.

Hertog, M. G., Feskens, E. J., Hollman, P. C., Katan, M.B. and Kromhout, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet.* **342**:1007-1011.

Higashimoto, M., Yamato, H., Kinouchi, T. and Ohnishi, Y. (1998). Inhibitory effects of citrus fruits on the mutagenicity of 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid treated with nitrite in the presence of ethanol.

Mutat Res. **415(3)**:219-226.

Hoensch, H. P. and Kirch, W. (2005) Potential role of flavonoids in the prevention of intestinal neoplasia: a review of their mode of action and their clinical perspectives. *Int J Gastrointest Cancer.* **35(3):**187-195.

Horvatha, G., Wessjohannb, L., Bigirimanac, J., Monicac, H., Jansend, M., Guiseza, Y., Caubergsa, R. and Horemans, N. (2006). Accumulation of tocopherols and tocotrienols during seed development of grape (Vitis vinifera L. cv. Albert Lavallée). *Plant Physiology and Biochemistry*. **44**:724–731.

Hosseinimehr, S. J. and Karami, M. (2005). Citrus extract modulates genotoxicity induced by cyclophosphamide in mice bone marrow cells. *J Pharm Pharmacol.* **57(4):**505-509.

Imao, K., Wang, H., Komatsu, M. and Hiramatsu, M. (1998). Free radical scavenging activity of fermented papaya preparation and its effect on lipid peroxide level and superoxide dismutase activity in iron-induced epileptic foci of rats. *Biochem Mol Biol Int.* **45(1):**11-23.

Janisch, K. M., Olschläger, C., Treutter, D. and Elstner, E. F. (2006). Simulated digestion of Vitis vinifera seed powder: polyphenolic content and antioxidant properties. *J Agric Food Chem.* **54(13):**4839-4848.

Jayaprakasha, G. K., Girennavar, B. and Patil, B. S. (2007). Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different in vitro model systems. *Bioresour Technol*. **99(10):**4484-4494.

Jime´nez-Escrig, A., Rinco, M., Pulido, R. and Saura-Calixto, F. (2001). Guava fruit (Psidium guajava L.) as a new source of antioxidant dietary fiber. *J. Agric. Food Chem.* **49**: 5489-5493.

Joseph JA, Shukitt-Hale B. and Denisova NA (1999). Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with blueberry, spinach or strawberry dietary supplementation. *J Neurosci* **19**:8114 –8121

Joseph, J. A., Shukitt-Hale, B. and Casadesus, G. (2005) Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr.* **81**(suppl): 313S–316S.

Joseph, J. A., Shukitt-Hale, B. and Denisova, N. A. (1998). Long-term dietary strawberry, spinach or vitamin E supplementation retards the onset of agerelated neuronal signal-transduction and cognitive behavioral deficits. J Neurosci. **18:**8047-8055.

Kahkonen, M. P., Hopia, A. I. and Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *J Agric Food Chem*. **49(8):**4076-4082.

Kahle, K., Kraus, M. and Richling, E. (2005). Polyphenol profiles of apple juices. *Mol Nutr Food Res.* **49(8)**:797-806.

Kammerer, D., Claus, A., Carle, R. and Schieber, A. (2004). Polyphenol Screening of Pomace from Red and White Grape Varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. . *J. Agric. Food Chem.* **52**:4360-4367.

Kedage, V.V., Tilak, J. C., Dixit, J. B., Devasagayam, T. P. and Mhatre, M. (2007). A study of antioxidant properties of some varieties of grapes (Vitis vinifera L.). *Crit Rev Food Sci Nutr.* **47(2):**175-185.

Kim, A. J. and Park, S. (2006). Mulberry extract supplements ameliorate the inflammation-related hematological parameters in carrageenan-induced arthritic rats. J. Med. Food. 9(3):431-435.

Kirszberg, C., Esquenazi, D., Alviano, C. S. and Rumjanek, V. M. (2003). The effect of a catechin-rich extract of *Cocos nucifera* on lymphocytes proliferation. *Phytother Res.***17(9)**:1054-1058.

Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B. and Yankova, T. (2006). Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from bulgarian herbs. *Phytother Res.* **20(11):**961-965.

Knödler, M., Conrad, J., Wenzig, E. M., Bauer, R., Lacorn, M., Beifuss, U., Carle, R. and Schieber, A. (2008). Anti-inflammatory 5-(11'Z-heptadecenyl)- and 5-(8'Z,11'Z-heptadecadienyl)-resorcinols from mango (*Mangifera indica* L.) peels. *Phytochemistry.* **69(4):**988-993.

Kohlmeier, L., Kark, J. D., Gomez-Garcia, E., Martin, B. C., Steck, S. E., Kardinaal, A. F. M., Ringstad, J., Thamm, M., Masaev, V., Riemersma, R., Martin-Moreno, J. M., Huttunen J. K. and Kok, F. J. (1997). Lycopene and myocardial infarction risk in the EURAMIC Study. *Am J Epidemiol.* **146:**618-626.

Kojima, H., Konishi, H. and Kuroda, Y. (1992). Effects of l-ascorbic acid on the mutagenicity of ethyl methanesulfonate in cultured mammalian cells. *Mutat. Res.* **266:**85-91.

Konan, N. A., Bacchi, E. M., Lincopan, N., Varela, S. D. and Varanda, E. A. (2007). Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardium occidentale* L.). *J Ethnopharmacol.* **110(1):**30-38.

Kvesitatdze, G. I., Kalandiia, A. G., Papunidze, S. G. and Vanidze, M. R. (2001). Use of HPLC for identification and quantitative determination of ascorbic acid in kiwi fruit. *Prikl Biokhim Mikrobiol.* **37(2)**:243-246.

Lala, G., Malik, M., Zhao, C., He, J., Kwon, Y., Giusti, M. M. and Magnuson, B. A. (2006). Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. *Nutr Cancer.* **54(1)**:84-93.

Lampe, J. W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am J Clin Nutr.* **70(suppl):**475S-490S.

Lester, G. E. (2008). Antioxidant, Sugar, Mineral, and Phytonutrient Concentrations across Edible Fruit Tissues of Orange-Fleshed Honeydew Melon (Cucumis melo L.). *J Agric Food Chem.* **56(10)**:3694-3698.

Lester, G. E., Hodges, D. M, Meyer, R. D. and Munro, K. D. (2004). Pre-extraction preparation (fresh, frozen, freeze-dried, or acetone powdered) and long-term storage of fruit and vegetable tissues: effects on antioxidant enzyme activity. *J Agric Food Chem.* **21;52(8)**:2167-2173.

Leu, S. J., Lin, Y. P., Lin, R. D., Wen, C. L., Cheng, K. T., Hsu, F. L. and Lee, M. H. (2006). Phenolic constituents of Malus doumeri var. formosana in the field of skin care. *Biol Pharm Bull.* **29(4)**:740-745.

Lichtenthaler, R., Rodrigues, R. B., Maia, J. G., Papagiannopoulos, M., Fabricius, H. and Marx, F. (2005). Total oxidant scavenging capacities of Euterpe oleracea Mart. (Açaí) fruits. *Int J Food Sci Nutr.***56(1)**:53-64.

Lohiya N. K., Manivannan, B., Goyal S. and Ansari, A. S. (2008). Sperm motility inhibitory effect of the benzene chromatographic fraction of the chloroform extract of the seeds of Carica papaya in langur monkey, Presbytis entellus entellus. *Asian J Androl.* **10(2):**298-306

Lohiya, N. K. and Goyal, R. B. (1992). Antifertility investigations on the crude chloroform extract of *Carica papaya* Linn. seeds in male albino rats. *Indian J Exp Biol.* **30(11):**1051-1055.

Lohiya, N. K., Goyal, R. B., Jayaprakash, D., Ansari, A. S. and Sharma, S. (1994). Antifertility effects of aqueous extract of Carica papaya seeds in male rats.

Planta Med. **60(5):**400-404.

Lohiya, N. K., Pathak, N., Mishra, P. K. and Manivannan, B. (1999). Reversible contraception with chloroform extract of Carica papaya Linn. seeds in male rabbits. *Reprod Toxicol.* **13(1):**59-66.

Lunec, J., Holloway, K. A., Cooke, M. S., Faux, S., Griffiths, H. R. and Evans, M. D. (2002). Urinary 8-oxo-2-deoxyguasinose: redox regulation of DNA repair in vivo? *Free Radic. Biol. Medic.* **33:**875-885.

Madeira, S. V. F., Resende, A. C., Ognibene, D. T., Vieira de Sousa, M. A. and Soares de Moura, R. (2005). Mechanism of the endothelium-dependent vasodilator effect of an alcohol-free extract obtained from a vinifera grape skin. *Pharmacological Research.* **52:**321-327.

Maffei Facinó, R., Carini, M., Aldini, G., Berti, F., Rossoni, G., Bombardelli, E. and Morazzoni, P. (1996). Procyanidines from Vitis vinifera seeds protect rabbit heart from ischemia/reperfusion injury: antioxidant intervention and/or iron and copper sequestering ability. *Planta Med.* **62(6):**495-502.

Mahattanatawee, K., Manthey, J. A., Luzio, G., Talcott, S. T., Goodner, K. and Baldwin, E. A. (2006). Total antioxidant activity and fiber content of select Florida-grown tropical fruits. *J Agric Food Chem.* **54(19):**7355-7363.

Mantena, S. K., Jagadish, Badduri, S. R., Siripurapu, K. B. and Unnikrishnan, M. K. (2003). In vitro evaluation of antioxidant properties of Cocos nucifera Linn. water. *Nahrung.* **47(2)**:126-131.

Mehdipour, S., Yasa, N., Dehghan, G., Khorasani, R., Mohammadirad, A. and Abdollahi, R. M. (2006). Antioxidant potentials of Iranian Carica papaya juice in vitro and in vivo are comparable to alpha-tocopherol. *Phytother Res.* **20(7):**591-594.

Melo Cavalcante A. A., Rubensam, G., Picada, J. N., Gomes da Silva, E., Fonseca Moreira, J. C. and Henriques, J. A. (2003). Mutagenicity, antioxidant potential, and antimutagenic activity against hydrogen peroxide of cashew (Anacardium occidentale) apple juice and cajuina. *Environ Mol Mutagen.* **41(5)**:360-369.

Mercadante, A. Z., Steck, A. and Pfander, H. (1999). Carotenoids from guava (Psidium guajava L.): isolation and structure elucidation. *J Agric Food Chem.* **47(1)**:145-151.

Mezadri, T., Fernandez-Pachon, M. S., Villano, D., Garcia-Parrilla, M. C. and Troncoso, A. M. (2006). The acerola fruit: composition, productive characteristics and economic importance. *Arch Latinoam Nutr.* **56(2)**:101-109.

Middleton Jr., E. and Kandaswami, C. (1992). Effects of flavonoids on immune and inflammatory cell functions. *Biochem. Pharma.* **43:**1167-1179.

Miean, K.H. and Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *J Agric Food Chem.* **49(6):**3106-3112.

Miyake, Y., Mochizuki, M., Okada, M., Hiramitsu, M., Morimitsu, Y. and Osawa, T. (2007). Isolation of antioxidative phenolic glucosides from lemon juice and their suppressive effect on the expression of blood adhesion molecules. *Biosci Biotechnol Biochem.* **71(8)**:1911-1919.

Miyazawa, M., Okuno, Y., Fukuyama, M., Nakamura, S. and Kosaka, H. (1999). Antimutagenic activity of polymethoxyflavonoids from Citrus aurantium. *J Agric Food Chem.* **47(12):**5239-5244.

Mourvaki, E., Gizzi, S., Rossi, R. and Rufini, S. (2005). Passionflower fruit-a "new" source of lycopene? J. Med. Food. **8(2)**:279-284.

Mullen, W., McGinn, J., Lean, M. E., MacLean, M. R., Gardner, P., Duthie, G. G., Yokota, T. and Crozier, A. (2002). Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *J Agric Food Chem.* **50(18):**5191-5196.

Mutsuga, M., Ohta, H., Toyoda, M. M. and Goda, Y. (2001). Comparison of carotenoid components between GM and non-GM papaya. *Shokuhin Eiseigaku Zasshi.* **42(6)**:367-373.

Nagao, M., Morita, N., Yahagi, T.,Shimizu, M., Kuroyanagi, M., Fukuoka, M., Yoshihira, Y., Natori, S., Fujino, T. and Sugimura, T. (1981). Mutagenicities of 61 flavonoids and 11 related compounds. *Environ. Mutagen.* **3:**401-419.

Nakagawa, T., Yokozawa, T., Satoh, A. and Kim, H. Y. (2005). Attenuation of renal ischemia-reperfusion injury by proanthocyanidin-rich extract from grape seeds. *J Nutr Sci Vitaminol (Tokyo).* **51(4):**283-286.

Nakamura, Y., Nakayama, Y., Ando, H., Tanaka, A., Matsuo, T., Okamoto, S., Upham, Chang, B. L., Trosko, C. C., Park, J. E. and Sato, K. (2008). 3-Methylthiopropionic Acid Ethyl Ester, Isolated from Katsura-uri (Japanese pickling melon, Cucumis melo var. conomon), Enhanced Differentiation in Human Colon Cancer Cells. *J Agric Food Chem.* **14;56(9):**2977-2984.

Nalini, N., Sabitha, K., Chitra, S., Viswanathan, P. and Menon, V. P. (1997). Histopathological and lipid changes in experimental colon cancer: effect of coconut kernal (Cocos nucifera Linn.) and (Capsicum annum Linn.) red chilli powder. *Indian J Exp Biol.* **35(9)**:964-971.

Narasimhamurthy, K., Muralidhara, R. and Raina, P. L. (1999). Absence of in vivo mutagenic potency of heated and fried oils in mice. *Indian J Exp Biol.* **37(1)**:50-55.

Nassiri-Asl, M., Shariati-Rad, S. and Zamansoltani, F. (2007). Anticonvulsant effects of aerial parts of *Passiflora incarnata* extract in mice: involvement of benzodiazepine and opioid receptors. *BMC Complement Altern Med.* **7:** 26-34.

National Toxicology Program (1990). NTP Toxicology and Carcinogenesis Studies of d-Limonene (CAS No. 5989-27-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies). *Natl Toxicol Program Tech Rep Ser.* **347**:1-165.

Navarro, A., Gomez, C., Sanchez-Pino, M. J., Gonzalez, H., Bandez, M. J., Boveris, A. D. and Boveris, A. (2005). Vitamin E at high doses improves survival, neurological performance and brain mitochondrial function in aging male mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289:**R1392–R1399.

Nayak, S. B., Pinto Pereira, L. and Maharaj, D. (2007). Wound healing activity of Carica papaya L. in experimentally induced diabetic rats. *Indian J Exp Biol.* **45(8)**:739-743.

Nelson, B. C., Putzbach, K., Sharpless, K. E. and Sander, L. C. (2007). Mass Spectrometric Determination of the Predominant Adrenergic Protoalkaloids in Bitter Orange (*Citrus aurantium*). *J. Agric. Food Chem.* **55***:* 9769–9775.

Nogata Y., Ohta, H., Sumida, T. and Sekiya, K. (2003). Effect of extraction method on the concentrations of selected bioactive compounds in mandarin juice. *J Agric Food Chem.* **51(25)**:7346-7351.

Nunez Selles, A. J., Velez Castro, T. H., Aguero-Aguero, J., Gonzalez-Gonzalez, J., Naddeo, F., De Simone, F. and Rastrelli, L. (2002). Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (Mangifera indica L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. *J Agric Food Chem.* **50(4):**762-766.

Ornelas-Paz, J., Yahia, E. M. and Gardea-Bejar, A. (2007). Identification and quantification of xanthophyll esters, carotenes, and tocopherols in the fruit of seven Mexican mango cultivars by liquid chromatography-atmospheric pressure chemical ionization-time-of-flight mass spectrometry [LC-(APcI(+))-MS]. *J Agric Food Chem.* **55(16):**6628-6635.

Osato, J. A., Santiago, L. A., Remo, G. M., Cuadra, M. S. and Mori, A. (1993). Antimicrobial and antioxidant activities of unripe papaya. *Life Sci.* **53(17)**:1383-1389.

Panetta, J., Smith, L. J. and Boneh, A. (2004). Effect of high-dose vitamins, coenzyme Q and high-fat diet in paediatric patients with mitochondrial diseases. *J. Inherit. Metab. Dis.* **27:**487-498.

Pelegrini, P. B., Murad, A. M., Silva, L. P., Dos Santos, R. C., Costa, F. T., Tagliari, P. D., Bloch Jr, C., Noronha, E. F., Miller, R. N. and Franco, O. L. (2008). Identification of a novel storage glycine-rich peptide from guava (Psidium guajava) seeds with activity against Gram-negative bacteria. *Peptides.* Epub ahead of print.

Pellati, F., Benvenuti, S. and Melegari, M. (2004). High-performance liquid chromatography methods for the analysis of adrenergic amines and flavanones in Citrus aurantium L. var. amara. *Phytochem Anal*,**15(4):** 220-225.

Percival, S. S., Talcott, S. T., Chin, S. T., Mallak, A. N., Lounds-Singleton, A. and Pettit-Moore, J. (2006). Neoplastic Transformation of BALB/3T3 Cells and Cell Cycle of HL-60 Cells are Inhibited by Mango (Mangifera indica L.) Juice and Mango Juice Extracts. *J Nutr.* **136(5):**1300-1304.

Petta, T. B., de Medeiros, S. R., do Egito, E. S. and Agnez-Lima, L. F. (2004). Genotoxicity induced by saponified coconut oil surfactant in prokaryote systems. *Mutagenesis.* **19(6)**:441-444.

Portnoy, V., Benyamini, Y., Bar, E., Harel-Beja, R., Gepstein, S., Giovannoni, J. J., Schaffer, A., Burger, J., Tadmor, Y., Lewinsohn, E. and Katzir, N. (2008). The molecular and biochemical basis for varietal variation in sesquiterpene content in melon (Cucumis melo L.) rinds. *Plant Mol Biol.* **66(6):**647-661.

Prior, R. L., Cao, G. and Martin, A.(1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium* species. *J Agric Food Chem.* **46:**2586-2593.

Rababah, T. M., Ereifej, K. I. and Howard, L. (2005). Effect of ascorbic acid and dehydration on concentrations of total phenolics, antioxidant capacity, anthocyanins, and color in fruits. *J Agric Food Chem.* **53(11)**:4444-4447.

Rahmat, A., Abu Bakar, M. F., Faezah, N. and Hambali, Z. (2004). The effects of consumption of guava (psidium guajava) or papaya (carica papaya) on total antioxidant and lipid profile in normal male youth. *Asia Pac J Clin Nutr.* 13(Suppl):S106.

Rai P.K., Singh, S. K., Kesari, A. N. and Watal, G. (2007). Glycaemic evaluation of *Psidium guajava* in rats. *Indian J Med Res.* **126**:224-227.

Ribeiro, M. R., Humberto de Queiroz, J., Lopes, M. E., Milagres Campos, F. and Pinheiro Sant'ana, H. M. (2007). Antioxidant in Mango (*Mangifera indica* L.) pulp. *Plant Foods for Human Nutrition.* **62**:13–17.

Rimbach, G., Minihane, A. M., Majewicz, J., Fischer, A., Pallauf, J., Virgli, E. and Weinberg, P. D. (2002). Regulation of cell signaling by vitamin E. *Proc. Nutr. Soc.* **61:**415-425.

Rincón, A. M., Vásquez, A. M. and Padilla, F. C. (2005). Chemical composition and bioactive compounds of flour of orange (*Citrus sinensis*), tangerine (*Citrus reticulata*) and grapefruit (*Citrus paradisi*) peels cultivated in Venezuela. *Arch Latinoam Nutr*. **55(3)**:305-310.

Rocha A. P., Carvalho, L. C., Sousa, M. A., Madeira, S. V., Sousa, P. J., Tano, T., Schini-Kerth, V. B., Resende, A. C. and Soares de Moura, R. (2007). Endothelium-dependent vasodilator effect of Euterpe oleracea Mart. (Açaí) extracts in mesenteric vascular bed of the rat. *Vascul Pharmacol.* **46(2):**97-104.

Rodrigues R.B., Lichtenthaler, R., Zimmermann, B. F., Papagiannopoulos, M., Fabricius, H., Marx, F., Maia, J. G. and Almeida, O. (2006). Total oxidant scavenging activity of Euterpe oleracea Mart. (acai) seeds and identification of their polyphenolic compounds. *J Agric Food Chem*. **54(12)**:4162-4167.

Rodriguez, J., Di Pierro, D., Gioia, M., Monaco, S., Delgado, R., Coletta, M. and Marini, S. (2006). Effects of a natural extract from Mangifera indica L, and its active compound, mangiferin, on energy state and lipid peroxidation of red blood cells. *Biochim Biophys Acta*. **1760(9):**1333-1342.

Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A. R. and O'Brien, N. M. (2006). Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. *Int J Food Sci Nutr*. **57(3-4)**:219-228.

Saffi, J., Sonego, L., Varela, Q. D. and Salvador, M. (2006) Antioxidant activity of L-ascorbic acid in wild-type and superoxide dismutase deficient strains of Saccharomyces cerevisiae. *Redox Rep*. **11(4)**:179-184.

Saiko, P., Szakmary, A., Jaeger, W. and Szekeres, T. (2008) Resveratrol and its analogs: Defense against cancer, coronary disease and neurodegenerative maladies or just a fad? *Mut. Res*. **658:**68–94

Sairam, K., Hemalatha, S., Kumar, A., Srinivasan, T., Ganesh, J., Shankar, M. and Venkataraman, S. (2003). Evaluation of anti-diarrhoeal activity in seed extracts of Mangifera indica. *J Ethnopharmacol.* **84(1):**11-15.

Sandhya, V. G. and Rajamohan, T. (2006). Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats. J. Med. Food. **9(3)**:400-407.

Sanoner, P., Guyot, S., Marnet, N., Molle, D. and Drilleau, J. P. (1999). Polyphenol profiles of French cider apple varieties (Malus domestica sp.). *J Agric Food Chem.* **47(12)**:4847-4853.

Schauss A. G., Wu, X., Prior, R. L., Ou, B., Huang, D., Owens, J., Agarwal, A., Jensen, G. S., Hart, A. N. and Shanbrom, E. J. (2006). Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, Euterpe oleraceae mart. (acai). *Agric Food Chem*. **54(22):**8604-8610.

Schieber, A., Berardini, N. and Carle, R. (2003). Identification of flavonol and xanthone glycosides from mango (Mangifera indica L. Cv. "Tommy Atkins") peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Agric Food Chem.* **51(17):**5006-5011.

Shafiee, M., Carbonneau, M. A., Urban, N., Descomps, B. and Leger, C. L. (2003). Grape and grape seed extract capacities at protecting LDL against oxidation generated by Cu2+, AAPH or SIN-1 and at decreasing superoxide THP-1 cell production. A comparison to other extracts or compounds. *Free Radic Res.* **37(5)**:573-584.

Silalahi, J. (2002) Anticancer and health protective properties of citrus fruit components. *Asia Pac J Clin Nutr.* **11**:79–84.

Singh, U. P., Singh, D. P., Singh, M., Maurya, S., Srivastava, J. S., Singh, R. B. and

Singh, S. P. (2004). Characterization of phenolic compounds in some Indian mango cultivars. *Int J Food Sci Nutr.* **55(2):**163-169.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *Am J Enol Viticult.* **16**:144–158.

Soares de Moura,R.,Miranda, D. Z., Pinto,A. C. A., Sicca,R. F., Souza,M. A. V., Rubenich ,L. M. S., Carvalho,L. C. R. M., Rangel, B. M., Tano, T., Madeira, S. V. F. and Resende, A. C. (2004). Mechanism of the endothelium-dependent vasodilation and the antihypertensive effect of brazilian red wine. *J Cardiovasc Pharmacol*. **44:**302-309

Spada, P. D. S., Nunes de Souza, G. G., Bortolini, G. V., Henriques, J. A. P. and Salvador, M. (2008). Antioxidant, mutagenic, and antimutagenic activity of frozen fruits. *J. Med. Food.* **11(1)**:144-151.

Srinivasan, K. (2007). Black pepper and its pungent principle-piperine: a review of diverse physiological effects. *Crit Rev Food Sci Nutr.* **47(8):**735-48.

Stagos, D., Kazantzoglou, G., Magiatis, P., Mitaku, S., Anagnostopoulos, K. and Kouretas, D. (2005). Effects of plant phenolics and grape extracts from Greek varieties of Vitis vinifera on Mitomycin C and topoisomerase I-induced nicking of DNA. *Int J Mol Med.* **15(6):**1013-1022.

Sun, J., Chu, Y. F., Wu, X. and Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *J Agric Food Chem.* **50(25):**7449-7454.

Suntornsuk, L., Gritsanapun, W., Nilkamhank, S. and Paochom, A. (2002) Quantitation of vitamin C content in herbal juice using direct titration. *J Pharm Biomed Anal.* **28(5)**:849-855.

Taguri, T., Tanaka, T. and Kouno, I. (2004). Antimicrobial Activity of 10 Different Plant Polyphenols against Bacteria Causing Food-Borne Disease. *Biol. Pharm. Bull.* **27(12):**1965-1969.

Tang, X. and Edenharder, R. (1997). Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene by vitamins, porphyrins and related compounds, and vegetable and fruit juices and solvent extracts. *Food Chem Toxicol.* **35(3-4)**:373-378.

Tapiero, H., Townsend, D. M. and Tew, K. D. (2004) The role of carotenoids in the prevention of human pathologies. *Biomed Pharmacother.* **8:**100–110.

Thimothe, J., Bonsi, I. A., Padilla-Zakour, O. I. and Koo, H. (2007). Chemical characterization of red wine grape (Vitis vinifera and Vitis interspecific hybrids) and pomace phenolic extracts and their biological activity against Streptococcus mutans. *J Agric Food Chem.* **55(25):**10200-10207.

Trevisan, M. T. S., Pfundstein, B., Haubner, R., Würtele, G., Spiegelhalder, B. and Bartsch, H., Owen, R. W. (2006). Characterization of alkyl phenols in cashew (Anacardium occidentale) products and assay of their antioxidant activity. *Food Chem Toxicol.* **44(2)**: 188-197.

Valcheva-Kuzmanova, S. V. and Belcheva, A. (2006). Current knowledge of Aronia melanocarpa as a medicinal plant. *Folia Med.* **48(2):**11-7.

Viljanen, K., Kylli, P., Kivikari, R. and Heinonen, M. (2004). Inhibition of protein and lipid oxidation in liposomes by berry phenolics. *J Agric Food Chem.* **52(24):**7419-7424.

Vouldoukis, I., Conti, M., Krauss, P., Kamate, C., Blazquez, S., Tefit, M., Mazier, D., Calenda, A. and Dugas, B. (2004). Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress. *Phytother Res.* **18(12):**957-962.

Voutilainen, S., Nurmi, T., Mursu, J. and Rissanen, T. (2006) Carotenoids and cardiovascular health. *Am J Clin Nut.* **83**:1265–1271.

Vrhovsek, U., Rigo, A., Tonon, D. and Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *J Agric Food Chem.* **52(21)**:6532-6538.

Wada, L. and Ou, B. (2002). Antioxidant activity and phenolic content of Oregon caneberries. *J Agric Food Chem.* **50(12):**3495-3500.

Wang, H., Cao, G. and Prior, R. (1996). Total antioxidant capacity of fruits. *J Agric Food Chem.* **44:**701-705.

Wang, S. Y. and Jiao, H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J Agric Food Chem.* **48(11):**5677-5684.

Wen, L., Wrolstad, R. E. and Hsu, V. L. (1999). Characterization of sinapyl derivatives in pineapple (Ananas comosus [L.] Merill) juice. *J Agric Food Chem.* **47(3)**:850-853.

Wijeratne, S. S. K., Abou-Zaid, M. M. and Shahidi, F. (2006). Antioxidants polyphenols in almond and its coproducts. *J Agric Food Chem.* **54**:312–318.

Yang, H., Protiva, P., Cui, B., Ma, C., Baggett, S., Hequet, V., Mori, S., Weinstein, I. B. and Kennelly, E. J. (2003). New bioactive polyphenols from Theobroma grandiflorum ("cupuacu"). *J Nat Prod.* **66**(11):1501-1504.

Yilmaz, Y. and, Toledo, R. T. (2004). Major Flavonoids in Grape Seeds and Skins: Antioxidant Capacity of Catechin, Epicatechin, and Gallic Acid. *J. Agric. Food Chem.* **52**:255-260.

Zadernowski, R., Naczk, M. and Nesterowicz, J. (2005). Phenolic acid profiles in some small berries. *J Agric Food Chem.* **53(6)**:2118-2124.

Zafirov, D., Bredy-Dobreva, G., Litchev, V. and Papasova, M. (1990). Antiexudative and capillaritonic effects of procyanidines isolated from grape seeds (V. Vinifera). *Acta Physiol Pharmacol Bulg.* **16(3):**50-54.

3.5 DISCUSSÃO GERAL

Apesar da diversidade na produção de frutas e de ser o maior exportador mundial de suco de laranja, o Brasil não apresenta tradição no consumo de sucos de frutas industrializados. Segundo o Instituto Brasileiro de Frutas (IBRAF, 2007), o consumo per capita de sucos no país é equivalente a 6 litros/hab/ano, bastante inferior ao dos Estados Unidos (45 litros/hab/ano), Alemanha (59 litros/hab/ano) e França (63 litros/hab/ano).

O suco de fruta tem, ainda, como forte concorrente os refrigerantes, que apresentam preço final mais baixo, massificando o consumo. Por outro lado, as recomendações para ingestão de bebidas saudáveis e funcionais têm impulsionado à indústria de frutas, que tem procurado desenvolver produtos mais econômicos, como por exemplo, as polpas de fruta congeladas. Particular interesse tem sido dado às frutas tropicais brasileiras, que vem despertando o interesse de vários países, inclusive Europa e Estados Unidos (IBRAF, 2007).

A produção de frutas congeladas representa uma importante fonte de renda para o país, já que as indústrias podem produzir as polpas de frutas na época da safra e revendê-las de acordo com a necessidade do mercado, evitando as perdas decorrentes do armazenamento e/ou transporte de frutas *in natura*. O mercado de frutas congeladas vem crescendo consideravelmente, já que as polpas são utilizadas não somente para preparo de sucos, mas também, na formulação de iogurtes, doces, biscoitos, bolos, sorvetes e alimentos infantis (Bueno *et al.*, 2002).

Estudos epidemiológicos têm demonstrado a associação entre dietas ricas em frutas e verduras e a diminuição de diversas doenças, incluindo diabetes, doenças cardiovasculares, neurodegenerativas, câncer e osteoporose (para revisão, ver

D'Archivio, 2007). Estes efeitos podem ser decorrentes, pelo menos, em parte, da reconhecida atividade antioxidante das frutas *in natura*. Considerando que não existem dados sobre a atividade biológica de frutas congeladas, este trabalho teve como objetivo avaliar a atividade antioxidante, mutagênica e antimutagênica de 23 polpas/sucos de frutas congelados. A seleção das amostras levou em consideração a disponibilidade destes produtos para o consumidor e incluiu tanto as frutas mais consumidas pela população, como também as frutas tropicais brasileiras.

Para a determinação da concentração de polpa de fruta a ser utilizada nos ensaios, tomou-se como base a recomendação do fabricante para preparação de suco, ou seja, uma diluição aquosa de 40% (p/v). Essa concentração foi utilizada para a determinação da atividade antioxidante *in vitro* (DPPH, SOD- e CAT-*like* e nos ensaios em homogenizado de cérebros de ratos). Para os ensaios *in vivo* (mutagênese e antimutagênese) utilizou-se a maior concentração não citotóxica à levedura *Saccharomyces cerevisiae* (20% p/v) e mais duas concentrações que foram de 5 e 10% (p/v).

Todas as amostras estudadas pertenciam ao mesmo lote de fabricação e foram armazenadas a -12^{o} C, sendo descongeladas previamente a realização de cada ensaio. Os teores de carboidratos, lipídios e proteínas, bem como o pH e acidez total de cada fruta são mostrados no Capítulo 1. Como esperado, as 23 frutas congeladas apresentaram teores de proteínas e lipídios baixos (valores médios de 0,42 e 0,08%, respectivamente) e concentrações variadas de carboidratos, com um mínimo de 0,10% para a polpa de coco até 17,8% para a tangerina. Não foi encontrada correlação entre esses dados e a atividade biológica descrita para cada fruta neste trabalho.

Além dos macronutrientes, verificou-se que as polpas, mesmo congeladas apresentaram quantidades significativas de polifenóis, carotenóides totais e vitamina C

(Capítulo 1, Tabela 2). Os valores de polifenóis totais variaram de 1,49 para a maçã até 22,83 mg% (em equivalentes de catequina) para o maracujá. O maior teor de carotenóides totais foi encontrado na pitanga (1,87 mg%) e o menor na graviola (0,01 mg%). Os valores de vitamina C apresentaram a maior variação, com um valor mínimo de 1,19 na maçã até 224,57 mg% para a acerola.

De modo geral, especialistas da área de nutrição tem preferido recomendar a utilização de sucos preparados a partir de frutas *in natura*, considerados mais ricos em compostos bioativos. No entanto, até o momento, os dados acerca da influência do congelamento na composição de macro e microelementos de frutas congeladas são escassos. O congelamento de acerola reduziu em 3% o conteúdo de vitamina C presente na fruta *in natura* (Yamashita *et al.*, 2003). Já para limão (Pedrão *et al.*, 1999) e manga (Brunini *et al.*, 2002) não foram observadas reduções significativas de ácido ascórbico após o congelamento. Por outro lado, framboesas congeladas em nitrogênio líquido perderam 50% do seu teor original de vitamina C, fato atribuído à forma de congelamento (Beekwilder *et al.*, 2005).

A estabilidade dos carotenóides, estudada apenas em polpa de acerola, mostrou uma redução significativa destes compostos (70%) após onze meses de congelamento da fruta fresca (Agostini-Costa *et al.*, 2003). Em relação aos polifenóis, os dados são contraditórios. Em acerola, verificou-se perda de 14% do teor de antocianidinas (Agostini-Costa *et al.*, 2003) após o congelamento, ao contrário do suco de laranja, o qual não mostrou alteração no conteúdo de polifenóis totais (Franke *et al.*, 2004). Estes resultados mostram a necessidade de mais estudos acerca da estabilidade de compostos bioativos após o congelamento, avaliando, não só as diferentes formas de processamento, mas, também, o tempo de armazenamento dos produtos.

Até o momento, não existem estudos mostrando a influência do congelamento no teor de minerais das frutas. Neste trabalho foram determinados 14 minerais (PIXE) em cada polpa de fruta (Capítulo 2, Tabelas 1, 2 e 3) e os valores encontrados são semelhantes aos observados em frutas *in natura* (Rizzon *et al.*, 2004). Estes resultados compõem o primeiro banco de informações acerca dos teores de minerais em 23 diferentes frutas e constituem-se numa ferramenta imprescindível ao cálculo do percentual de ingestão diária de minerais recomendada (National Academy of Sciences, 1997) para a manutenção da saúde da população.

Mesmo supondo-se uma possível perda de compostos bioativos durante o processamento/congelamento das polpas, verificou-se que todas elas apresentaram atividade antioxidante (Capítulo 1, Tabela 3). Dezessete frutas mostraram atividade antioxidante similar a do ácido ascórbico no ensaio que mede a capacidade de varredura do radical livre DPPH. Todas as polpas congeladas foram capazes de reagir com o peróxido de hidrogênio (atividade CAT-*like*) e 16 delas apresentaram atividade SOD-*like*, ou seja, foram capazes de dismutar o radical $O_2^{\bullet-}$. Importante salientar, que cada ensaio mostrou uma classificação diferente para a atividade antioxidante das frutas, tornando-se difícil determinar qual é mais antioxidante, já que o tipo de ensaio realizado influi no resultado obtido. A capacidade de uma amostra de doar átomos de hidrogênio (atividade redox) é avaliada no teste do $DPPH^{\bullet}$ (Yamaguchi *et al.*, 1998). Já os ensaios SOD e CAT-*like* são específicos para avaliar a presença de compostos capazes de reagir com superóxido (Bannister & Calabrese, 1987) e peróxido de hidrogênio, respectivamente (Aebi, 1984). Sabe-se que alguns polifenóis, como a catequina (Silalahi, 2002) e a quercetina (Ferguson, 2001; Halliwell & Gutteridge, 2007) são excelentes varredores de espécies reativas de oxigênio. Neste sentido, seria interessante quantificar os compostos fenólicos majoritários presentes nas frutas congeladas e sua influência na atividade biológica. No entanto, deve-se levar em conta, também, que a ação sinérgica entre os compostos que fazem parte de uma mistura complexa pode ser mais importante que a de um composto isolado (Moure *et al*., 2001).

Embora não existam estudos sobre atividade antioxidante de frutas congeladas, várias frutas *in natura* (para revisão, ver Capítulo 4 e Introdução, Tabela 1) e extratos obtidos com solventes orgânicos (Wang *et al.*, 1996; Silalahi, 2002; Edenharder, 2002) mostraram efeitos biológicos importantes, os quais foram atribuídos à presença de polifenóis, carotenóides e vitaminas.

De forma similar ao observado neste trabalho, a atividade antioxidante de frutas *in natura* também depende do ensaio realizado (Tabela 2). Comparando-se os resultados obtidos em quatro testes que medem a atividade antioxidante *in vitro* (FRAP, TRAP, TEAC e DPPH) verificou-se que a framboesa, tanto *in natura* quanto congelada, foi a fruta que mostrou a maior atividade antioxidante. Abacaxi, melão e morango apresentaram resultados semelhantes, tanto para a fruta *in natura*, quanto congelada. Deve-se considerar, no entanto, que os ensaios FRAP, TRAP e TEAC foram realizados com frutas produzidas na Itália (Pelegrini *et al.*, 2003), enquanto que o DPPH$^\bullet$ com frutas produzidas no Brasil.

Além da atividade antioxidante, doze frutas (coco, cupuaçu, graviola, maçã, manga, laranja, mamão, pêssego, framboesa, uva, goiaba e tangerina) apresentaram efeito antimutagênico em células de *S. cerevisiae* tratadas com peróxido de hidrogênio (Capítulo 1, Tabela 5). Este efeito mostrou correlação positiva com a atividade CAT-*like* observada (r = 0,400; $p \leq 0,05$), sugerindo que a capacidade de reação com o peróxido de hidrogênio é um mecanismo importante para evitar danos ao DNA. O peróxido de hidrogênio é um forte oxidante, podendo provocar inclusive quebras duplas

Tabela 2. Atividade antioxidante de frutas *in natura* e congeladas.

Amostras	Frutas *in natura*			Frutas congeladas
	FRAP	TEAC	TRAP	DPPH
Abacaxi	4[*]	3	3	3
Framboesa	1	1	1	1
Kiwi	6	5	6	1
Laranja	3	4	4	1
Melão	8	7	8	3
Morango	2	2	2	1
Pêssego	7	6	7	1
Tangerina	5	8	5	2
Referências	Pelegrini *et al.*, 2003			Spada *et al.*, 2008- Capítulo 1

FRAP: ferric reducing-antioxidant power; TEAC: trolox equivalent antioxidant capacity; TRAP: total radical-trapping antioxidant parameter.

[*] Os números referem-se à classificação de cada fruta no respectivo ensaio, sendo a maior capacidade antioxidante representada pelo número 1.

no DNA (Henriques *et al.*, 2001; Halliwell & Gutteridge, 2007). Sabe-se que o peróxido de hidrogênio participa da reação de Fenton, formando radical hidroxila (Halliwell & Gutteridge, 1986), o qual pode danificar o DNA (Picada *et al.*, 2003, Halliwell & Gutteridge, 2007).

Curiosamente, apesar dos efeitos benéficos observados, quatro frutas (açaí, caju, kiwi e morango), foram capazes de induzir danos ao DNA em todas as concentrações avaliadas (5, 10 e 15% p/v - Capítulo 1). É importante, salientar, no entanto, que em baixas concentrações (até 1% p/v) estas frutas não apresentaram atividade mutagênica em leveduras (dados não mostrados), minimizando, portanto, o risco de mutagenicidade ao homem. Outros sucos de frutas, como caju (Melo Cavalcante *et al.*, 2003) e laranja

(Franke *et al.*, 2004) também se mostraram, concomitantemente, antioxidantes e mutagênicos no teste de Ames. Estudos em mamíferos, avaliando, entre outros fatores, a absorção e metabolização hepática, seriam importantes para esclarecer melhor estes efeitos, aparentemente, contraditórios.

Neste trabalho, observou-se (Capítulo 1, Tabela 4) correlação positiva entre a atividade mutagênica na levedura *S. cerevisiae* e o teor de polifenóis ($r = 0{,}688$; $p \leq 0{,}05$); carotenóides ($r = 0{,}654$; $p \leq 0{,}01$) e vitamina C ($r = 0{,}640$; $p \leq 0{,}05$) para os revertentes *his* (mutação *locus* específica). Nos revertentes *hom* (mutação por deslocamento de quadro de leitura), foi observada correlação positiva entre o efeito mutagênico e a concentração de carotenóides ($r = 0{,}701$; $p \leq 0{,}05$) e de vitamina C ($r = 0{,}752$; $p \leq 0{,}05$). No meio seletivo *lis* (mutação *locus* específica) verificou-se correlação positiva entre o efeito mutagênico e a concentração com carotenóides ($r = 0{,}793$; $p \leq 0{,}01$). Embora outros estudos sejam necessários, parece haver certa especificidade dos compostos antioxidantes em relação aos diferentes tipos de mutação ao DNA. O teor de carotenóides está associado tanto a mutações por deslocamento do quadro de leitura quanto *locus* especifica, ao contrário do observado para polifenóis.

Embora exista um número maior de trabalhos mostrando efeitos antioxidante (para revisão, ver Gallicchio, 2008) e antimutagênico (para revisão, ver Ferguson & Philpott, 2008) dos carotenóides, recentemente verificou-se que alguns retinóides são capazes de induzir quebras duplas de DNA em leucócitos humanos (Valli *et al.*, 2008). Cerca de 200 polifenóis dos 8.000 conhecidos, mostraram-se mutagênicos em bactérias (Rueff *et al.,* 1992; Ferguson, 2001). Altas concentrações de vitamina C também podem induzir efeitos mutagênicos (Norkus *et al.*, 2003), os quais tem sido atribuídos a geração do radical hidroxila, pela reação de Fenton, quando em presença de Fe e Cu divalentes (Ferguson, 2001; Halliwell, 2001; Kaya *et al.*, 2002). As 4 frutas que mostraram-se mutagênicas apresentaram teores de vitamina C altos, variando de 15,7 a 77 mg%. No entanto, outras frutas com altas concentrações de vitamina C (abacaxi, acerola, graviola, laranja, maracujá e tangerina) não apresentaram atividade mutagênica. Essas frutas, com exceção da tangerina, no entanto, apresentaram baixos teores de ferro, o que poderia ter minimizado possíveis efeitos mutagênicos. De fato, observou-se uma correlação positiva entre os teores Fe (Capítulo 2) com a atividade mutagênica (Capítulo 1). As correlações entre a atividade mutagênica e os níveis de Fe foram $r = 0{,}580$ para mutações no locus *his*; $r = 0{,}680$ para mutações no locus *lis;* e $r = 0{,}902$ para mutações no locus *hom,* todas para $p \leq 0{,}05$.

Os minerais desempenham importantes funções no organismo, atuando como co-fatores enzimáticos, na estabilização da membrana plasmática, e participando na manutenção da homeostase celular (Halliwell & Gutteridge, 2007; Prá *et al.*, 2008). Mesmo congeladas, as 23 frutas estudadas neste trabalho (Capitulo 2, Tabelas 1, 2 e 3) mostraram quantidades significativas de macro e microminerais. No entanto, com exceção do ferro, nenhum outro mineral mostrou correlação com as atividades biológicas estudadas.

Entre as várias frutas nativas brasileiras, o açaizeiro (*Euterpe oleracea* Mart.), uma palmeira tropical da Amazônia, tem chamado a atenção do mercado nacional e internacional. Seus frutos são utilizados na produção da polpa de açaí, um alimento muito consumido pela população, principalmente da região Norte do País (Santos *et al.*, 1999), e exportado principalmente para a Europa, Estados Unidos e Austrália (IBRAF, 2007). No entanto, até o momento existem poucos estudos acerca da atividade biológica do açaí. Em vista disso, selecionou-se esta fruta para o estudo da atividade antioxidante em homogeneizado de cérebro de ratos (Capítulo 3). Este tipo de ensaio, embora não avalie a absorção, distribuição e metabolização das amostras ensaiadas, tem sido bastante utilizado (Evelson, 2001; Bavaresco, 2006; Beskow, 2008) para o estudo de diversos compostos.

O açaí mostrou-se capaz de reduzir os danos oxidativos, tanto em lipídios como em proteínas (Capítulo 3, Figura 1), corroborando a atividade antioxidate *in vitro* descrita no Capítulo 1. Entre todas as frutas estudadas, o açaí apresentou a maior atividade SOD-*like* (Capítulo 1, Tabela 3). De fato, no tratamento dos homogenizados de tecidos de rato, o açaí foi capaz de diminuir a atividade da enzima superóxido dismutase a níveis inferiores ao observado nos controles (tratamentos sem presença de fruta). O açaí apresenta altos teores de compostos fenólicos (Capítulo 4), principalmente, ciandina3-glucosídeo e a cianidina3-rutinosídeo (Schauss *et al.*, 2006), os quais poderiam estar associados à dismutação do radical superóxido observada neste trabalho.

Os dados apresentados nesta tese formam o maior banco de dados acerca dos teores de carboidratos, lipídios, proteínas, polifenóis, carotenóides, vitamina C e minerais de 23 polpas/sucos de frutas congeladas. Além disso, verificou-se que todas as frutas estudadas apresentaram importante atividade antioxidante, sendo, 12 delas, também antimutagênicas. Estes resultados são importantes, também, para a fruticultura brasileira, um dos segmentos econômicos de maior destaque no País e que vem ganhando espaço no mercado internacional, com a exportação de frutas tropicais, subtropicais e de clima temperado. Em 1998, foi criado o programa *Brazilian Fruit,* coordenado pelo Instituto Brasileiro de Frutas, a Agência de Promoção de Exportações e Investimentos (APEX-Brasil) e as associações do setor. As ações do *Brazilian Fruit* objetivam promover o consumo de frutas e seus derivados no mercado nacional e internacional, incluindo a União Européia, os Estados Unidos, e os novos mercados, como Países Asiáticos, do Leste Europeu, Países Árabes e Países da América Latina como México, Chile e Argentina. Desta forma, espera-se, para os próximos anos, um aumento considerável no consumo de frutas e derivados produzidos em nosso País e, como conseqüência, uma diminuição na incidência de doenças associadas à dieta, como, por exemplo, câncer e aterosclerose (Ahmed, 2004).

4. CONCLUSÕES

Os dados obtidos neste trabalho permitem concluir que:

1. As polpas/sucos de frutas congeladas apresentaram em sua composição baixos teores de lipídios (0 a 0,67 mg%) e proteínas (0,15 a 0,65 mg%). O menor conteúdo de carboidratos foi encontrado no coco (0,10 mg%) e o maior na tangerina (17,80 mg%).
2. Mesmo após o congelamento, as frutas mostraram um conteúdo significativo de polifenóis (de 0,15 a 2,28 mg de caquina %), carotenóides (de 0,01 a 1,87 mg%) e ácido ascórbico (de 1,19 a 224,57 mg%).
3. Todas as frutas apresentaram Mg, Cl, P, Ca, Fe e K; 91,3% mostraram S e Si; Mn foi encontrado em 65,2% das amostras e os demais minerais ocorreram em menos de 30% das amotras avaliadas.
4. Todas as 23 frutas congeladas apresentaram atividade antioxidante frente ao radical livre DPPH$^\bullet$. Para o açaí, maçã, amora, coco, cupuaçu, kiwi, manga, laranja, mamão, pêssego, framboesa, uva, goiaba, graviola, morango e pitanga este efeito foi semelhante ao da vitamina C.
5. Todas as frutas mostraram atividade CAT-*like* e 56% dessas SOD-*like*, sendo essa última mais pronunciada nas polpas de açaí, caju, coco, cupuaçu, kiwi, limão, laranja, uva e pitanga.
6. O açaí foi capaz de reverter os danos oxidativos em lipídios e proteínas induzidos pelo peróxido de hidrogênio em homogeneizados de córtex, cerebelo e hipocampo de ratos Wistar.
7. Açaí, caju, kiwi e morango mostraram-se mutagênicas para a levedura

Saccharomyces cerevisiae, quando ensaiadas em altas concentrações (5, 10 e 15% p/v).

8. Doze polpas de frutas (maçã, coco, cupuaçu, manga, laranja, mamão, pêssego, framboesa, goiaba, graviola, uva e tangerina) mostraram-se antimutagênicas nos ensaios com a levedura *S cerevisiae.* Este efeito apresentou correlação positiva com a atividade CAT-*like*.

5. PERSPECTIVAS

Como continuidade deste trabalho, pretende-se:

- Avaliar o efeito do processamento (congelamento/liofilização) das frutas na sua composição e atividade biológica.
- Determinar os compostos fenólicos majoritários e carotenóides presentes nas frutas congeladas (cromatografia líquida de alta eficiência), verificando a influência de cada composto na atividade biológica da frutas.
- Estudar a mutagenicidade das polpas de açaí, caju, kiwi e morango *in vivo* (em cultura de células de mamíferos), a fim de entender melhor a relação existente entre atividade mutagênica e antioxidante.
- Avaliar a possível atividade quimiopreventiva das frutas congeladas, utilizando-se, como modelo, linhagem de células tumorais.
- Realizar diferentes bioensaios, utilizando as polpas em dietas, curvas de sobrevivência, avaliação comportamental, teste de memória, desenvolvimento embrionário.

6. REFERÊNCIAS BIBLIOGRÁFICAS

Abdelrahim, S. I., Almagboul, A. Z., Omer, M. E., Elegami, A. (2002). Antimicrobial activity of Psidium guajava L. **Fitoterapia** 73(7-8):713-715.

Aebi, H. (1984). Catalase in vitro. **Methods Enzymol**.105:121–126.

Agostini-Costa, T.S., Abreu, L.N., Rosseti, A.G. Efeito do congelamento e do tempo de estocagem da polpa de acerola sobre o teor de carotenóides. **Rev. Bras. Frutic.** 25:56-58.

Ahmed, F. E. (2004) Effect of diet, life style, and other environmental/chemopreventive factors on colorectal cancer development, and assessment of the risks. **J. Environ. Sci. Health C. Environ. Carcinog. Ecotoxicol. Rev.** 22(2):91–147.

Bala, S. and Grover, I. S. (1989). Antimutagenicity of some citrus fruits in Salmonella typhimurium. **Mutat. Res.** 222(3):141-8.

Bannister, J.V., Calabrese, L. (1987). Assays for SOD. **Methods Biochem. Anal.** 32:279–312.

Bavaresco, C.S., Chiarani, F., Wajner, M., Netto, C.A., Wyse, A.T. (2006) Intrastriatal hypoxanthine administration affects Na+,K+-ATPase, acetylcholinesterase and catalase activities in striatum, hippocampus and cerebral cortex of rats. **Int. J. Dev. Neurosci.** 24(7):411-7.

Beekwilder, J., Hall, R.D., de Vos, C.H. (2005) Identification and dietary relevance of antioxidants from raspberry. **Biofactors** 23(4):197-205.

Ben-Yehoshua, S., Rodov, V., Nafussi, B., Feng, X., Yen, J., Koltai, T., Nelkenbaum, U. (2008). Involvement of limonene hydroperoxides formed after oil gland injury in the induction of defense response against Penicillium digitatum in lemon fruit. **J Agric. Food Chem.** 56(6):1889-1895.

Beskow, A.P., Fernandes, C.G., Leipnitz, G., da Silva, L. de B., Seminotti, B., Amaral, A.U., Wyse, A.T., Wannmacher, C.M., Vargas, C.R., Dutra-Filho, C.S., Wajner, M. (2008) Influence of ketone bodies on oxidative stress parameters in brain of developing rats in vitro. **Metab. Brain Dis**. 23(4):411-425.

Boeira, J.M., Viana, A.F., Picada, J.N., Henriques, J.A. (2002) Genotoxic and recombinogenic activities of the two beta-carboline alkaloids harman and harmine in *Saccharomyces cerevisiae*. **Mutat. Res.** 500(1-2):39-48.

BRASIL. Ministério da Agricultura e do Abastecimento. Instrução Normativa No 1 de 7 de janeiro de 2000, Diário Oficial da União No 6, Brasília 10 de janeiro de 2000. Seção 1, p.54-58. Regulamento técnico geral para fixação dos padrões de identidade e qualidade para polpas de fruta.

Brunini, M. A.; Durigan, J.F.; Oliveira, A.L. (2002). Avaliação das alterações em polpa de manga ‘tommy-atkins’ congeladas. **Rev. Bras. Frutic**. 24(3):651-653,

Bueno, S. M., Lopes, M. R. V., Graciano, R.A.S., Fernandes, E.C.B., Garcia-Cruz, C.H. (2002) Avaliação da qualidade de Polpas de Frutas Congeladas. **Rev. Inst. Adolfo Lutz** 62(2):121-126, 2002

Burton, G. W., Ingold, K. U. (1984). β-Carotene: an unusual type of lipid antioxidant. **Science** 224: 569-573.

Caregnato, F.F., Koller, C.E., MacFarlane, G.R., Moreira, J.C. (2008) The glutathione antioxidant system as a biomarker suite for the assessment of heavy metal exposure and effect in the grey mangrove, Avicennia marina (Forsk.) **Vierh. Mar. Pollut. Bull.** 56(6):1119-27.

Castenmiller, J.J.M., Lauridsen, S.T., Dragsted, L.O., Van HET Hof, K.H., Linssen, J.P.H., West, C.E. (1999). β-Carotene does not change markers of enzymatic and nonenzymatic antioxidant activity in human blood. **J. Nutr.** 129: 2162-2169.

Castillo, J., Benavente-García, O., Lorente, J., Alcaraz, M., Redondo, A., Ortuño, A. and Del Rio, J. A. (2000). Antioxidant activity and radioprotective effects against chromosomal damage induced in vivo by X-rays of flavan-3-ols (Procyanidins) from grape seeds (Vitis vinifera): comparative study versus other phenolic and organic compounds. **J. Agric. Food Chem.** 48(5):1738-1745.

Combs, G.F. (1998). The vitamins, fundamental aspects in nutrition and health. Academic Press. San Diego. 2.ed.

D'Archivio, M., Filesi, C., Di Benedetto, R., Gargiulo, R., Giovannini, C., Masella, R. (2007) Polyphenols, dietary sources and bioavailability. **Ann. Ist. Super Sanità.** 43(4): 348-361.

Dal-Pizzol, F., Klamt, F., Dalmolin, R.J., Bernard, E.A., Moreira, J.C. (2001) Mitogenic signaling mediated by oxidants in retinol treated Sertoli cells. **Free Radic Res.** 35(6):749-755.

De Flora, S. (1998) Mechanisms of inhibitors of mutagenesis and carcinogenesis. **Mutat. Res.** 402(1-2):151-158.

De Flora, S., Izzotti, A., D'Agostini, F., Balansky, R.M., Noonan, D., Albini, A. (2001) Multiple points of intervention in the prevention of cancer and other mutation-related diseases. **Mutat. Res.** 1(480-481):9-22.

De Freitas, J.M., Meneghini, R. (2001) Iron and its sensitive balance in the cell. **Mutat. Res.** 475(1–2):153–159.

De Tullio, M.C., Arrigoni, O. (2004) Hopes, disillusions and more hopes from vitamin C. **Cell. Mol. Life Sci.** 61(2):209-219.

Deters, A. M., Schroder, K. R. and Hensel, A. (2005). Kiwi fruit (Actinidia chinensis

L.) polysaccharides exert stimulating effects on cell proliferation via enhanced growth factor receptors, energy production, and collagen synthesis of human keratinocytes, fibroblasts, and skin equivalents. **J. Cell. Physiol.**202(3):717-722.

Devi, S. A., Jolitha, A. B. and Ishii, N. (2006). Grape Seed Proanthocyanidin Extract (GSPE) and antioxidant defense in the brain of adult rats. **Med. Sci. Monit**. 12(4): 124-129.

Deyhim, F., Lopez, E., Gonzalez, J., Garcia, M. and Patil, B. S. (2006). Citrus juice modulates antioxidant enzymes and lipid profiles in orchidectomized rats. **J. Med. Food**. 9(3):422-426.

Dreosti, I.E. (2001) Zinc and the gene. **Mutat. Res.** 475(1-2):161-167.

Edenharder, R., Kurz, P., John, K., Burgard, S. and Seeger, K. (1994). In vitro effect of vegetable and fruit juices on the mutagenicity of 2-amino-3-methylimidazo[4,5-f] quinoline, 2-amino-3,4-dimethylimidazo [4,5-f] quinoline and 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline. **Food Chem. Toxicol.** 32(5):443-459.

El-Ashmawy, I. M., Saleh, A. and Salama, O. M. (2007). Effects of marjoram volatile oil and grape seed extract on ethanol toxicity in male rats. **Basic Clin. Pharmacol. Toxicol.** 101(5):320-327.

Emeruwa, A. C. (1982). Antibacterial substance from Carica papaya fruit extract. **J Nat Prod.** 45(2):123-127.

Espín, J.C.; Soler-Rivas, C. & Wichers, H.J. (2000). Characterization of the total free scavenger capacity of vegetable oils and oil fraction using 2,2 diphenyl –1-picrylhydrazyl radical. **J. Agric. Food Chem**. 48: 648-656.

Evelson, P., Travacio, M., Repetto, M., Escobar, J., Llesuy, S., Lissi, E. (2001) Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. **Arch. Biochem. Biophys**. 388:261–266

Ferguson, L.R. (2001). Role of plant polyphenols in genomic stability. **Mutat. Res.** 475: 89-111.

Ferguson, L.R., Philpott, M. (2008) Nutrition and mutagenesis. **Annu Rev. Nutr.** 28:313-29.

Flagg, E.W., Coates, R.J., Greenberg, R.S. (1995). Epidemiologic studies of antioxidants and cancer in human. **J. Am. Coll. Nutr.** 14:415-419.

Flynn, A. (2003) The role of dietary calcium in bone health. **Proc. Nutr. Soc.** 62(4):851-858.

Fotherby, M.D., Williams, L.A., Foster, P., Craner, G.A., Ferns, G.A. (2000). Effects of vitamin C on ambulatory blood pressure and plasma lipid in older persons. **J. Hyperten.** 18: 411-415.

Franke, S. I.R., Pra, D., Dias, J. F. (2006) Influence of orange juice in the levels and in the genotoxicity of iron and copper. **Food Chem. Toxicol**. 44(3):425–435.

Franke, S.I.R., K. Ckless, Silveira, J.D., Rubensam, G., Brendel, M., Erdtmann, B., Henriques, J.A.P. (2004). Study of antioxidant and mutagenic activity of different orange juices. **Food Chem.** 88:45-55.

Fukumoto, L.R. & Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. **J. Agric. Food Chem**. 48(8): 3597-3604.

Gallicchio, L., Boyd, K., Matanoski, G., Tao, X.G., Chen, L., Lam, T.K., Shiels, M., Hammond, E., Robinson, K.A., Caulfield, L.E., Herman, J.G., Guallar, E., Alberg, A.J. (2008) Carotenoids and the risk of developing lung cancer: a systematic review. **Am. J. Clin. Nutr**. 88(2):372-383.

Gambera, L., Campanella, G., Piomboni, P., Serafini, F., Morgante, G., De Leo, V. (2007). Association of antioxidants and natural immune activators in the treatment of astheno-teratospermia and abacterial leukocytosis. **Minerva Ginecol.** 59(5):473-

479.

Garg, S. K., Saksena, S. K. and Chaudhury, R. R. (1970). Antifertility screening of plants. VI. Effect of five indigenous plants on early pregnancy in albino rats. **Indian J. Med. Res.** 58(9):1285-1289.

Gey, K. F. (1998) Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. **Biofactors**. 7(1-2):113-174.

Green, I. R., Tocoli, F. E., Lee, S. H., Nihei, K. I. and Kubo, I. (2007). Design and evaluation of anacardic acid derivatives as anticavity agents. **Eur. J. Med. Chem**. 16(4):123-138.

Grover, I. S., Bala, S. (1993). Studies on antimutagenic effects of guava (Psidium guajava) in Salmonella typhimurium. **Mutat. Res.** 300(1):1-3.

Halliwell B. (2001). Vitamin C and genomic stability. **Mutat. Res.** 475(1-2):29-35.

Halliwell, B., Gutteride, J.M.C. (2007). Free Radicals in Biology and Medicine. 4rd. ed. New York: Editora Oxford.

Halliwell, B., Gutteridge, J.M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. **Arch. Biochem. Biophys**. 246:501-514.

Hanamura, T., Hagiwara, T. and Hirokazu, K. (2005). Strutural and Functional Characterization of polyphenols isolated from Acerola fruit. **Bios. Biotechnol. Biochem.** 69(2):280-286.

Harborne, J. B., Williams, C. A. (2000). Advances in flavonoid research since 1992. **Phytochemistry** 55(6):481-504.

Harris, E.D. (1992). Regulation of antioxidant enzymes. **FASEB** 6:2675-2683.

Hartwig, A. (2001) Role of magnesium in genomic stability. **Mutat. Res.** 475(1-2):113-

121.

Hassimoto, N.M.A., Genovese, M.I., Lajolo, F.M. (2005) Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruits pulps. **J. Agric. Food Chem.** 53:2928–2935.

Henriques, J.A.P., Dafré, A.L., Picada, J.N., Maris, A.F., Salvador, M. (2001). Espécies reativas de oxigênio e avaliação de antioxidantes em sistemas biológicos. In: Biotecnologia na Agricultura e na Agroindústria. Guaíba: Editora Agropecuária, 463p.

Heo, H.J., Lee, C.Y. (2005) Strawberry and its anthocyanins reduce oxidative stress-induced apoptosis in PC12 cells. **J. Agric. Food Chem.** 23;53(6):1984-9.

Herraiz, T., Galisteo, J. (2003). Tetrahydro-beta-carboline alkaloids occur in fruits and fruit juices: activity as antioxidants and radical scavengers. **J. Agric. Food Chem.** 51(24):7156-7161.

Hertog, M. G.; Hollman, P. C.; Katan, M. B.; Kromhout, D. (1993) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. **Nutr. Cancer** 20:21–29.

Higashimoto, M., Yamato, H., Kinouchi, T. and Ohnishi, Y. (1998). Inhibitory effects of citrus fruits on the mutagenicity of 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid treated with nitrite in the presence of ethanol. **Mutat. Res.** 415(3):219-226.

Hoelzl, C., Bichler, J., Ferk, F., Simic, T., Nersesyan, A., Elbling, L., Ehrlich, V., Chakraborty, A., Knasmüller, S. (2005). Methods for the detection of antioxidants which prevent age related diseases: a critical review with particular emphasis on human intervention studies. **J. Physiol. Pharmacol.** 56 (2):49-64.

Hoover, L. L., Burton, E. G., Brooks, B. A., Kubalak, S. W. (2008) The expanding role for retinoid signaling in heart development. **Sci. World J.** 25(8):194-211.

Hosseinimehr, S. J. and Karami, M. (2005). Citrus extract modulates genotoxicity induced by cyclophosphamide in mice bone marrow cells. **J. Pharm. Pharmacol.** 57(4):505-509.

Imao, K., Wang, H., Komatsu, M. and Hiramatsu, M. (1998). Free radical scavenging activity of fermented papaya preparation and its effect on lipid peroxide level and superoxide dismutase activity in iron-induced epileptic foci of rats. **Biochem. Mol. Biol. Int**. 45(1):11-23.

Instituto Brasileiro de Frutas (IBRAF) http://www.ibraf.org.br/ em outubro/2008

Ishige, K., Schubert, D., Sagara, Y. (2001) Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. **Free Radic. Biol. Med.** 30:433–446.

Janisch, K. M., Olschläger, C., Treutter, D. and Elstner, E. F. (2006). Simulated digestion of Vitis vinifera seed powder: polyphenolic content and antioxidant properties. **J. Agric. Food Chem.** 54(13):4839-4848.

Jayaprakasha, G. K., Girennavar, B. and Patil, B. S. (2007). Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different in vitro model systems. **Bioresour Technol.** 99(10):4484-4494.

Jenkins, D. J., Kendall, C.W., Marchie, A., Jenkins, A. L., Augustin, L. S., Ludwig, D. S., Barnard, N. D., Anderson, J. W. (2003). Type 2 diabetes and the vegetarian diet. **Am. J. Clin. Nutr.** 78(3 Suppl):610S-616S.

Jime´nez-Escrig, A., Rinco, M., Pulido, R. and Saura-Calixto, F. (2001). Guava fruit (Psidium guajava L.) as a new source of antioxidant dietary fiber. **J. Agric. Food Chem**. 49: 5489-5493.

Johnson , I. T. (2007) Phytochemicals and cancer. **Proc. Nutr. Soc.** 66: 207–215

Jones, G. P., Briggs, D. R., Wahlquvist, L. M., Flentje, S. B. J. (1990) Dietary fiber content of Australian foods. **Food Aust**. 42(3):143-145.

Kahkonen, M. P., Hopia, A. I. and Heinonen, M. (2001). Berry phenolics and their antioxidant activity. **J. Agric. Food Chem.** 49(8):4076-4082.

Kahle, K., Kraus, M. and Richling, E. (2005). Polyphenol profiles of apple juices. **Mol. Nutr. Food Res.** 49(8):797-806.

Kappel, V.D., Costa, G.M., Scola, G., Silva, F.A., Landell, M.F, Valente, P., Souza, D.G., Vanz, D.C., Reginatto, F.H., Moreira, J.C. (2008) Phenolic content and antioxidant and antimicrobial properties of fruits of Capsicum baccatum L. var. pendulum at different maturity stages. **J. Med. Food** 11(2):267-74.

Katsube, N.; Iwashita, K.; Tsushida, T.; Yamaki, K.; Kobori, M. (2003) Induction of apoptosis in cancer cells by bilberry (Vaccinium myrtillus) and the anthocyanins. **J. Agric. Food Chem**. 51:68-75.

Kaya, B., Creus, A., Velázquez, A., Yanikoğlu, A., Marcos, R. (2002). Genotoxicity is modulated by ascorbic acid. Studies using the wing spot test in Drosophila. **Mutat. Res.** 520(1-2):93-101.

Kedage, V.V., Tilak, J. C., Dixit, J. B., Devasagayam, T. P. and Mhatre, M. (2007). A study of antioxidant properties of some varieties of grapes (Vitis vinifera L.). **Crit. Rev. Food Sci. Nutr.** 47(2):175-185.

Kim, A. J. and Park, S. (2006). Mulberry extract supplements ameliorate the inflammation-related hematological parameters in carrageenan-induced arthritic rats. **J. Med. Food** 9(3):431-5.

Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B. and Yankova, T. (2006). Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from bulgarian herbs. **Phytother. Res.** 20(11):961-965.

Klamt, F., Pizzol, F. D., Dalmolin, R., Roehrs, R., Henriques, J.A.P., Andrade, H.H.R., Ramos, A.L.L.P., Saffi, J., Moreira, J.C. (2003). Genotoxicity, recombinogenicity and cellular preneoplasic transformation induced by vitamin A supplementation. **Mut. Res**. 539: 117-125.

Knödler, M., Conrad, J., Wenzig, E. M., Bauer, R., Lacorn, M., Beifuss, U., Carle, R. and Schieber, A. (2008). Anti-inflammatory 5-(11'Z-heptadecenyl)- and 5-(8'Z,11'Z-heptadecadienyl)-resorcinols from mango (Mangifera indica L.) peels. **Phytochemistry** 69(4):988-993

Konan, N. A., Bacchi, E. M., Lincopan, N., Varela, S. D. and Varanda, E. A. (2007). Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (Anacardium occidentale L.). **J. Ethnopharmacol.** 110(1):30-38.

Lala, G., Malik, M., Zhao, C., He, J., Kwon, Y., Giusti, M. M. and Magnuson, B. A. (2006). Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. **Nutr. Cancer** 54(1):84-93.

Lester, G. E. (2008). Antioxidant, sugar, mineral, and phytonutrient concentrations across edible fruit tissues of orange-fleshed honeydew melon (Cucumis melo L.). **J. Agric. Food Chem.** 56(10):3694-8.

Lester, G. E., Hodges, D. M, Meyer, R. D. and Munro, K. D. (2004). Pre-extraction preparation (fresh, frozen, freeze-dried, or acetone powdered) and long-term storage of fruit and vegetable tissues: effects on antioxidant enzyme activity. **J. Agric. Food Chem.** 21;52(8):2167-2173.

Leu, S. J., Lin, Y. P., Lin, R. D., Wen, C. L., Cheng, K. T., Hsu, F. L. and Lee, M. H. (2006). Phenolic constituents of Malus doumeri var. formosana in the field of skin care. **Biol. Pharm. Bull.** 29(4):740-745.

Li, B.W., Cardozo, M.S. (1994). Determination of total dietary fiber in foods and products with little or no starch, non-enzymatic-gravimetric method: collaborative study. **J. AOAC Int.** 77(3): 687-689.

Lichtenthaler, R., Rodrigues, R. B., Maia, J. G., Papagiannopoulos, M., Fabricius, H. and Marx, F. (2005). Total oxidant scavenging capacities of Euterpe oleracea Mart. (Açaí) fruits. **Int. J. Food Sci. Nutr.** 56(1):53-64.

Linder, M.C. (2001) Copper and genomic stability in mammals. **Mutat. Res.** 475(1–2):141–152.

Liu, Z.Q., Ma, L.P., Liu, Z.L. (1998). Making vitamin C lipophilic enhances its protective effect against free radical induced peroxidation of low density lipoprotein. **Chen. Phys. Lipids.** 95:49-57.

Lohiya N. K., Manivannan, B., Goyal S. and Ansari, A. S. (2008). Sperm motility inhibitory effect of the benzene chromatographic fraction of the chloroform extract of the seeds of Carica papaya in langur monkey, Presbytis entellus entellus. **Asian J. Androl.** 10(2):298-306.

Lopes, M.I.L., Saffi, J., Echeverrigaray, S., Henriques, J.A.P., Salvador, M. (2004) Mutagenic and antioxidant activities of Croton lechleri sap in biological systems. **J. Ethno-Pharm.** 95: 437-445.

Lutsenko, D., Lindahl, T., Wood, R.D. (2002). Quality contol by DNA repair. **Sciense** 286: 1897-1905.

Madeira, S. V. F., Resende, A. C., Ognibene, D. T., Vieira de Sousa, M. A. and Soares de Moura, R. (2005). Mechanism of the endothelium-dependent vasodilator effect of an alcohol-free extract obtained from a vinifera grape skin. **Pharm. Res.** 52:321-327.

Mahan, L. K.; Stump, S. E.(2005) Krause- Alimentos, nutrição e dietoterapia. 11ª ed. São Paulo: Editora Roca.

Mahattanatawee, K., Manthey, J. A., Luzio, G., Talcott, S. T., Goodner, K. and

Baldwin, E. A. (2006). Total antioxidant activity and fiber content of select Florida-grown tropical fruits. **J. Agric. Food Chem.** 54(19):7355-7363.

Matsuura , F.C.A.U., Costa, J.I.P., Folegatti, M.I.S. (2004) Marketing de banana: preferências do consumidor quanto aos atributos de qualidade dos frutos. **Rev. Bras. Frutic.** 26(1): 14-25.

Mehdipour, S., Yasa, N., Dehghan, G., Khorasani, R., Mohammadirad, A. and Abdollahi, R. M. (2006). Antioxidant potentials of Iranian Carica papaya juice in vitro and in vivo are comparable to alpha-tocopherol. **Phytother. Res**. 20(7):591-594.

Mello, A.C., Santos, S.C. (1999). Taninos. In: Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R. Ed. UFSC. Farmacognosia da planta ao medicamento. 3.ed. Porto Alegre. 527p.

Melo Cavalcante A. A., Rubensam, G., Picada, J. N., Gomes da Silva, E., Fonseca Moreira, J. C.; Henriques, J. A. (2003). Mutagenicity, antioxidant potential, and antimutagenic activity against hydrogen peroxide of cashew (Anacardium occidentale) apple juice and cajuina. **Environ. Mol. Mutagen.** 41(5):360-9.

Melo, E.A., Lima, V.L.A.G., Nascimento, P.P. Temperatura no armazenamento de pitanga. **Sci. Agric.** 57(4):629-634.

Miyazawa, M., Okuno, Y., Fukuyama, M., Nakamura, S. and Kosaka, H. (1999). Antimutagenic activity of polymethoxyflavonoids from Citrus aurantium. **J. Agric. Food Chem.** 47(12):5239-5244.

Moure, A., Cruz, J.M., Franco, D., Domínguez, J.M., Sineiro, J., Dominguez, H., Núñez, M.J., Parajó, J.C. (2001). Natural antioxidants from residual sources. **Food Chem.**72:145-171.

Mullen, W., McGinn, J., Lean, M. E., MacLean, M. R., Gardner, P., Duthie, G. G., Yokota, T. and Crozier, A. (2002). Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. **J. Agric. Food Chem.** 50(18):5191-5196.

National Academy of Sciences (1997). Institute of Medicine. Food and Nutrition Board. em http://www.iom.edu/CMS/3708.aspx em outubro/2008.

Nalini, N., Sabitha, K., Chitra, S., Viswanathan, P. and Menon, V. P. (1997). Histopathological and lipid changes in experimental colon cancer: effect of coconut kernal (Cocos nucifera Linn.) and (Capsicum annum Linn.) red chilli powder. **Indian J. Exp. Biol.** 35(9):964-971.

Narasimhamurthy, K., Muralidhara, R. and Raina, P. L. (1999). Absence of in vivo mutagenic potency of heated and fried oils in mice. **Indian J. Exp. Biol**. 37(1):50-55.

Nassiri-Asl, M., Shariati-Rad, S. and Zamansoltani, F. (2007). Anticonvulsant effects of aerial parts of Passiflora incarnata extract in mice: involvement of benzodiazepine and opioid receptors. **BMC Complement. Altern. Med.** 7: 26-29.

National Toxicology Program (1990). NTP Toxicology and Carcinogenesis Studies of d-Limonene (CAS No. 5989-27-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies). **Natl .Toxicol. Program. Tech. Rep. Ser.** 347:1-165.

Nayak, S. B., Pinto Pereira, L. and Maharaj, D. (2007). Wound healing activity of Carica papaya L. in experimentally induced diabetic rats. **Indian J. Exp. Biol.** 45(8):739-743.

Ness, A.R., Powles, J.W. (1997) Fruit and vegetables, and cardiovascular disease: a review. **Int. J. Epidemiol.** 26:1–13.

Norkus, E.P., Kuenzig, W., Conney, A.H. (1983) Studies on the mutagenic activity of ascorbic acid in vitro and in vivo. **Mutat. Res.** 117(1-2):183-191.

Ocké, M.C., Bueno-de-Mesquita, H.B., Feskens, E.J.M., Van Staveren, W.A., Kromhout, D. (1997). Repeated measurements of vegetables, fruits, β-carotene and vitamins C and E in relation to lung cancer. **Am. J. Epidemiol**. 45:145-358.

Osato, J. A., Santiago, L. A., Remo, G. M., Cuadra, M. S. and Mori, A. (1993). Antimicrobial and antioxidant activities of unripe papaya. **Life Sci.** 53(17):1383-1389.

Palozza, P., Luberto, C., Calviello, G., Ricci, P., Bartolli, G.M. (1997).Antioxidant and prooxidant role of beta-carotene in murine normal and tumor thymocyte: effects of oxygen partial pressure. **Free Radic. Biol. Med**. 22:1065-1073.

Parry, E.M., Parry, J.M. (1984). The assay of genotoxicity of chemicals using the budding yeast *Saccharomyces cerevisiae*. In: Mutagenicity testing: A practical approach, Venitt, S., Parry, S.M. (Eds), ORL Press, Oxford, Washington DC.

Pedrão, M.R.; Beleia, A.; Modesta, R.C.D.; Prudêncio-Ferreira, S.M. (1999). Estabilidade físico-química e sensorial do suco de limão tahiti natural e adoçado, congelado. **Ciênc. Tecnol. Aliment.** 19(2): 282-286.

Pelegrini, P. B., Murad, A. M., Silva, L. P., Dos Santos, R. C., Costa, F. T., Tagliari, P. D., Bloch Jr, C., Noronha, E. F., Miller, R. N. and Franco, O. L. (2008). Identification of a novel storage glycine-rich peptide from guava (Psidium guajava) seeds with activity against Gram-negative bacteria. **Peptides**. 17(2): 132-142.

Percival, S. S., Talcott, S. T., Chin, S. T., Mallak, A. N., Lounds-Singleton, A. and Pettit-Moore, J. (2006). Neoplastic Transformation of BALB/3T3 Cells and Cell Cycle of HL-60 Cells are Inhibited by Mango (Mangifera indica L.) Juice and Mango Juice Extracts. **J. Nutr.** 136(5):1300-1304.

Petta, T. B., de Medeiros, S. R., do Egito, E. S. and Agnez-Lima, L. F. (2004). Genotoxicity induced by saponified coconut oil surfactant in prokaryote systems.

Mutagenesis 19(6):441-4.

Picada, J.N., Kern, A.L., Ramos, L.P., Saffi, J. (2003). O estresse oxidativo e as defesas antioxidantes. In: Silva, J., Erdtmann, B., Henriques, J.A.P. Genética Toxicológica. Porto Alegre.

Prá, D., Franke, S.I., Giulian, R., Yoneama, M.L., Dias, J.F., Erdtmann, B., Henriques, J. A. (2008). Genotoxicity and mutagenicity of iron and copper in mice. **Biometals** 21(3):289-297.

Pungartnik, C., Viau, C., Picada, J., Caldeira-de-Araújo, A., Henriques, J.A., Brendel, M. (2005) Genotoxicity of stannous chloride in yeast and bacteria. **Mutat. Res.** 583(2):146-157.

Rababah, T. M., Ereifej, K. I. and Howard, L. (2005). Effect of ascorbic acid and dehydration on concentrations of total phenolics, antioxidant capacity, anthocyanins, and color in fruits. **J. Agric. Food Chem.** 53(11):4444-4447.

Rabello-Gay, M.N.; Rodrigues, M.A.L.A.R. & Monteleone Neto, R. (1991). Mutagênese Teratogênese e Carcinogênese: métodos e critérios de avaliação. Sociedade Brasileira de Genética, São Paulo.

Rao, A.V. (2002). Lycopene, tomatoes and the prevention of coronary hart disease. Exp. Radic. **Biol. Med.** 22:1065-1073.

Raspor, P., Plesnicar, S., Gazdag, Z., Pesti, M., Miklavcic, M., Lah, B., Logar-Marinsek, R., Poljsak, B. (2005) Prevention of intracellular oxidation in yeast: the role of vitamin E analogue, Trolox (6-hydroxy-2,5,7,8-tetramethylkroman-2-carboxyl acid). **Cell. Biol. Int.** 29(1):57-63.

Reinhold, J. G., Ismail-Beigi, F. & Faradji, B. (1975) Fibre vs. phytate as determinants of availability of calcium, zinc and iron by fibre in breadstuffs. **Nutr. Rep. Int.** 12: 1-8.

Riccioni, G., Mancini, B., Di Ilio, E., Bucciarelli, T., D'Orazio, N. Protective effect of lycopene in cardiovascular disease. (2008) **Eur. Rev. Med. Pharmacol. Sci**.12(3):183-90.

Rice-Evans, C.A.; Miller, N.J.; Bolwell, P.G.; Bramley, P.M.; Pridham, J.B. (1995). The relative antioxidants activities of plant-derived polyphenolic flavonoids. **Free Rad. Res.** 22(4): 375-383.

Rizzon, L.A., Beranrdi, J., Miele, A. (2005). Características analíticas dos sucos de maçã gala, golden delicious e Fuji. **Ciênc. Tecnol. Aliment.** 25(4):750-756.

Rocha A. P., Carvalho, L. C., Sousa, M. A., Madeira, S. V., Sousa, P. J., Tano, T., Schini-Kerth, V. B., Resende, A. C. and Soares de Moura, R. (2007). Endothelium-dependent vasodilator effect of Euterpe oleracea Mart. (Açaí) extracts in mesenteric vascular bed of the rat. **Vascul. Pharmacol.** 46(2):97-104.

Rodrigues RB, Lichtenthäler R, Zimmermann BF, Papagiannopoulos M, Fabricius H, Marx F, Maia JG, Almeida O (2006). Total oxidant scavenging capacity of Euterpe oleracea Mart. (açaí) seeds and identification of their polyphenolic compounds. **J. Agric. Food Chem.** 54:4162-4167.

Rodriguez, J., Di Pierro, D., Gioia, M., Monaco, S., Delgado, R., Coletta, M. and Marini, S. (2006). Effects of a natural extract from Mangifera indica L, and its active compound, mangiferin, on energy state and lipid peroxidation of red blood cells. **Biochim. Biophys. Acta** 1760(9):1333-1342.

Rojas, E., Herrera, L.A., Poirier, L.A., Ostrosky-Wegman, P. (1999) Are metals dietary carcinogens? **Mutat. Res.** 443(1-2):157-181.

Rueff, J., Laires, A., Gaspar, J., Borba, H., Rodrigues, A. (1992) Oxygen species and the genotoxicity of quercetin. **Mutat. Res.** 265(1):75-81.

Saffi, J., Sonego, L., Varela, Q.D., Salvador, M. (2006) Antioxidant activity of L-ascorbic acid in wild-type and superoxide dismutase deficient strains of Saccharomyces cerevisiae. **Redox Rep**. 11(4):179-184.

Sairam, K., Hemalatha, S., Kumar, A., Srinivasan, T., Ganesh, J., Shankar, M. and Venkataraman, S. (2003). Evaluation of anti-diarrhoeal activity in seed extracts of Mangifera indica. **J. Ethnopharmacol**. 84(1):11-15.

Salgado, S.M., Guerra, N.B., Melo Filho, A.B. (1999). Polpa de fruta congelada: efeito do processamento sobre o conteúdo de fibra alimentar. **Rev. Nutr**. 12(3):303-308.

Sanchez-Moreno, C., Cano, M.P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F. (2006) Mediterranean vegetable soup consumption increases plasma vitamin C and decreases F2-isoprostanes, prostaglandin E2 and monocyte chemotactic protein-1 in healthy humans. **J. Nutr. Biochem.** 17:183–189.

Sandhya, V. G. and Rajamohan, T. (2006). Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats. **J. Med. Food** 9(3):400-407.

Santos, G.B., Açaí: Aspectos químicos e farmacológicos, 2001. 150p. Dissertação de Mestrado em Ciências Farmacêuticas – Faculdade de Farmácia, UFRJ, Rio de Janeiro.

Schauss, A. G., Wu, X., Prior, R.L., Ou, B., Huang, D., Owens, J., Agarval, A., Jensen, G.S., Hart, A.N., Shanbro, E. (2006) Antioxidant capacity and other bioactivities of the freeze-dried amazonian palm berry Euterpe oleraceae Mart. (acai). **J. Agric. Food Chem.** 54:8604-8610.

Seifried, H. E., Anderson, D. E., Fisher, E.I., Milner, J.A. (2007) A review of the interaction among dietary antioxidants and reactive oxygen species. **J. Nutr. Biochem.** 18(9):567-579.

Shafiee, M., Carbonneau, M. A., Urban, N., Descomps, B. and Leger, C. L. (2003). Grape and grape seed extract capacities at protecting LDL against oxidation generated by Cu2+, AAPH or SIN-1 and at decreasing superoxide THP-1 cell production. A comparison to other extracts or compounds. **Free Radic. Res**. 37(5):573-584.

Shils, M.E., Olson, J. A., Shike, M. (1994) Modern nutrition in health and disease. 8a ed. Philadelphia, Lea & Febiger.

Silalahi, J. (2002). Anticancer and health protective properties of citrus fruit components. **Asia Pac. J. Clin. Nutr.** 11:79–84.

Da Silva, K.V., Henriques, J. A. (1987). Induction of the cytoplasmic 'petite' mutation in pso mutants of Saccharomyces cerevisiae by photoaddition of furocoumarins or by ultraviolet radiation. **Mutagenesis** 2(3):187-197.

Singh, M., Arseneault, M., Sanderson, T., Murthy, V., Ramassamy, C. (2008). Challenges for research on polyphenols from foods in Alzheimer's disease: bioavailability, metabolism, and cellular and molecular mechanisms. **J. Agric. Food Chem.** 56(13):4855-73.

Soares de Moura, R., Miranda, D. Z., Pinto, A. C. A., Sicca, R. F., Souza, M. A. V., Rubenich, L. M. S., Carvalho, L. C. R. M., Rangel, B. M., Tano, T., Madeira, S. V. F., Resende, A. C. (2004). Mechanism of the endothelium-dependent vasodilation and the antihypertensive effect of brazilian red wine. **J. Cardiovasc. Pharmacol.** 44:302-309

Soares, D.G., Andreazza, A.C., Salvador, M. (2003). Sequestering ability of butylated hydroxytoluene, propyl gallate, resveratrol, and vitamins C and E against ABTS, DPPH, and hydroxyl free radicals in chemical and biological systems. **J. Agric. Food Chem.** 51:1077-1080.

Spada, P.D., de Souza, G.G., Bortolini, G.V., Henriques, J.A., Salvador, M. (2008). Antioxidant, mutagenic, and antimutagenic activity of frozen fruits. **J. Med. Food.**

11(1):144-151

Spada, P.K.W. D.S., Salvador M. (2005) Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. **J. Agric. Food Chem.** 53(12):4757-4761.

Stagos, D., Kazantzoglou, G., Magiatis, P., Mitaku, S., Anagnostopoulos, K. and Kouretas, D. (2005). Effects of plant phenolics and grape extracts from Greek varieties of Vitis vinifera on Mitomycin C and topoisomerase I-induced nicking of DNA. **Int. J. Mol. Med**. 15(6):1013-1022.

Stoclet, J.C., Chataigneau, T., Ndjaye, M., Oak, M.-H., Bedoui, J.E., Chataigneaus, M., Schni-Kerth, V.B., (2004). Vascular protection by dietary polyphenols. **Eur. J. Pharmacol**. 500: 299–313.

Taguri, T., Tanaka, T., Kouno, I. (2004) Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. **Biol. Pharm. Bull**. 27(12):1965-1969.

Tang, X., Edenharder, R. (1997). Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene by vitamins, porphyrins and related compounds, and vegetable and fruit juices and solvent extracts. **Food Chem. Toxicol.** 35(3-4):373-378.

Thimothe, J., Bonsi, I. A., Padilla-Zakour, O. I. and Koo, H. (2007). Chemical characterization of red wine grape (Vitis vinifera and Vitis interspecific hybrids) and pomace phenolic extracts and their biological activity against Streptococcus mutans. **J. Agric. Food Chem.** 55(25):10200-10207.

Toit, D.R., Volsteedt, Y., Apostolides, Z. (2001). Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. **Elsevier** 166: 63-69.

Trevisan, M. T. S., Pfundstein, B., Haubner, R., Würtele, G., Spiegelhalder, B. and Bartsch, H., Owen, R. W. (2006). Characterization of alkyl phenols in cashew (Anacardium occidentale) products and assay of their antioxidant activity. **Food Chem. Toxicol.** 44(2): 188-197.

Trueba, G.P., Sánches, M. (2001). Los flavonoides como antioxidantes naturales. **Acta Farma. Bonaerense** 20: 297-306.

Valli, C., Paroni, G., Di Francesco, A.M., Riccardi, R., Tavecchio, M., Erba, E., Boldetti, A., Gianni, M., Fratelli, M., Pisano, C., Merlini, L., Antoccia, A., Cenciarelli, C., Terao, M., Garattini, E. (2008) Atypical retinoids ST1926 and CD437 are S-phase-specific agents causing DNA double-strand breaks: significance for the cytotoxic and antiproliferative activity. **Mol. Cancer Ther.** 7(9):2941-54.

Van Poppel, G., Van Der Berg, H. (1997). Vitamins and cancer, **Cancer Lett**. 114, 195-202.

Viljanen, K., Kylli, P., Kivikari, R. and Heinonen, M. (2004). Inhibition of protein and lipid oxidation in liposomes by berry phenolics. **J. Agric. Food Chem.** 52(24):7419-7424.

Vouldoukis, I., Conti, M., Krauss, P., Kamate, C., Blazquez, S., Tefit, M., Mazier, D., Calenda, A. and Dugas, B. (2004). Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress. **Phytother Res.** 18(12):957-962.

Wada, L. and Ou, B. (2002). Antioxidant activity and phenolic content of Oregon caneberries. **J. Agric. Food Chem.** 50(12):3495-3500.

Wang, H., Cao, G., Prior, R.L. (1996) Total antioxidant capacity of fruits. **J. Agric. Food Chem.** 44:701–705.

Wang, J., Mazza, G. (2002) Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor alpha in LPS/IFN-gamma-activated RAW 264.7 macrophages. **J. Agric. Food Chem.** 50(15):4183-9.

Wang, S. Y. and Jiao, H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. **J. Agric. Food Chem.** 48(11):5677-5684.

Wheeler, G.L., Jones, M., Sminorff, N. (1998). The biosynthetic pathway of vitamin C in higher plants. **Nature** 383: 365-369.

Yamaguchi, T.; Takamura, H.; Matoba, T.C. & Terao, J. (1998). HPLC method for evaluation of the free radical- scavenging activity of foods by using 1,1 diphenyl-2 picrylhydrazyl. **Biosci. Biotechnol. Biochem**. 62(6): 1201-1204.

Yamashita, F., Benassi, M.T., Tonzar, A.C., Moriya, S.; Fernandes, J.G. (2003). Produtos de acerola: estudo da estabilidade de vitamina C. **Ciênc. Tecnol. Aliment**. 23(1): 92-94.

Yang, H., Protiva, P., Cui, B., Ma, C., Baggett, S., Hequet, V., Mori, S., Weinstein, I. B. and Kennelly, E. J. (2003). New bioactive polyphenols from Theobroma grandiflorum ("cupuacu"). **J. Nat. Prod.** 66(11):1501-4.

Yeh, C.T., Yen, G.C. (2005) Effect of sulforaphane on metallothionein expression and induction of apoptosis in human hepatoma HepG2 cells. **Carcinogenesis** 2005 26(12):2138-48.

Yong, L.C., Brown, C.C., Schatzkin, A., Dresser, C.M., Slesinski, M.J., Cox, C.S., Taylor, P.R. (1997). Intake of vitamins E, C and A and risk of lung cancer. The NHANES I epidemiologic followup study. **Am. J. Epidemiol**. 38:146-231.

Zimmermann, F.K.; Van Borstel, R.C.; Von Halle, E.S.; Parry, J.M.; Siebert, G.; Zetterberg, G.; Barale, R. & Loprieno, N. (1984). Testing chemicals for genetic activity weith *Saccharomyces cerevisiae*: a report the U.S. environmental protection agency geno-tox program mutation. **Mutat. Res.** 133: 199-244.

7. ANEXO

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