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EFEITO DO PROCESSO DE OBTENÇÃO DO CATCHUP SOBRE SEUS COMPOSTOS ANTIOXIDANTES, CAPACIDADE SEQÜESTRANTE DO RADICAL DPPH$^{\bullet}$ E COR

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SUMÁRIO

RESUMO

O Brasil é o nono produtor mundial de tomates sendo os primeiros lugares em produção ocupados pelos Estados de Goiás, São Paulo e Minas Gerais. Os tomates são um dos vegetais mais versáteis podendo ser consumidos frescos ou como produtos processados (suco, catchup, molhos e sopas). Os tomates contêm compostos bioativos, como os carotenóides (principalmente, licopeno), ácido ascórbico e flavonóides (rutina como predominante), com propriedades antioxidantes e outras funções importantes para a saúde humana. Uma vez que a composição desses compostos nos tomates pode ser afetada por processamentos térmicos, o estudo do efeito do processamento dos tomates torna-se de grande interesse. O objetivo geral deste trabalho foi verificar o efeito do processo de obtenção do catchup sobre seus compostos antioxidantes (fenólicos totais, flavonóides totais, licopeno, β-caroteno, ácido L-ascórbico, rutina, quercetina, naringenina e kaempferol), capacidade seqüestrante do radical $DPPH^{\bullet}$ (% de inibição do radicl livre e capacidade antioxidante equivalente em vitamina C - VCEAC) e cor (parâmetros CIELab). Os tomates frescos (*Lycopersicon esculentum* cv. AP533) e seus produtos derivados (polpa de tomate e catchup) foram coletados em três diferentes datas de processamento industrial. Ao longo do trabalho, outros objetivos específicos surgiram e estes também foram avaliados: (i) variação dos compostos antioxidantes, atividade antioxidante e cor em tomates colhidos em cinco datas diferentes, e (ii) comparação da composição dos compostos antioxidantes, atividade antioxidante e cor entre dois tipos comerciais de catchup. Os tomates colhidos em Setembro 2006 (final) apresentaram teores significativamente ($p < 0,05$) maiores em fenólicos totais, licopeno e rutina, e menor teor em ácido L-ascórbico. Conseqüentemente, a atividade antioxidante equivalente em vitamina C (VCEAC) foi também significativamente ($p < 0,05$) menor. A partir desses resultados pudemos inferir que esses frutos provavelmente estavam em um estádio mais avançado de maturação do que os demais. A cor vermelha desses tomates foi significativamente ($p < 0,05$) mais intensa e viva do que a dos demais. O processo de obtenção do catchup resultou na significativa ($p < 0,05$) diminuição dos teores em flavonóides totais (84 %), fenólicos totais (63 %), licopeno (92 %), ácido L-ascórbico (81 %), rutina (72 %) e kaempferol (87 %), e da atividade antioxidante expressa em VCEAC (89 %). O catchup apresentou uma cor vermelho-amarronzada mais viva e escura do que a do tomate provavelmente devido ao escurecimento não-enzimático e degradação do licopeno. O teor de polpa de tomate utilizado na produção dos dois tipos comerciais de catchup influenciou nos teores de seus compostos antioxidantes, atividade antioxidante e cor. O catchup produzido com 4 % a menos de polpa de tomate apresentou teores significativamente ($p < 0,05$) menores em fenólicos totais, flavonóides totais, ácido ascórbico e rutina, e conseqüentemente uma menor ($p < 0,05$) capacidade seqüestrante do radical DPPH• e VCEAC. A cor vermelha deste catchup foi menos intensa, mais apagada e clara do que a do outro tipo.

ABSTRACT

Brazil is the ninth world producer of tomatoes being the first in production occupied by the states of Goias, Sao Paulo and Minas Gerais. Tomatoes are one of the most versatile vegetable can be eaten fresh or as processed products (juice, ketchup, sauces and soups). Tomatoes contain bioactive compounds such as carotenoids (mainly, lycopene), flavonoids and ascorbic acid (as rutin predominant), with antioxidant properties and other important functions to human health. Since the composition of these compounds in tomatoes may be affected by thermal process, the study of the effect of the processing of tomatoes becomes of great interest. The general objective of this study was to evaluate the effect of the process of obtaining the catchup on their antioxidant compounds (total phenolics, total flavonoids, lycopene, β-carotene, L-ascorbic acid, rutin, quercetin, naringenin and kaempferol), the radical capacity seqüestrante $DPPH^{\bullet}$ (% free radical inhibition and vitamin C equivalent antioxidant capacity - VCEAC) and color (parameters CIELab). The fresh tomatoes (*Lycopersicon esculentum* cv. AP533) and their sub-products (tomato pulp and ketchup) were collected on three different dates of industrial processing. Throughout the work, other specific goals have emerged and they were also evaluated: (i) changes in antioxidant compounds, antioxidant activity and color in tomatoes harvested in five different dates, and (ii) compared the composition of antioxidant compounds, antioxidant activity and color between two types of commercial ketchup. The tomatoes harvested in September 2006 (late) showed levels significantly ($p < 0.05$) higher in total phenolics, lycopene and rutin, and lower levels of L-ascorbic acid. Consequently, the in vitamin C equivalent antioxidant capacity (VCEAC) was also significantly ($p < 0.05$) lower. From these results we could infer that these fruits were probably in a more advanced stage of maturity than other. The red color of tomatoes was significantly ($p < 0.05$) more intense and vivid than other. The ketchup manufacture resulted in significant ($p < 0.05$) decrease in levels of total flavonoids (84 %), total phenolics (63 %), lycopene (92 %), L-ascorbic acid (81 %), rutin (72 %) and kaempferol (87 %), and in antioxidant activity expressed as VCEAC (89 %). The ketchup presented a reddish-brown color more vivid and darker than that of tomato probably due to non-enzymatic browning and lycopene degradation. The tomato pulp content used in the production of two types of commercial catchup influenced in their antioxidant compounds levels, antioxidant activity and color. The ketchup produced with less than 4 % of tomato pulp showed levels significantly ($p < 0.05$) lower in phenolic total, total flavonoids, ascorbic acid and rutin, and consequently a lower ($p < 0.05$) $DPPH^{\bullet}$ scavenging capacity and VCEAC. The red color of this ketchup was less intense, more grayish and very pale than the other type.

INTRODUÇÃO GERAL

As frutas e os vegetais são fontes de antioxidantes naturais (carotenóides, flavonóides e ácido ascórbico) que apresentam inúmeras atividades biológicas importantes para a saúde humana tais como a inibição do desenvolvimento de doenças cardíacas e de certos tipos de câncer (GARDNER et al., 2000).

Os efeitos benéficos promovidos à saúde por esses antioxidantes naturais têm aumentado o interesse sobre a sua composição nos diferentes alimentos. A estabilidade desses compostos durante os processamentos térmicos de frutas e de vegetais é outro fator importante visto poderem ser degradados por oxidação. Entretanto, estudos dos efeitos dos tratamentos térmicos industriais utilizados na produção de alimentos sobre a atividade dos antioxidantes presentes ainda são poucos (EWALD et al., 1999).

Os tomates são um dos vegetais mais versáteis podendo ser consumidos frescos ou como produtos processados. Os tomates e seus produtos são ricos em nutrientes como carotenóides (principalmente licopeno), flavonóides (naringenina e rutina como predominantes), ácido ascórbico, vitamina E, folato, potássio e fibras (GAHLER et al., 2003; SAHLIN et al., 2004; TOOR e SAVAGE, 2005).

Os carotenóides são pigmentos naturais, amplamente distribuídos na natureza e utilizados principalmente como corantes na indústria alimentícia. Entretanto, tem-se observado que os carotenóides possuem outras atividades além da pró-vitamínica A como a antioxidante, proteção contra degeneração macular relacionada à idade e cataratas, redução do risco de doenças cardiovasculares e atuação sobre certos tipos de câncer.

Os flavonóides, outra classe de pigmentos naturais, são tão importantes quanto à dos carotenóides por apresentarem inúmeras atividades benéficas à saúde humana, tais como a antioxidante, antiinflamatória, antitrombótica e antialérgica (MIEAN e MOHAMED, 2001; LE GALL et al., 2003).

O ácido ascórbico também é um antioxidante natural que pode prevenir o desenvolvimento de doenças cardíacas e de certos tipos de cânceres (GARDNER et al., 2000). Adicionalmente, atua em sinergismo com os carotenóides e flavonóides na promoção de uma barreira efetiva contra a oxidação celular (YOUNG e LOWE, 2001; MIEAN e MOHAMED, 2001).

O tomate, fruto do tomateiro, pertence à família Solanaceae e ao gênero *Solanum*, é conhecido botanicamente como *Lycopersicum esculentum.* Originário das Cordilheiras dos Andes, na América do Sul, foi levado para o sul da Europa pelos espanhóis (FEAGRI, 2007).

A tomaticultura nacional é importante economicamente devido à exportação anual de mais de quatro milhões de toneladas de tomates e também, pelo fruto ser uma das hortaliças mais consumidas no mundo, precedida apenas pela batata e pela cebola. O Brasil é o 9º maior produtor de tomates (3,5 milhões de toneladas ao ano), o 12º em área cultivada e o 4º em produtividade média sendo responsável por 3 % da produção mundial em 1 % da área plantada no mundo. Nos últimos anos, as exportações de tomate vêm apresentando crescimento gradativo com um aumento de 23 % no preço obtido pelo produto nas negociações, de 28 % no volume e de 57 % no valor de vendas. As maiores participações na produção nacional, por estado, são Goiás (23 %), São Paulo (21 %) e Minas Gerais (18 %). Da produção nacional de tomate, 65 % destinam-se ao consumo *in natura* e 35 % para o processamento industrial (CARVALHO et al., 2005; EPAGRI-CEPA, 2007). Dentre os principais cultivares utilizados no processamento se encontra o IPA-6, Viradouro, AP533, Heinz (9553, 9665 e 9992), H 7155N, Hypeel 108, Malinta, Calroma, RPT 1570 e Calmazano com maturação entre 100-125 dias (EMBRAPA, 2008).

Desta forma, o estudo do efeito do processamento térmico de tomates na produção de polpa de tomate e de catchup sobre a composição dos carotenóides, flavonóides e ácido ascórbico, e sobre a atividade antioxidante tornam-se importantes.

OBJETIVOS

Os objetivos deste trabalho são:

- **Geral:** Estudar o efeito do processo de obtenção do catchup sobre seus compostos antioxidantes (fenólicos totais, flavonóides totais, licopeno, β-caroteno, ácido L-ascórbico, rutina, quercetina, naringenina e kaempferol), atividade antioxidante (capacidade seqüestrante do radical $DPPH^{\bullet}$ e VCEAC) e cor.
- **Específicos:** (i) Determinar a composição dos carotenóides e flavonóides em tomates frescos e seus produtos processados (polpa de tomate e catchup) por cromatografia líquida de alta eficiência (CLAE), (ii) Avaliar a influência da data de colheita sobre os compostos antioxidantes, atividade antioxidante e cor em tomates, e (iii) Comparar a composição dos compostos antioxidantes, atividade antioxidante e cor entre dois tipos comerciais de catchup.

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Capítulo 1

Revisão Bibliográfica.

1. COMPOSTOS BIOATIVOS COM PROPRIEDADES ANTIOXIDANTES PRESENTES EM TOMATES

O conteúdo dos compostos bioativos presentes nos tomates pode ser afetado pela variedade, localização climática ou geográfica da produção (temperatura, luminosidade, irrigação, nutrientes no solo), estádio de maturação (quanto maior o grau de maturação, maior a concentração desses nutrientes), colheita e pós-colheita, processamento e estocagem (RODRIGUEZ-AMAYA, 1999; CANO et al., 2003; DUMAS et al., 2003; RAFFO et al., 2006).

Há evidencias de que os vegetais podem proteger o organismo humano contra doenças crônicas cardio e cérebro-vascular, oculares, neurológicas, e certos tipos de cânceres, propriedades atribuídas aos seus constituintes antioxidantes. Nos tomates, os principais são os carotenóides (principalmente licopeno), flavonóides (rutina como predominante), ácido ascórbico (vitamina C), α-tocoferol (vitamina E), ácidos fenólicos, glutationa e outros compostos não identificados (GAHLER et al., 2003; SAHLIN et al., 2004; TOOR e SAVAGE, 2005).

Dessa forma, os compostos bioativos antioxidantes, naturalmente presentes nos alimentos, têm despertado interesse devido aos efeitos em relação à prevenção de doenças e propriedades biológicas importantes à saúde humana.

1.1. Agentes Antioxidantes

Os antioxidantes são compostos que em baixa concentração, quando comparada ao substrato oxidável, atrasam ou previnem a oxidação de lipídios ou outras moléculas por capturarem radicais livres e formas tóxicas do oxigênio, evitando o início ou propagação das reações em cadeia de oxidação (YOUNG e LOWE, 2001; DEGÁSPARI e WASZCZYNSKYJ, 2004).

Os radicais livres são átomos ou grupos de átomos, com um elétron desemparelhado ($R^{\bullet}$), sendo altamente reativos e cujos produtos de suas reações geram outros radicais livres (reação em cadeia). Os radicais, tais como o mercaptoetanol tiol ($HOCH_2CH_2S^{\bullet}$), metanosufonil ($CH_3SO_2^{\bullet}$), glutationa tiol ($GS^{\bullet}$) e dióxido de nitrogênio ($NO_2^{\bullet}$), são gerados normalmente no metabolismo e intensificados após exposição ao cigarro, estresse, luz solar e poluição (YOUNG e LOWE, 2001).

As formas tóxicas do oxigênio compreendem o oxigênio singleto ($^1O_2$) e as espécies reativas do oxigênio, $O_2^{\bullet-}$ (radical ânion superóxido), $HOO^{\bullet}$ (radical peroxil originário de peróxido de hidrogênio), $LOO^{\bullet}$ (radical peroxil lipídico originário de lipídios peróxidos) e $HO^{\bullet}$ (radical hidroxil).

O estresse oxidativo é o resultado do desequilíbrio entre a peroxidação e a antioxidação, com maior produção de espécies reativas de oxigênio e menor produção de antioxidantes, caracterizando-se, principalmente, pela peroxidação da membrana lipídica celular (AW et al., 1991; EVELO et al., 1992).

As frutas, os vegetais e os condimentos contêm numerosos fitoquímicos, além dos compostos fenólicos, como os carotenóides, o ácido ascórbico e os tocoferóis que apresentam significante capacidade antioxidante (DEGÁSPARI e WASZCZYNSKYJ, 2004).

As substâncias antioxidantes podem ser classificadas em dietéticas, intra e extracelulares. Podemos ainda ser denominadas de preventivas, pois inibem a formação de radicais livres; de varredoras, por impedirem a destruição celular através de ação direta dos radicais livres nas células; e de reparadoras, ao proporcionarem a reconstituição da membrana celular lesada e ainda remover o dano ao DNA (BURK, 1983; CLARK, 2002).

Dentre os antioxidantes dietéticos temos o zinco e o selênio, que atuam como preventivos, e a vitamina C, os carotenóides e os flavonóides que compõem o grupo dos varredores (BURK, 1983).

De acordo com Huang et al. (2005), o comportamento cinético dos compostos antioxidantes pode ser classificado em rápido (< 5 min), intermediário (5-30 min) e lento (> 30 min); por exemplo, o ácido ascórbico apresenta um comportamento rápido (1,15 min) e a rutina um lento (103 min).

Atividade (capacidade ou potencial) antioxidante é um parâmetro amplamente utilizado (isolado ou com outros) para caracterizar diferentes matrizes como os vegetais, vinhos, óleos, chás, etc. Inúmeros métodos são utilizados na medição desta atividade sendo os preferidos, pela facilidade, rapidez e sensibilidade, aqueles com compostos cromógenos de natureza radicalar que simulam espécies oxigênio (e nitrogênio) reativas como $ABTS^{+}$ e $DPPH^{\bullet}$ (1,1-difenil-2-picrilhidrazila) (ARNAO, 2000).

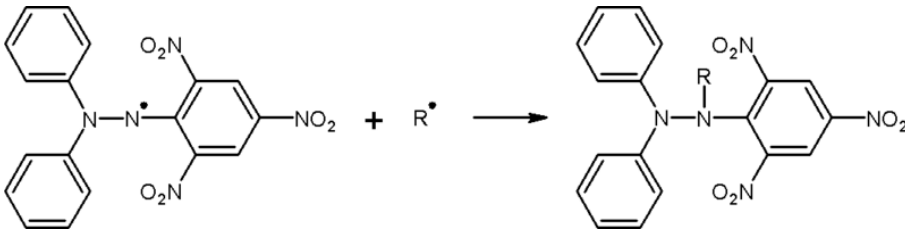
O $DPPH^{\bullet}$ é um radical livre adquirido diretamente sem preparo enquanto que, o $ABTS^{+}$ é gerado por reações enzimáticas ou químicas. O $DPPH^{\bullet}$ é um radical muito estável, solúvel em meio orgânico (especialmente alcoólico) e insolúvel em meio aquoso, e apresentando pico de absorbância a 515 nm (ε = 12,5 mM^{-1} cm^{-1}) em meio metanólico (ARNAO, 2000).

O $DPPH^{\bullet}$ apresenta coloração violeta e na presença de doadores de hidrogênio (antioxidantes seqüestrantes de radicais livres) se reduz tornando-se amarelo o que é monitorado pelo decréscimo na absorbância durante a reação ou até atingir-se um platô (Figura 1). Esta reação é amplamente utilizada para testar a habilidade de compostos em seqüestrarem radicais livres ou doadores de hidrogênio, e assim, avaliar a atividade antioxidante de alimentos e extratos vegetais (YAMAGUCHI et al., 1998).

Usualmente, os métodos utilizados expressam a capacidade antioxidante dos alimentos testados como capacidade antioxidante equivalente em Trolox (TEAC) ou valor IC_{50} em unidades molares. O Trolox, potente antioxidante análogo hidrossolúvel da vitamina E, não é um composto naturalmente presente nos alimentos. Por outro lado, a vitamina C,

comumente reportada como majoritária é encontrada como nutriente e antioxidante em nossa dieta diária (KIM et al., 2002).

A capacidade antioxidante expressa em base molar como equivalente em Trolox ou IC_{50} é de difícil compreensão. Adicionalmente, as informações nutricionais dos alimentos são expressas em base úmida e não em unidades molares. Dessa forma, a capacidade antioxidante equivalente em vitamina C (VCEAC) calculada em base úmida (mg/100 g ou mg/ 100 mL) é mais adequada do que as mencionadas anteriormente (KIM et al., 2002).



1,1-difenil-2-picrilhidrazila
(DPPH)
violeta

DPPH : H
amarelo pálido

FIGURA 1. Esquema do seqüestro do DPPH$^{\bullet}$ pelo antioxidante (R$^{\bullet}$).

1.2. Compostos Fenólicos

Os compostos fenólicos são metabólitos secundários, comumente referidos como polifenóis, presentes em todas as plantas e na dieta humana também. Há mais de 8.000 estruturas fenólicas desde moléculas simples (ácidos fenólicos com anel C_6) até compostos altamente polimerizados como os taninos (KRIS-ETHERTON et al., 2002).

Os compostos fenólicos classificam-se em flavonóides e não-flavonóides. Os compostos fenólicos tipo não-flavonóides são classificados em (i) derivados das estruturas químicas C_6-C_1 específicas dos ácidos hidroxibenzóico, gálico e elágico, (ii) derivados das estruturas químicas C_6-C_3 específicas dos ácidos hidroxicinâmico, caféico e *p*-cumárico e (iii)

derivados das estruturas químicas C_6-C_2-C_6 específicas do *trans*-resveratrol, *cis*-resveratrol e *trans*-resveratrol-glucosídeo (DEGÁSPARI e WASZCZYNSKYJ, 2004).

Os derivados do ácido hidroxicinâmico são compostos que possuem um anel aromático com uma cadeia carbônica constituída por três carbonos ligados ao anel. Como exemplos têm-se os ácidos *p*-cumárico, ferúlico e caféico que aparecem nas plantas usualmente na forma esterificada, glicosilada ou ligada às proteínas (DEGÁSPARI e WASZCZYNSKYJ, 2004).

Os derivados do ácido hidroxibenzóico são compostos com o grupo carboxílico ligado ao anel aromático e destacam-se os ácidos protocatecuíco, vanílico, siríngico, gentísico, salicílico, gálico e elágico (DEGÁSPARI e WASZCZYNSKYJ, 2004).

Nos tecidos vegetais, os compostos fenólicos acumulam-se nos vacúolos como metabólitos intermediários os protegendo contra a radiação ultravioleta e o ataque de patógenos e predadores, e conferindo uma ação atrativa para a dispersão dos frutos (TOOR e SAVAGE, 2005).

Vários destes compostos exibem atividades biológicas benéficas aos seres humanos como a antioxidante, antimicrobiana, antiinflamatória, e ação vasodilatadora (KÄHKONEN et al., 2001), além de influenciarem na percepção da cor, adstringência e aroma dos alimentos. A atividade antioxidante é geralmente determinada pelo número de hidroxilas presentes na molécula (HARBORNE, 1973; DEGÁSPARI e WASZCZYNSKYJ, 2004).

Os tomates foram identificados como a fonte dietética mais importante de fenólicos para os humanos, com base no seu consumo médio, seguidos pelo milho e feijões (VINSON et al., 1998); estes compostos podem ser encontrados livres (solúveis) ou ligados às fibras (insolúveis) (FRUSCIANTE et al., 2007).

Os tomates contêm vários polifenóis (Figura 2) como os ácidos *p*-cumárico, ferúlico, gálico, cafêico e clorogênico (ANTEROLA e LEWIS, 2002). Ambos os últimos foram detectados no pericarpo e na pele de tomates (LONG et al., 2006). Dentre estes ácidos, o

clorogênico foi determinado com o majoritário com o teor médio de 0,71 mg/100 g em base úmida (BU) em tomates-cereja cv. Jennita (SLIMESTAD e VERHEUL, 2005).

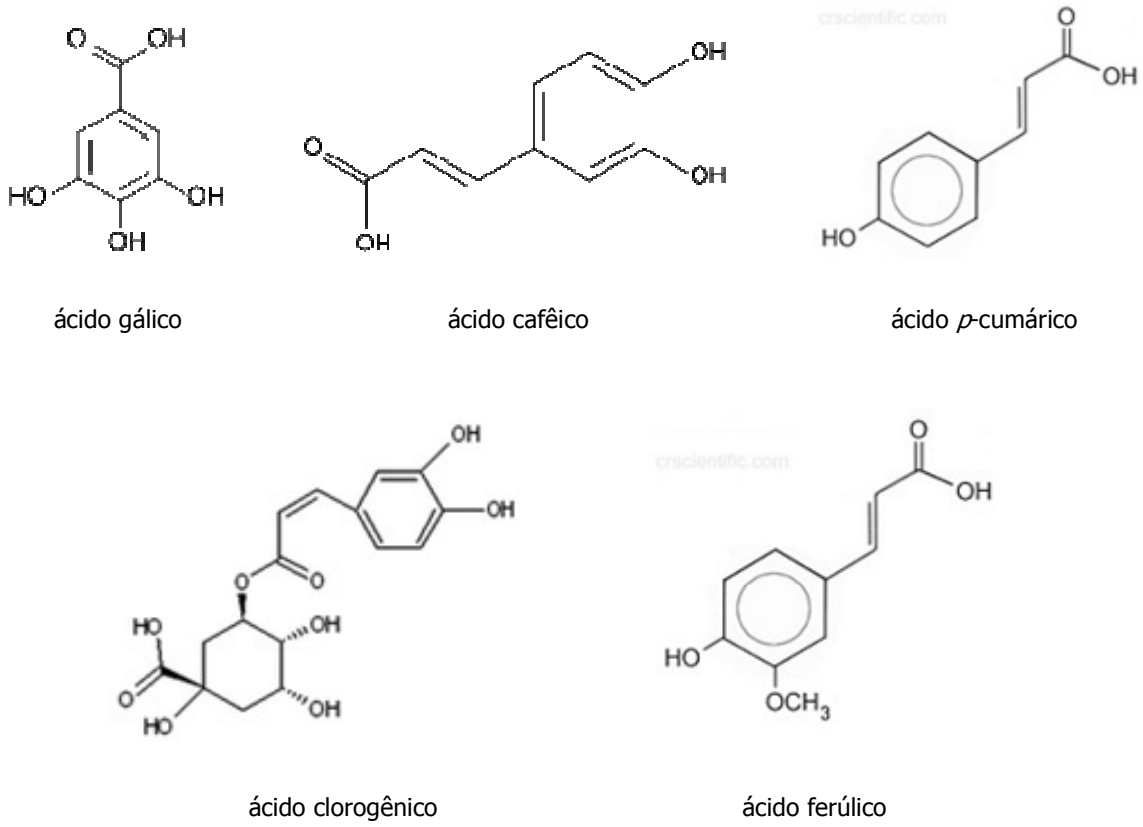


FIGURA 2. Principais ácidos fenólicos presentes em tomates.

Os compostos fenólicos aumentam substancialmente ao longo da maturação. Nos tomates verdes, os fenólicos constituem-se como minoritários (< 5%), enquanto que nos maduros variam entre 7-14 % no início do climatério aumentando para 21-26 % no pós-climatério (DUMAS et al., 2003; ARABBI et al., 2004). Dumas et al. (2003) também reportaram que tomates crescidos sob intensa luminosidade tiveram um teor de fenólicos solúveis aproximadamente duas vezes maior do que nos com pouca luminosidade.

Kahkonen et al. (1999) reportaram um teor de fenólicos totais acima de 200 mg de equivalente em ácido gálico/100 g de tomates em base seca (BS). Frusciante et al. (2007)

observaram variação nos teores de fenólicos totais em tomates de diferentes variedades, entre 2,04 (Cayambe) e 3,75 (Motelle) mg /100 g.

Os processamentos térmicos de tomates em torno de 80^{o}C são reportados por aumentarem o conteúdo de fenólicos totais devido ao incremento no número de grupos fenólicos livres como resultando da hidrólise de flavonóides glicosilados e/ou liberados das paredes celulares fenólicas. Na fruta intacta, os compostos fenólicos localizam-se nos vacúolos ficando separados das enzimas oxidativas que são inativadas com o processamento térmico e assim, evitando as perdas dos compostos fenólicos (CHANG et al., 2006; TOOR e SAVAGE, 2006).

Chang et al. (2006) verificaram um aumento no conteúdo de fenólicos totais dos tomates I-Tien-Hug e Sheng-Neu (liofilizados à – 50^{o}C, 5 pa por 24 h, secos ao ar quente à 80^{o}C por 2 h e posteriormente, à 60^{o}C por 6 h) provavelmente devido à liberação destes compostos da matriz celular durante o processamento. Gahler et al. (2003) também reportaram um aumento no teor dos fenólicos totais em decorrência do processamento térmico de tomates.

Odriozola-Serrano et al. (2009) avaliaram o efeito do processo de campo elétrico pulsado de alta intensidade HIPEF (35 kV/cm por 1500 μs) e da pasteurização (90 ºC por 30s ou 60s) sobre o teor de fenólicos do suco de tomate. Os autores reportaram que os processos HIPEF, pasteurização por 30s e 60s (8,9; 9,1 e 9 mg/100 mL BU, respectivamente) não alteraram significativamente o teor em fenólicos do suco de tomate fresco (9 mg/100 mL BU). Entretanto, perdas substanciais do ácido clorogênico, de 4,4 para 3,5-3,8 mg/100 mL BU, foram verificadas após 56 dias de estocagem do suco de tomate à 4ºC.

A degradação oxidativa de compostos fenólicos envolve a peroxidase (AMIOT et al., 1997) e assim, a degradação do ácido clorogênico durante a estocagem do suco de tomate pôde estar associada à atividade residual dessa enzima. Aguiló-Aguayo et al. (2008) reportaram uma atividade residual de 10 e 21 % peroxidase em sucos de tomate submetidos à

90 ºC por 60s e 30s, respectivamente. Já, os sucos submetidos ao HIPEF apresentaram uma redução de 97 % da atividade inicial da peroxidase.

Após 56 dias de estocagem do suco de tomate, o teor do ácido cafêico aumentou ligeiramente ao máximo de 0,51-0,57 mg/100 mL BU (ODRIOZOLA-SERRANO et al., 2009).

A hidroxilação do ácido *p*-cumárico em ácido cafêico acontece nos alimentos como conseqüência da inserção de uma segunda hidroxila no ácido *p*-cumárico provavelmente, catalisada pelas monofenol monoxigenases (MACHEIX et al., 1990). Dessa forma, o aumento no teor do ácido cafêico no suco de tomate após 28 dias de estocagem (0,42-0,48 mg/100 mL BU) pode estar diretamente associado com a atividade residual da hidroxilase que converte o ácido *p*-cumárico em ácido cafêico.

1.3. Flavonóides

Os flavonóides, classe de pigmentos naturais com mais de 5.000 metabólitos presentes nas frutas e nos vegetais, são tão importantes quanto os carotenóides (MIEAN e MOHAMED, 2001; KRIS-ETHERTON et al., 2002).

A estrutura química dos flavonóides consiste de dois anéis benzênicos incluindo um anel heterocíclico de seis membros contendo um átomo de oxigênio. Alguns grupos hidrofenólicos presentes podem estar livres (agliconas ou geninas), metilados ou ligados a açúcares (glicosídeos). Os principais flavonóides são distinguidos pelas diferenças no anel heterocíclico e pelos grupos ligados (OOGHE et al., 1994).

Os flavonóides estão divididos em (i) antocianinas, que podendo apresentar colorações azuis, vermelhas ou violeta, sendo encontradas em flores e frutas, e (ii) antoxantinas que podem ser incolores ou apresentar coloração amarela pálida (CAMPBELL et al., 1979).

As antoxantinas incluem: flavonas (apigenina, luteolina, diosmetina, tangeritina e nobelitina), flavanas (catequinas, epicatequinas, luteoferol, procianidina e teaflavinas), flavonóis (quercetina, rutina, miricetina e kaempferol), isoflavonas (daidzeína e genisteína), e flavanonas (hesperidina, narirutina, naringenina e neohesperidina) (LE GALL et al., 2003).

Os flavonóides desempenham vários efeitos benéficos à saúde humana, além das atividades antioxidante, antialérgica e antiinflamatória (HIRAI et al., 2007) como a inibição das enzimas prostaglandina sintetase, lipoxigenase e cicloxigenase (ambas as últimas, relacionadas a carcinogênese) e indução dos sistemas enzimáticos detoxificantes (MIEAN e MOHAMED, 2001). A atividade antioxidante diminui a velocidade de envelhecimento celular e protegendo as células contra a peroxidação lipídica, reação esta que pode resultar no desenvolvimento de doenças cardiovasculares, coronarianas e inflamações crônicas (LE GALL et al., 2003).

A atividade seqüestrante de radicais livres deve-se à excelente capacidade doadora de hidrogênios e de elétrons conferida pela sua configuração C_6-C_3-C_6. A estrutura considerada essencial (Figura 3) para o efetivo seqüestro radicalar é com hidroxilas nas posições 3'e 4' do anel B, e 3-OH do anel C. A dupla ligação conjugada com o grupo 4-keto, responsável pelo deslocamento do elétron do anel B, aumenta a capacidade seqüestrante. Também, a presença de ambos os grupos 3-OH e 5-OH concomitantemente com a função 4-carbonila e dupla ligação C2-C3 incrementa a atividade seqüestrante de radicais livres (AMIC et al., 2003).

Os tomates (Figura 4) contêm rutina (quercetina-3-*O*-rutinosídeo), chalconaringenina e kaempferol-3-*O*-rutinosídeo em sua composição sendo ambos os primeiros, majoritários (LE GALL et al., 2003). Nos vegetais, há o predomínio da quercetina glicosilada, mas também estão presentes a luteolina e a apigenina (CROZIER et al., 1997; MUIR et al., 2001; MIEAN e MOHAMED, 2001).

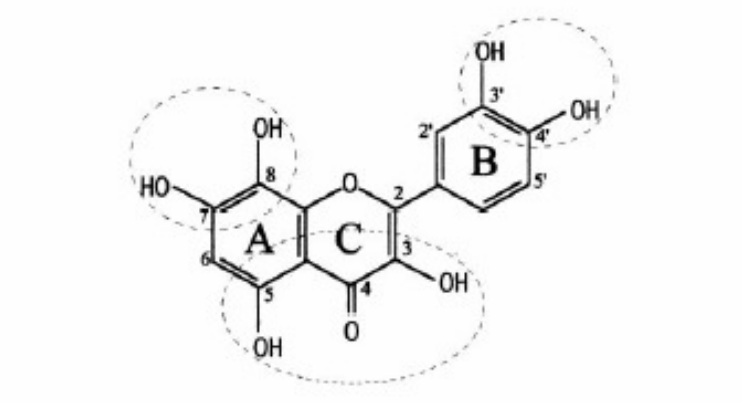


FIGURA 3. Características estruturais dos flavonóides com a atividade seqüestrante de radicais livres (AMIC et al., 2003).

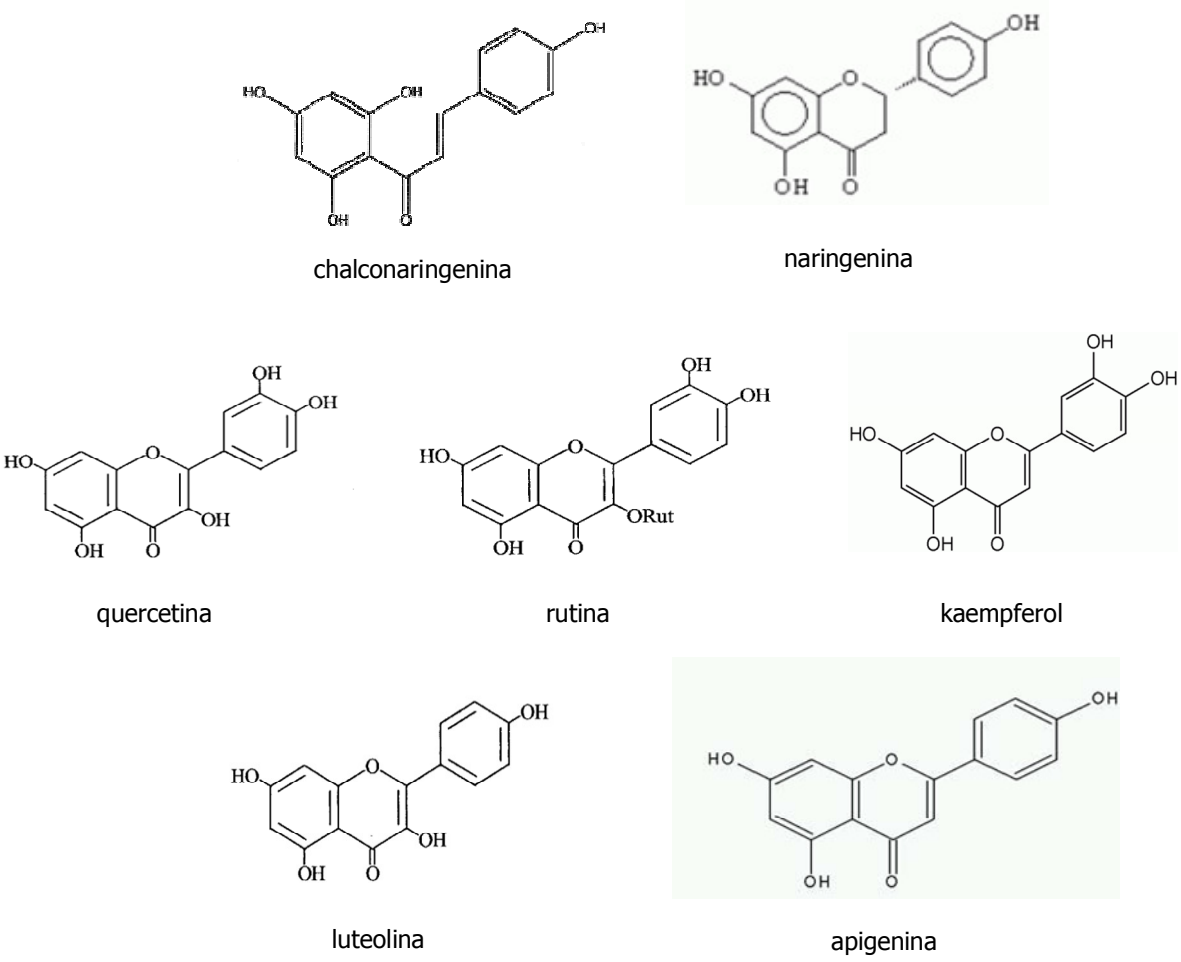


FIGURA 4. Estrutura química de alguns flavonóides identificados nos tomates.

Em várias espécies vegetais, o teor em flavonol pode aumentar em resposta à intensa luminosidade em especial, a radiação UV-B (BRANDT et al., 2006). Foi reportado que tomates-cereja produzidos sob intensa luminosidade acumularam o dobro do teor em fenólicos solúveis (rutina e ácido clorogênico) em comparação às plantas crescidas sob fraca iluminação (WILKENS et al., 1996).

A influência da sazonalidade sobre os componentes antioxidantes em tomates-cereja cv. Naomi F1 foi avaliada por Raffo et al. (2006) que verificaram variações nos teores da rutina (2-76 mg/100 g BU, base úmida), da naringenina (2-9 mg/100 g BU) e do ácido clorogênico (3-5 mg/100 g BU). Dentre estes, a rutina apresentou-se como predominante nos tomates.

Ao longo da maturação dos tomates, os teores de rutina e chalconaringenina aumentam sendo que no fruto verde, a chalcona encontra-se ausente (ARABBI et al., 2004). Slimestad e Verheul (2005) reportaram a chalconaringenina e a rutina como os flavonóides majoritários em tomates-cereja totalizando 0,5-1,0 mg/100 g BU.

Embora a literatura reporte mudanças na composição de flavonóides ocorrida durante o processamento de tomates, alguns estudos mostraram um aumento no teor destes. O processamento de tomates usualmente envolve tratamento térmico e homogeneização que aumentam a biodisponibilidade dos diferentes nutrientes presentes por romper a matriz celular. Entretanto, este pode, ao mesmo tempo, degradar esses micronutrientes (SAHLIN et al., 2004).

Stewart et al. (2000) avaliaram a ocorrência de flavonóis em tomates e seus produtos derivados (suco, sopa, pasta, catchup e purê) verificando que dentre estes o purê apresentou o maior teor médio (72 µg/g BU).

1.4. Carotenóides

Os carotenóides são uma classe de pigmentos naturais lipossolúveis, amplamente distribuídos na natureza, apresentando diversidade estrutural e numerosas funções biológicas importantes para a saúde humana.

Além da atividade pró-vitamínica A, desempenhada por alguns carotenóides (β-caroteno, α-caroteno, β-criptoxantina e γ-caroteno), têm-se reportado que os ativos, pró-vitamínicos A ou não, podem atuar ainda como antioxidantes, antiúlcera gástrica, contra doenças cardiovasculares, certos tipos de câncer, desordens neurológicas, fortalecer o sistema imunológico, atuar na degeneração macular relacionada à idade e cataratas (luteína e zeaxantina), na ativação genética e nos processos inflamatórios, por modularem a lipoxigenase (BRITTON et al., 1995; WOODALL et al., 1997; SETIAWAN, et al., 2001; HUMPHRIES e KHACHIK, 2003; GAMA e SYLOS, 2007).

Quimicamente, os carotenóides são substâncias tetraterpênicas formadas por oito unidades de isopreno. A ligação isoprênica sofre reversão na parte central da molécula e assim, os dois grupos metílicos centrais ficam separados por três carbonos. A estrutura do licopeno (Figura 5) é considerada a estrutura fundamental dos carotenóides, da qual podem ser derivadas outras formas estruturais por reação de hidrogenação, ciclização, oxidação ou a combinação dessas (BRITTON et al., 1995; PAIVA e RUSSELL, 1999; RODRIGUEZ-AMAYA, 1999; OLIVER e PALOU, 2000).

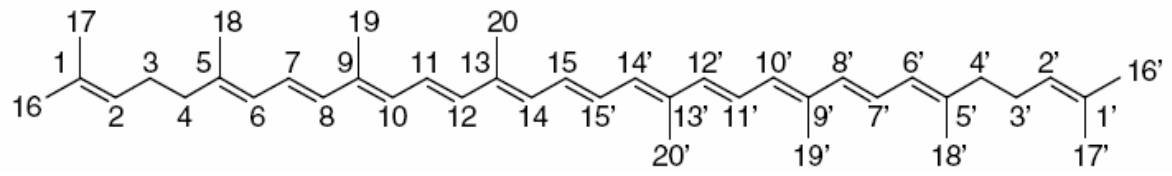


FIGURA 5. Estrutura do licopeno.

Os carotenóides são divididos em carotenos, compostos constituídos apenas por carbono e hidrogênio (β-caroteno e licopeno) e seus derivados oxigenados, as xantofilas cujos substituintes mais comumente são os grupos hidroxílicos (β-criptoxantina), carbonílicos (β-citraurina) e epóxidos (violaxantina).

As ligações duplas conjugadas que formam a parte central da molécula dos carotenóides lhes confere a sua forma, reatividade química, e suas propriedades biológicas e absorvedoras de luz, responsável pela coloração amarela, laranja ou vermelha conferida aos alimentos (ROCK, 1997; LEE et al., 2001).

Contudo, essa característica estrutural também promove sua instabilidade em relação à exposição à luz, alta temperatura, oxigênio, atividade de água, acidez, presença de metais, tipo de processamento, material de embalagem, condições de armazenagem devido à propensão à isomerização e oxidação (RODRIGUEZ-AMAYA, 1999; OLIVER e PALOU, 2000; SUBAGIO e MORITA, 2001).

Essa susceptibilidade à oxidação habilita os carotenóides da dieta a atuarem como antioxidantes nos sistemas biológicos. Essa característica estrutural possibilita a sua incorporação dentro das membranas biológicas e por outro lado, influi na sua interação com espécies oxigênio reativas. A efetividade dos carotenóides como antioxidantes também depende da sua interação com outros coantioxidantes em especial, as vitaminas E e C (YOUNG e LOWE, 2001), sendo que esta última os reciclam.

A atividade antioxidante dos carotenóides nos sistemas biológicos é influenciada por: (i) estrutura (tamanho, peso, posição e número de substituintes) e a forma física da molécula (agregado ou monômero, configuração *cis* ou *trans*), (ii) localização ou sítio de ação dentro da célula, (iii) potencial de interação com outros coantioxidantes, (iv) concentração do carotenóide e (v) pressão parcial do oxigênio (YOUNG e LOWE, 2001). Os carotenóides com 9 ou mais ligações duplas conjugadas são capazes de seqüestrar o oxigênio singleto com

aumento desta atividade dependendo do número de ligações duplas conjugadas sendo o licopeno, o mais eficiente seguido pelo β-caroteno (BOHM et al., 2002).

Os tomates apresentam em sua composição como majoritário o licopeno (79-88 %), em menor concentração (FIGURA 6) o β-caroteno (5-8 %) e em traços, a fitoeno, fitoflueno, neurosporeno, neoxantina, violaxantina, anteraxantina, luteína, licoxantina α-criptoxantina, β-criptoxantina, α-caroteno, δ-caroteno, ζ-caroteno (WILBERG e RODRIGUEZ-AMAYA, 1995; BEN-AMOTZ e FISHLER, 1998; LONG et al., 2006; GAMA et al., 2006).

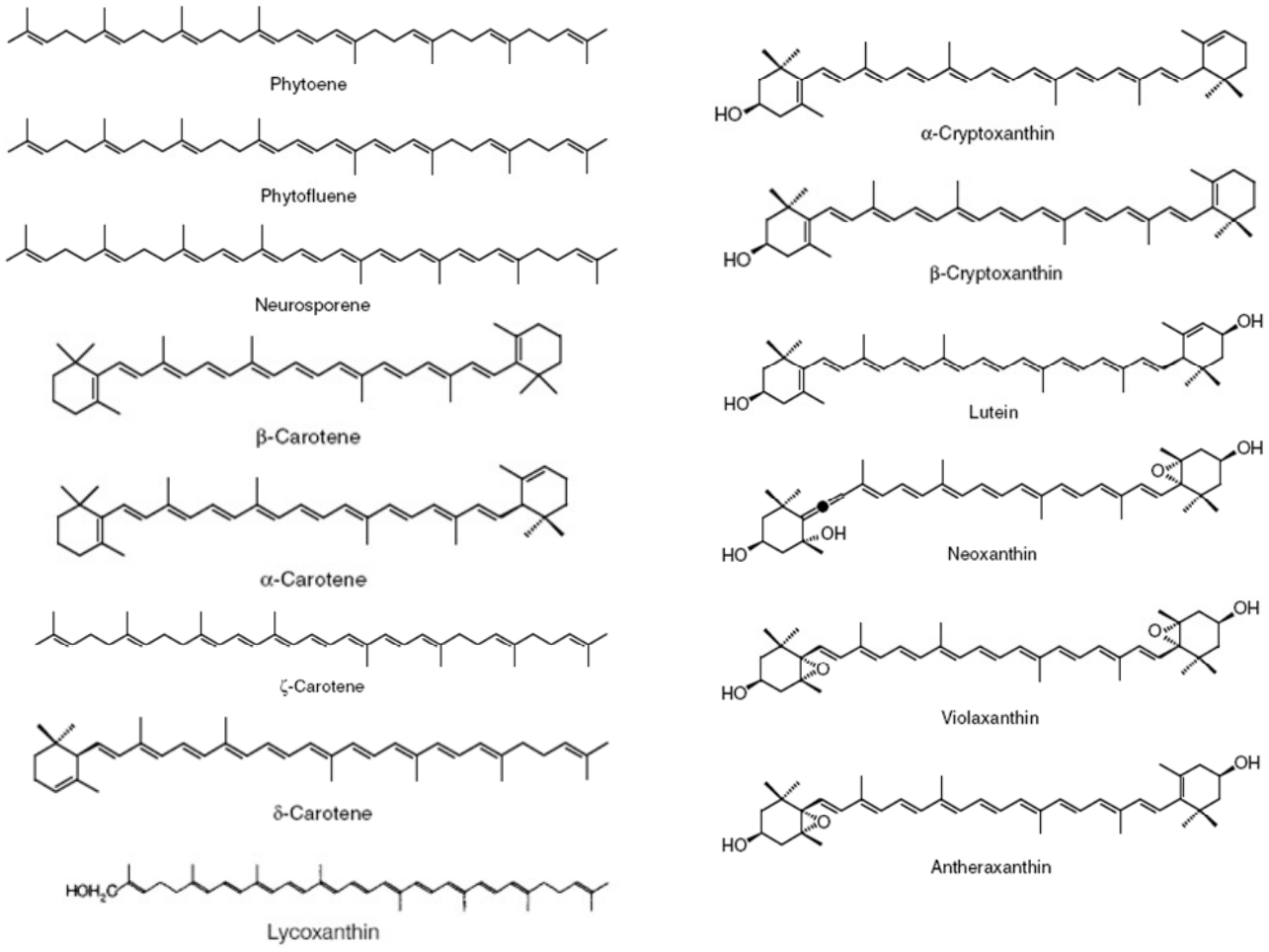


FIGURA 6. Estrutura química de outros carotenóides identificados em tomates em menores teores (RODRIGUEZ-AMAYA, 1999).

Dentre os carotenóides presentes nos tomates, o licopeno é o que desperta interesse por causa de suas propriedades biológicas e físico-químicas, especialmente àquelas relacionadas aos seus efeitos antioxidantes (STAHL e SIES, 1996). Há evidências de que este pigmento desempenha um efeito protetor contra doenças cardiovasculares, cânceres de próstata, esôfago, estômago, cólon e reto, e radiação ultravioleta e fumaça do cigarro. Adicionalmente, este pigmento se mostrou um potente inibidor da proliferação e do crescimento de células tumorais, em culturas celulares e em modelos animais (KRIS-ETHERTON et al., 2002).

O tomate possui alto teor de carotenóides, em especial de licopeno cujo teor é maior em frutos maduros. Pesquisas demonstram que o consumo de tomate pode reduzir em cerca de 50% o risco de câncer de próstata, além de outras evidências na atuação em câncer de esôfago, mama, pulmão e pele. Atuando como antioxidante protege o organismo humano contra os radicais livres. O tomate *in natura* tem entre 3 e 8 mg licopeno/100 g, enquanto que o processado apresenta um teor em torno de 11 mg licopeno/100 g e a pasta enlatada de 30 mg licopeno/100 g (FEAGRI, 2007).

Embora os tratamentos térmicos possam liberar o licopeno de sua matriz celular, perdas significativas de seu conteúdo têm sido reportadas durante a desidratação industrial de tomates devido à presença de oxigênio e principalmente, exposição à luz resultando na isomerização e oxidação do licopeno (SAHLIN et al., 2004; TOOR e SAVAGE, 2005).

Por outro lado, Abushita et al. (2000) observaram alteração somente no teor do β-caroteno após a produção de pasta de tomate, apesar da elevada temperatura de processamento e tempo prolongado, e pouca alteração no teor do licopeno.

Gärtner et al. (1997) investigaram a entrada do licopeno nos quilomicrôns humanos após ingestão de uma dose única desse pigmento proveniente de tomates frescos e de pasta de tomate. Os mesmos verificaram que a biodisponibilidade desse pigmento foi maior a partir da pasta do que dos tomates frescos.

1.5. Ácido Ascórbico (Vitamina C)

A vitamina C é conhecida como ácido ascórbico (AA) na forma reduzida e ácido dehidroascórbico (DHAA) na forma oxidada (DUTRA-DE-OLIVEIRA e MARCHINI, 1998).

O acido ascórbico (I) é uma cetolactona de seis carbonos que se oxida facilmente e de modo reversível a ácido dehidroascórbico (II) que apresenta cerca de 60 % das propriedades da vitamina C.

A atividade biológica da vitamina C se perde quando o ácido dehidroascórbico se transforma pela quebra irreversível do anel lactônico em ácido 2,3-dicetogulónico (III) (ROJAS e GERSCHENSON, 1997; UDDIN et al., 2002; GIANNAKOUROU et al., 2003) (Figura 7).

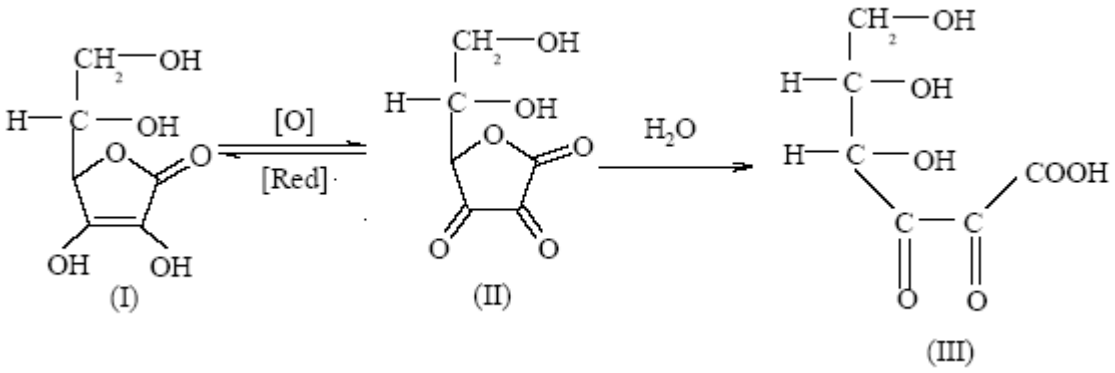


FIGURA 7. Mecanismo de conversão do ácido L-ascórbico em ácido dehidroascórbico.

O ácido ascórbico é necessário na produção e manutenção do colágeno, atuando como antiescorbútico, participa na formação da hidroxiprolina, reduz o ferro férrico a ferroso no trato intestinal, facilita a absorção de zinco e cobre, auxilia na eliminação de chumbo, mercúrio, vanádio, cádmio e níquel, aumenta a resistência orgânica às infecções por proteger células e tecidos do ataque de patógenos, e também, pode inibir o desenvolvimento de doenças cardíacas e de certos tipos de cânceres (MILANESIO et al., 1997, DUTRA-DE-OLIVEIRA e MARCHINI, 1998).

Todas as suas ações fisiológicas e bioquímicas da vitamina C devem-se a ação doadora de elétrons provenientes da dupla ligação entre o segundo e terceiro carbonos da sua molécula, o que lhe confere excelente atividade antioxidante. Devido a essa propriedade antioxidante, a vitamina C é utilizada industrialmente como agente preservativo de alimentos prevenindo a deterioração pela auto-oxidação dos ácidos graxos insaturados.

Nos sistemas biológicos, o ácido ascórbico atua ainda em sinergismo com os carotenóides, flavonóides e vitamina E, na promoção de uma barreira efetiva contra a oxidação celular e também, reciclando-os (YOUNG e LOWE, 2001; MIEAN e MOHAMED, 2001).

As principais fontes de vitamina C para os seres humanos são as frutas e os vegetais onde o ácido ascórbico encontra-se principalmente na forma livre e, também, unida às proteínas. Nos tomates, o conteúdo de ácido ascórbico varia de acordo com o cultivar e as condições de cultivo, entre 14 e 44 mg/100 g (GARDNER et al., 2000; GAHLER et al., 2003).

O conteúdo em ácido ascórbico nas frutas e nos vegetais é um índice de maturação e a sua determinação torna-se interessante no controle de qualidade (ÖZGUR e SUNGUR, 1995). O teor em ácido ascórbico é influenciado pelas condições ambientais da produção em especial, pela intensidade luminosa (MARTINEZ-VALVERDE et al., 2002) cuja exposição favorece o acúmulo dessa vitamina nos tomates. Já, uma adubação rica em nitrogênio (N) solúvel pode causar decréscimo no teor de vitamina C por razões indiretas uma vez, que o suprimento de N aumenta a densidade das folhas que por sua vez diminuem a incidência luminosa sobre os frutos (DUMAS et al., 2003).

Como o ácido ascórbico é uma vitamina hidrossolúvel de fácil oxidação pelo calor, e catalisada pelo cobre e pH alcalino, perdas significativas desta vitamina podem ocorrer durante o preparo e o cozimento dos alimentos (DUTRA-DE-OLIVEIRA e MARCHINI, 1998; GAHLER et al., 2003; SAHLIN et al., 2004). Tais perdas são altamente dependentes da

temperatura e do tempo de aquecimento utilizados no processamento dos tomates (GAHLER et al. 2003; CHANG et al., 2006; TOOR e SAVAGE, 2006). Gahler et al. (2003) e Toor e Savage (2005) reportaram perdas consideráveis de ácido ascórbico após a obtenção industrial de suco, molho, catchup e sopa de tomates devido às altas temperaturas, exposição à luz e ao ar. Abushita et al. (2000) verificaram perdas de ácido ascórbico com o processamento de tomates na obtenção da pasta (-16%) e extrato de tomate (-38%). Toor e Savage (2005) reportaram perdas entre 17-27% de ácido ascórbico em tomates secos à 42^{o}C por 18 h. Chang et al. (2006) observaram um decréscimo de 8 % no teor de ácido ascórbico após a liofilização de tomates. O armazenamento dos tomates também podem resultar em perdas expressivas da vitamina C contribuindo para a variação do seu conteúdo no fruto (CHITARRA, 1994).

2. FATORES QUE INTERFEREM NOS PARÂMETROS DE COR CIELAB E NO TEOR DE PIGMENTOS EM TOMATES

A cor é uma sensação provocada no observador pelos comprimentos de onda da luz produzida pela fonte de luz e modificada pelo objeto sobre os órgãos da visão (olhos). Assim, as cores resultam da interação entre a fonte de luz, o objeto e o sistema visual humano (observador).

A percepção humana das cores primárias (vermelho, azul, amarelo) e de suas combinações (laranja, verde, púrpura, etc.) torna-se complexa uma vez que, diferenças nas eter de luz, observadores, tamanhos do objeto, planos de fundo (*background*) e direções do olhar alteram a *tonalidade*, *saturação* e *luminosidade* da cor (LÓPEZ CAMELO e GÓMEZ, 2004; COLOR GLOSSARY, 2008).

Essas três diferentes sensações (tonalidade, saturação e luminosidade) são responsáveis pela natureza tridimensional da cor sendo denominadas de estímulos cromáticos (MÉNDELEZ-MARTINEZ et al., 2005).

2.1. Medição da Cor pelo Sistema CIELAB

A fim de evitar as diferenças naturais entre as percepções humanas da cor, a CIE (*Comission Internationale de l'Eclairage, International Commission on Illumination* – Comissão Internacional de Iluminação que determina padrões de cores e de iluminação) em 1976, desenvolveu o sistema CIELAB (Sistema Lab Color ou Espaço Lab Color) de medição de cor através dos parâmetros (L^*, a^* e b^*) empregando-se colorímetros triestímulos (COLOR GLOSSARY, 2008; KONICA MINOLTA, 2008).

Os colorímetros triestímulos examinam uma área extensa da amostra, medindo a cor real e a correlacionando com a observada visualmente, utilizando filtros de cor especializados e detectores luminosos que simulam a sensibilidade humana para a cor. O olho humano é capaz de detectar acima de 10 milhões de tonalidades diferentes de cor e o colorímetro triestímulo pode quantificá-las.

Uma descrição mais precisa e uniforme cor é auferida com a medição dos parâmetros L^* (luminosidade), a^* (índice de saturação vermelho) e b^* (índice de saturação amarelo) que matematicamente combinados permitem calcular outros índices de cor como o Chroma e o ângulo Hue (ARIAS et al., 2000) e também, as razões a^*/b^* e $(a^*/b^*)^2$.

Os parâmetros do sistema CIELAB se localizam dentro de uma esfera de cor definida pelos três eixos perpendiculares L^*, a^* e b^* (Figura 8).

A luminosidade (L^*) pode ser definida como a capacidade de um objeto refletir ou transmitir luz resultando em uma cor luminosa (100 – branco) ou escura (zero – preto) (ARIAS et al., 2000; COLOR GLOSSARY, 2008; KONICA MINOLTA, 2008).

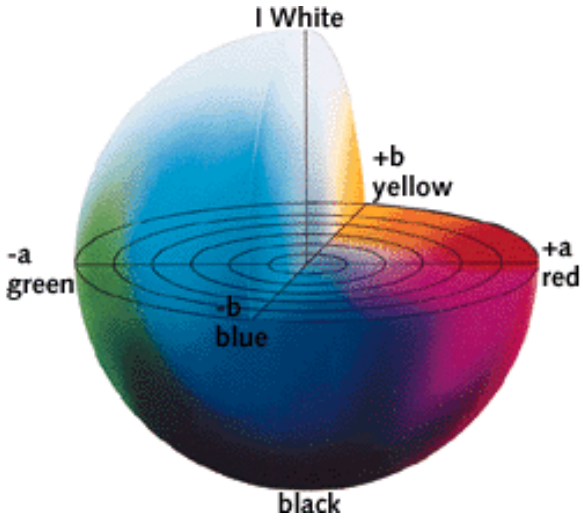


FIGURA 8. Parâmetros *L**, *a** e *b** do sistema de cor CIELAB (KONICA MINOLTA, 2008).

A cromaticidade é composta pelas coordenadas *a** (vermelho-verde: valor positivo representa vermelho e negativo, verde) e *b** (amarelo-azul: valor positivo representa amarelo e negativo, azul). Tal relaciona-se à refletância ou transmitância da luz visível em comprimentos de onda específicos (ARIAS et al., 2000; COLOR GLOSSARY, 2008; KONICA MINOLTA, 2008).

A saturação (*C** ou Chroma) define a vivacidade (próximo de 60) ou opacidade da cor (próximo de zero) (COLOR GLOSSARY, 2008; KONICA MINOLTA, 2008) sendo calculado através da equação $C^* = (a^{*2} + b^{*2})^{1/2}$ (Figura 2) (ARIAS et al., 2000).

Hue ou ângulo Hue relaciona-se às diferenças de absorbância em diferentes comprimentos de onda permitindo distinguir colorações de mesma luminosidade (Figura 9), calculado a partir de $\tan^{-1}(b^*/a^*)$, quando $a^* > 0$ e $b^* \geq 0$, e $180 + \tan^{-1}(b^*/a^*)$, quando $a^* < 0$ (ARIAS et al., 2000).

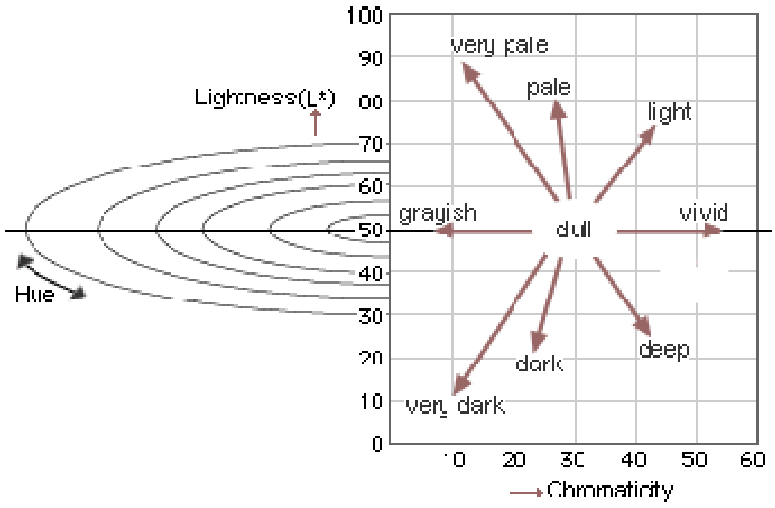


FIGURA 9. Representação da luminosidade (*L**), Chroma (*C**) e Hue (KONICA MINOLTA, 2008).

O valor hue (Figura 10) de 180^{o} representa o verde puro e 0^{o}, vermelho puro; quanto mais próximo de zero for este valor mais vermelho será a cor do alimento como a do tomate maduro (COLOR GLOSSARY, 2008; KONICA MINOLTA, 2008).

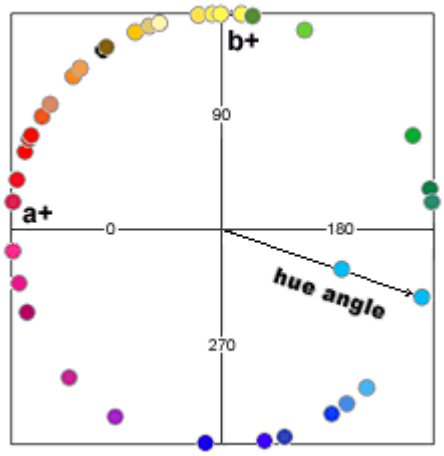


FIGURA 10. Representação do ângulo Hue e as tonalidades □eterminer□□ts.

2.2. Classificação dos Tomates

A divisão dos tomates em grupos caracteriza os seus tipos que podem ser divididos em quatro grupos de acordo com o formato, coloração, durabilidade e apresentação.

Os tomates podem ser identificados primeiramente, pelo formato, o qual pode estar relacionado à sua finalidade de uso, de acordo com a classificação: (i) Grupo Santa Cruz – formato oblongo, uso tradicional na culinária em molhos e saladas; (ii) Grupo Caqui – redondo, uso em saladas e lanches; (iii) Grupo Saladete – redondo, uso em saladas; (iv) Grupo Italiano – oblongo tipicamente alongado, uso em molhos e saladas; (v) Grupo Cereja – redondo ou oblongo com tamanho reduzido, uso em aperitivos, saladas, etc. (FEAGRI, 2007).

Normalmente os consumidores relacionam os tomates com a coloração vermelha, mas com o aparecimento de novas variedades, atualmente existe a disposição dos consumidores um grupo de coloração de tomates o qual é determinado pela cor final ou do fruto maduro. Entretanto, os tomates de coloração laranja e amarela não são comuns no Brasil. Quando os tomates vermelhos são submetidos a altas temperaturas os frutos podem demonstrar coloração amarela a qual é uma característica encontrada facilmente nos meses mais quentes do ano, principalmente no Norte e Nordeste (FEAGRI, 2007).

A classificação em grupo de durabilidade está relacionada à vida pós-colheita ou vida útil do tomate em condições normais de conservação e este grupo apresenta-se como (i) Longa vida: Esta é uma denominação utilizada para os tomates de cultivares que possuem uma vida pós-colheita mais prolongada, permanecendo firmes por um período maior de tempo. Muitas vezes é utilizado para transporte em longas distâncias; ex., cv Alambra, Bona, Carmem, Densus, Stylus, Avansus; (ii) Normal: Os tomates que possuem esta característica possuem menor vida-útil, durando menos, mas, em geral, são mais saborosos que os tomates longa vida; ex.: cv Momotaro, Olympo, Cereja, Andréa, Colibri, Fanny, Fanny Ty, Madarin, Escala, Débora, Kindio, Santa Clara. Já, em relação à apresentação, o tomate poderá ser

apresentado ao consumidor na forma normal ou em penca (racimo), ou seja, na forma em que é colhido sendo que a apresentação em penca é uma característica de algumas variedades (FEAGRI, 2007).

2.3. Fases de Cor e Maturação dos Tomates

O ciclo vital dos frutos engloba as etapas de fertilização, formação, crescimento, maturação e senescência. Na maturação, que se inicia com o fruto ainda não colhido e termina com o início da senescência, o fruto encontra-se na máxima qualidade comestível, em pleno crescimento. Nesta etapa ocorre o desenvolvimento das sementes, mudanças na cor (a cada dois dias entre cada fase), na taxa respiratória, na permeabilidade dos tecidos, na textura, e na química dos carboidratos, ácidos orgânicos, proteínas, fenólicos, pigmentos, pectinas, etc., produção de substâncias voláteis e etileno, e formação de ceras na casca do fruto (CHITARRA, 1990).

Na senescência, o crescimento do fruto cessa e os processos químicos de envelhecimento substituem as trocas químicas de amadurecimento e em um curto espaço de tempo resulta na perecibilidade aumentando a probabilidade de morte do fruto por desidratação e invasão de microrganismos (CHITARRA, 1990).

A cor dos tomates é um importante fator que permite □eterminer a maturação dos frutos, afeta a decisão de compra dos consumidores e também é um atributo de qualidade para as indústrias produtoras de tomates (ARIAS et al., 2000).

A cor é determinada pela pigmentação da pele e da polpa dos tomates. A pele dos frutos rosados é incolor e sua polpa, vermelha enquanto que, nos tomates vermelhos, a pele é amarela e a polpa, vermelha. Algumas variedades de tomates como os utilizados em processamento industrial apresentam uma pele vermelha intensa devido ao acúmulo de

licopeno. Usualmente, os consumidores preferem os frutos vermelhos (BRANDT et al., 2006).

As clorofilas e os carotenóides são os responsáveis pela coloração dos tomates. Nos estádios iniciais, as clorofilas fornecem a cor verde e com o amadurecimento, estas são degradadas e os carotenóides sintetizados. Nos tomates, os carotenóides principais são o licopeno (79-88 %), pigmento majoritário e responsável pela cor vermelha, e o β-caroteno que representa cerca de 7 % do teor total de carotenóides. O teor do licopeno aumenta com a maturação dos tomates quando os cloroplastos transformam-se em cromoplastos e a sua síntese incrementa resultando ao aparecimento da cor vermelha (ARIAS et al., 2000).

Os estádios de maturação dos tomates se refletem na mudança da cor externa do fruto que podem ser classificados de acordo com o seu grau de maturação nas seguintes fases de cor (i) Verde (*Mature green*): frutos maduros com a superfície verde que pode variar de claro a escuro; (ii) Intermediário (*Breaker*): aparecimento da cor amarela, rosa ou vermelha em não mais que 10 % da superfície do fruto; (iii) Virando (*Turning*): entre 10 e 30 % da superfície do fruto apresenta uma mudança da cor verde para amarelo-esverdeado, rosa ou a combinação dessas cores; (iv) Rosa (*Pink*): entre 30 e 60 % da superfície do fruto apresenta cor rosa ou vermelha; (v) Vermelho Claro (*Red*): entre 60 e 90 % da superfície do fruto apresenta cor vermelho-rosada ou vermelha, e (vi) Vermelho (*Deep red*): mais de 90 % da superfície do fruto apresenta cor vermelha (YAMAGUCHI, 1983).

O desenvolvimento da cor dos tomates é sensível à temperatura sendo mais eficiente entre 12 e 30^{o}C. Tomates amadurecidos sob altas temperaturas (> 30^{o}C) apresentam uma cor amarela devido à inibição da síntese do licopeno e acúmulo de carotenóides amarelos e laranjas. Por outro lado, em temperaturas abaixo de 12^{o}C, o acúmulo de licopeno não é evidenciado, devido a não degradação da clorofila (ESKIN, 1989; LÓPEZ CAMELO e GÓMEZ, 2004).

Quando o tomate encontra-se na fase *Rosa*, com 30-60 % da sua superfície vermelha, ocorre o pico climatérico da respiração. A perda da cor verde e o aparecimento da vermelha são utilizados como indicativos do grau de maturidade dos tomates que quando maduro, apresenta a típica coloração vermelha. Após a colheita, a cor, a textura e o sabor dos frutos são alterados (BRANDT et al., 2006).

2.4. Relação entre os Parâmetros CIELAB e o Teor de Licopeno em Tomates

Os parâmetros do sistema CIELAB fornecem dados quantificáveis e precisos sobre a cor de frutas e vegetais *in natura* e processados.

Atualmente, este sistema vem sendo empregado no estudo da influência da localidade geográfica ou climática da produção (GÓMEZ et al., 2001), dos estádios de maturação (ARIAS et al., 2000; LOPEZ CAMELO e GÓMEZ, 2004; CARVALHO et al., 2005; BRANDT et al., 2006), do cultivar, da colheita e pós-colheita (GIOVANELLI et al., 1999), do processamento (SAHLIN et al., 2004; KERKHOFS et al., 2005; SÁNCHEZ-MORENO et al., 2006) e da estocagem sobre a cor de tomates.

Segundo Giovanelli et al. (1999), a razão a^*/b^* é essencial no estudo dos carotenóides presentes em tomates por representar um índice de maturação simples e significativo e que as condições de maturação afetam em particular, o acúmulo de licopeno.

Arias et al. (2000) avaliaram a relação entre o teor de licopeno e as fases de maturação em tomates cv. Laura observando um aumento do valor a^* (de – 10 para + 26) como resultado da síntese do licopeno e degradação da clorofila, representada pela mudança da cor verde para vermelha. Logo, o valor L^* diminuiu nos estádios iniciais da maturação (fase *Verde*, 57) e depois, permaneceu constante refletindo o escurecimento dos tomates pelo aparecimento e intensificação da cor vermelha (fase *Vermelho*, 41). O valor b^* aumentou nos quatro estádios iniciais da maturação (da fase *Verde*, + 20, para a *Vermelho claro*, + 31) e

depois, decresceu (fase *Vermelho*, + 25), refletindo a síntese de β-caroteno e o subseqüente aumento do teor do licopeno. Segundo os mesmos, o valor *a** e as razões *a**/*b** e $(a^*/b^*)^2$ produziram correlações satisfatórias com as fases da maturação dos frutos e assim, com o teor de licopeno.

Os parâmetros colorimétricos foram avaliados em 12 variedades de tomates provenientes de várias localidades da Espanha por Gómez et al. (2001) que através da razão *a**/*b** separaram os frutos com na sua cor externa cujos resultados foram melhores que os do Índice de cor dos tomates (TCI).

Brandt et al. (2006) reportaram diferenças no teor de licopeno em tomates cv. Lemance F_1 ao longo da maturação sendo menor nos frutos nas fases *Verde* e *Intermediário* (0,1 e 5,5 mg/kg tomate fresco, respectivamente). Após a fase *Virando* (15,3 mg de licopeno/kg tomate fresco), o teor de licopeno foi aumentando rapidamente [fases *Rosa* (25,4 mg/kg) e *Vermelho claro* (39,8 mg/kg)] até um máximo de 59 mg/kg nos tomates da fase *Vermelho*.

Um comportamento similar do valor *a** e da razão *a**/*b** ao longo da maturação dos tomates que passaram da fase *Verde* para a *Vermelho*, indo de -10 (coordenada verde) para + 29 (coordenada vermelha), e de – 0,4 para + 1,4, respectivamente. A razão *a**/*b** produziu a melhor correlação com o teor de licopeno. Já, o valor *b** variou entre + 20 e + 30 (coordenada amarela) durante a maturação dos tomates indicando a presença de outros carotenóides nos fruto. Os autores concluíram que a cor é um indicativo do teor de licopeno e que melhor se correlaciona com a razão *a**/*b** (BRANDT et al., 2006). Os mesmos autores também verificaram que a biosíntese do licopeno foi influenciada pela temperatura ambiente que acima de 30^{o}C decresceu significativamente o seu teor.

López Camelo e Gómez (2004) compararam os índices de cor de tomates durante a maturação e verificaram que a razão *a**/*b**, similar ao observado por Brandt et al. (2006), e o Hue puderam ser utilizados como indicativo de amadurecimento. Os valores *L**, *a**e *b**

variaram durante a maturação dos tomates sendo o segundo parâmetro de cor, o mais importante por relacionar-se a degradação da clorofila e síntese de licopeno.

Uma vez que os carotenóides são susceptíveis à degradação frente a fatores como luz, alta temperatura, oxigênio, atividade de água, acidez e metais (RODRIGUEZ-AMAYA, 1999; SUBAGIO e MORITA, 2001), o processamento de tomates bem como a armazenagem pode resultar em perdas de licopeno e conseqüentemente, alterações de cor.

Wiese e Dalmasso (1994) verificaram um aumento no valor do ângulo Hue após o processamento e estocagem de suco de tomate sendo um indicatico da perda da cor vermelha. A melhor retenção da cor em produtos de tomates se dá em temperaturas mais brandas.

Shi e Le Maguer (2000) observaram diminuições dos valores L^*, a^* e da razão a^*/b^* com a secagem de tomates à 95^{o}C. Os mesmos reportam que tomates tratados osmoticamente apresentaram uma coloração vermelha mais intensa do que aqueles secos sob circulação de ar e sob vácuo, indicando o maior teor em licopeno.

Kerkhofs et al. (2005) avaliaram o efeito do processamento térmico (secagem à 42^{o}C por 48h, e posterior armazenagem à -18^{o}C) e a influência da variedade (Aranka, Encore e Flavourine) sobre a cor dos tomates. Ambos os fatores afetaram significativamente a cor do produto final. A secagem resultou em um decréscimo acentuado do valor a^* para os tomates Encore (de + 39 para + 21), e menor para Aranka (de + 23 para + 20) e Flavourine (de + 20 para + 16). As três variedades apresentaram uma diminuição de 25 % no valor da razão a^*/b^* que foi quase metade do observado por Shi et al. (1999) com tomates secos à 90^{o}C sugerindo que secagem até 42^{o}C reduz a degradação da cor em comparação às elevadas temperaturas.

Segundo Toor e Savage (2006), o decréscimo do valor a^* em produtos de tomate processados podem decorrer do escurecimento não-enzimático (reação de Maillard) que resulta no aparecimento da cor vermelha-amarronzada.

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Capítulo 2

Comparison of carotenoid content in tomato, tomato pulp and ketchup by liquid chromatography.

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COMPARISON OF CAROTENOID CONTENT IN TOMATO, TOMATO PULP AND KETCHUP BY LIQUID CHROMATOGRAPHY

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■ ABSTRACT: Although tomatoes are commonly consumed fresh, over 80 % the consumption of tomatoes is in the form of processed products such as tomato pulp, ketchup, juice and sauce. Research has indicated the potential health benefits of a diet rich in tomatoes and tomato products. The present study was carried out to determine the carotenoid content of fresh tomato, tomato pulp and ketchup by high performance liquid chromatography. The major differences among these products were in the concentration of some of the pigments. Tomato had all-trans-lycopene (1046-1099 µg/g DW), cis-lycopene (125-132 µg/g DW) and all-trans-β-carotene (45-59 µg/g DW) as principal carotenoids. Tomato pulp and ketchup had all-trans-lycopene (951-999 µg/g DW and 455-476 µg/g DW), all-trans-β-carotene (76-88 DW µg/g and 20-27 DW µg/g) and cis-lycopene (71-83 µg/g DW and 14-25 µg/g DW) as the main pigments, respectively. They also contained other carotenoids in much smaller amounts (lycoxanthin, zeaxanthin, anteraxanthin, lutein, γ-carotene, ζ-carotene and phytofluene).

■ KEYWORDS: Carotenoids; tomato; tomato pulp; ketchup; HPLC.

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1. Introduction

Tomato is certainly an important agricultural commodity worldwide. In Brazil, it is one of the most widely consumed foodstuffs, either fresh or processed, and it is the main source of carotenoids for much of the population [28,15].

Epidemiological studies have shown that increased consumption of tomato and tomato-based products may reduce the risk of certain types of cancer, such as prostate, lung and stomach cancer [12], and cardiovascular disease [25].

In a recent study, tomatoes ranked first as a source of lycopene (71.6%), second as a source of vitamin C (12.0%), pro-vitamin A carotenoids (14.6%) and β-carotene (17.2%), and third as a source of vitamin E (6.0%) [11].

One of the major phytochemicals in tomato products to which the anti-carcinogenic function has been attributed is lycopene [7,28]. Because of presence of long-chain conjugated double bonds, lycopene has been reported to possess antioxidant activity and is superior to lutein or β-carotene [18].

Other quantifiable carotenoids in fresh tomatoes and tomato products such as juice, paste, puree, and sauce include phytoene, phytofluene, ζ-carotene, neurosporene, γ-carotene, and β-carotene, but their concentrations are significantly lower than that of lycopene [26].

The ability of lycopene to act as a potent antioxidant is thought to be responsible for protecting cells against oxidative damage and thereby decreasing the risk of chronic diseases [21]. In addition to its antioxidant properties, lycopene has also been shown to induce cell to cell communication [29], and to modulate hormonal and immune systems and other metabolic pathways [9,1] which may also be responsible for the beneficial effects [21].

Carotenoids predominantly occur in their all-trans configuration, which is thermodynamically the more stable isomer. Apart from naturally occurring in plants, cis-isomers have been formed as a consequence of food processing and they possess different biological properties [24].

All-trans-lycopene may be converted to its cis configuration during food processing [16]. Several reports have demonstrated that the cis isomers of lycopene are absorbed into the body more easily and play a more important part in biological function than all-trans-lycopene [3,4].

The objective of this study was to estimate the carotenoid profiles and carotenoid contents of fresh tomato, tomato pulp and ketchup by HPLC.

2. Material and Methods

2.1. Material

Fresh tomatoes [*Lycopersicon esculentum* cv. AP 533, (1 kg)], tomato pulp (1.5 kg) and ketchup (1.5 kg) were supplied by Alimentos Predilecta LTDA (São Lourenço do Turvo Matão, Brazil). Before analysis, fresh tomatoes were homogenized in a Waring blender to obtain a representative sample. Five analyses were carried out in duplicate. To compare tomatoes and their products, the results were expressed on a dry weight basis.

2.2. Industrial Thermal Treatment

To obtain tomato pulp, fresh tomato was submitted to enzyme inactivation by heating (90^{o}C for 6 min) and concentrated in 3 stages, until the pulp reached 32^{o}Brix. The tomato pulp was sterilized at 105^{o}C for 2-3 min and then cooled to 40^{o}C. In the ketchup manufacturing process, the tomato pulp was heated at 100^{o}C for 5 min. Following addition of condiments, the product was heated at 85^{o}C for 10 min and cooled to 35^{o}C and bottled.

2.3. Methods

2.3.1. Extraction of carotenoids

The carotenoids present in the samples (5 g) were extracted with hexane-acetone (1:1, v/v) with celite in a mechanical Waring blender. The mixture was centrifuged and the supernatant reserved. The residue was further extracted and centrifuged until all color was removed, and the successive supernatants pooled. The pigments in this organic extract were then transferred to petroleum ether, washed with distilled water and concentrated, in a rotary evaporator, at a temperature not exceeding 35°C. Saponification was not carried out, to avoid losses, especially of the more polar carotenoids, and also because HPLC provides enough separation of chlorophyll and carotenoid peaks [19,2].

2.3.2. Determination of total carotenoids content

The total carotenoid content was determined in an aliquot of the petroleum ether extract, obtained as described above by measuring the absorbance at 470 nm in a Beckman UV/Vis spectrophotometer DU® 640. Total carotenoid content was calculated as described by Rodriguez-Amaya [22] using the lycopene absorption coefficient ($A^{1\%}_{1cm}$ = 3450) in petroleum ether, and expressed in fresh weight (FW) and dry weight (DW) basis.

2.3.3. HPLC analysis of carotenoids

Carotenoid pigments were analyzed by RP-HPLC using ternary gradient elution and a Symmetry C_{18} column (4.6 x 150 mm I.D., 3.5 μm) from Waters. The chromatography system was equipped with a Shimadzu LC-10AT VP solvent delivery system and SPD-M 10A VP photodiode array detector (DAD). The mobile phase consisted of acetonitrile:methanol:ethyl acetate containing 0.05% triethylamine flowing at 0.8 mL/min. A gradient was applied from 88:8:4 to 48:26:26 in 25 min, and back to the initial condition (30 min). Volume injection was 20 μL. Detection was at the wavelengths of maximum absorption (max plot). The samples and the solutions were filtered through a 0.22 μm membrane before injection.

The isolation and purification of standards was performed as in Kimura & Rodriguez-Amaya [14]. Natural sources, such as tomato (lycopene), carrot (β-carotene), butter collard greens, lettuce and watercress (lutein and zeaxanthin), and passion-fruit juice (ζ-carotene) were used to isolate the standards indicated as described in our previous work [10].

2.3.4. Identification and estimation of carotenoids

The individually isolated carotenoids in samples were identified by comparison of their HPLC retention times and diode array spectral characteristics with those of the standards and also literature values [17,15,6].

The identification of *cis*-carotenoids was based on the UV spectrum which exhibits a new maximum around 330-350 nm ('cis-peak'), the intensity of which depends on the localization of the cis-double bond and is greatest when the double bond is located near or at the center of the chromophore [5]. In addition, a hypsochromic shift in the λ_{max} is observed in cis-carotenoid spectra, compared to trans- isomers [24].

The results are expressed in relative percentage of total peak area taken into account and also in μg/g DW of lycopene.

3. Results and Discussion

The chromatographic profiles (Figure 1) of extracts of tomato pulp and ketchup are similar to that of tomato. The major differences among them appear to be in the concentration of some carotenoids presented in Table 1.

Because of the predominance of lycopene, the other pigment peaks appeared small. The chromatograms obtained demonstrated that many minor xanthophylls (oxygen-containing carotenoids) were eluted in the region before lycopene while the carotenes (hydrocarbon carotenoids) eluted after it.

In tomato, tomato pulp and ketchup, the carotenes predominate, constituting 86-91 % of the total carotenoids, with very high levels of lycopene (79-88 %), and the xanthophylls make up about 9-14 % of the total.

The data show that the amount of all-trans-lycopene comprised 82, 74 and 81 % of total carotenoids, while cis-lycopene represented 6, 6 and 5 %, in tomato, tomato pulp and ketchup, respectively. Thus, lycopene (cis + trans) accounted for 88, 80 and 87 % of the total carotenoid content of tomato, tomato pulp and ketchup, respectively.

Our data were consistent with previous studies. In samples of cherry tomatoes, lycopene was by far the main component of the carotenoid fraction, ranging from 76 to 85 % [20]. Sass-Kiss et al. [24] reported that lycopene in tomatoes accounted for 90-95 % of total carotenoids. Ishida & Chapman [13] showed that all-trans-lycopene comprised 69-100 % of the total carotenoids in ketchup. Davis et al. [8] demonstrated that lycopene constituted about 80 % of the total carotenoids in fresh red tomato tissues.

In fresh tomato, the principal carotenoids present were all-trans-lycopene (1046-1099 μg/g DW), cis-lycopene (125-132 μg/g DW), all-trans-β-carotene (45-59 μg/g DW), all-trans-lycoxanthin (17-23 μg/g DW), and cis-lutein (14-19 μg/g DW).

Tomato pulp had, mainly, all-trans-lycopene (951-999 μg/g DW), all-trans-β-carotene (76-88 μg/g DW), cis-lycopene (71-83 μg/g DW), cis-β-carotene (25-29 μg/g DW), and cis-lycoxanthin (19-23 μg/g DW).

Ketchup had all-trans-lycopene (455-476 μg/g DW), all-trans-β-carotene (20-27 μg/g DW), cis-lycopene (14-25 μg/g DW), all-trans-lycoxanthin (8-12 μg/g DW), and cis-β-carotene (8-11 μg/g DW) as the main pigments.

Therefore, the predominant carotenoid detected in all our samples was lycopene. They also contained β-carotene, lycoxanthin and lutein, in much smaller amounts. Anteraxanthin, zeaxanthin, γ-carotene, ζ-carotene and phytofluene were also identified in samples. These results were in agreement with others [17,13,19].

The total carotenoid content measured at 470 nm ranges were 1318-1382, 1289-1316 and 547-583 μg/g DW in fresh tomato, tomato pulp and ketchup, respectively. This content was in the ranges 67-71, 348-355 and 123-131 μg/g FW for tomato, tomato pulp and ketchup, respectively.

The total carotenoid content of fresh tomatoes cv. AP533 was higher than those reported by Tavares & Rodriguez-Amaya [27] with cv. Santa Clara (21-62 μg/g FW) due variety differences. The total carotenoid content of ketchup was close to reported by Tavares & Rodriguez-Amaya [27] which was in the ranges 106-139 μg/g FW and, by Ishida & Chapman [13] (59-183 μg/g FW).

The carotenoid profile and carotenoid content (DW) of fresh tomato, tomato pulp and ketchup estimated was relevant because they will allow to us evaluate the effect of thermal process on tomato pulp and ketchup manufacture in following stage of work.

4. Conclusions

The results obtained in the present study for tomatoes and their products agree with most of those reported in the literature. In tomato, tomato pulp and ketchup, while lycopene accounted for 88, 80 and 87 % of the total carotenoid content, β-carotene represented 5, 8 and 6 %, respectively. Note once again that the samples having the highest carotenoid contents were fresh tomato followed by tomato pulp. The lowest carotenoid content was verified in ketchup. However, ketchup is an excellent source of lycopene, responsible for the dark red appearance of the product.

GAMA, J.J.T.; TADIOTTI, A.C.; SYLOS, C.M. Comparação do teor de carotenóides em tomate, polpa de tomate e catchup por cromatografia líquida. **Alim. Nutr.**, Araraquara, v.17, n.4, p.353-358, out./dez. 2006.

RESUMO: Embora os tomates sejam comumente consumidos in natura, mais de 80 % do seu consumo ocorre na forma de produtos processados como polpa de tomate, catchup, suco e sopa. Estudos vêm demonstrando os potenciais benefícios de uma dieta rica em tomates e em seus produtos. O presente trabalho visou determinar o teor de carotenóides em tomate fresco, polpa de tomate e catchup por cromatografia líquida de alta eficiência. A principal diferença decorreu da concentração de alguns pigmentos. O tomate apresentou como carotenóides principais o all-trans-licopeno (1046-1099 µg/g BS), cis-licopeno (125-132 µg/g BS) e all-trans-β-caroteno (45-59 µg/g BS). Na polpa de tomate e no catchup, os pigmentos majoritários foram all-trans-licopeno (951-999 µg/g BS e 455-476 µg/g BS), all-trans-β-caroteno (76-88 µg/g BS e 20-27 µg/g BS) e cis-licopeno (71-83 µg/g BS e 14-25 µg/g BS), respectivamente. Estes também contiveram outros carotenóides em quantidades menores (licoxantina, zeaxantina, anteraxantina, luteína, γ-caroteno, ζ-caroteno e fitoflueno).

PALAVRAS-CHAVE: Carotenóides; tomate; polpa de tomate; catchup; CLAE.

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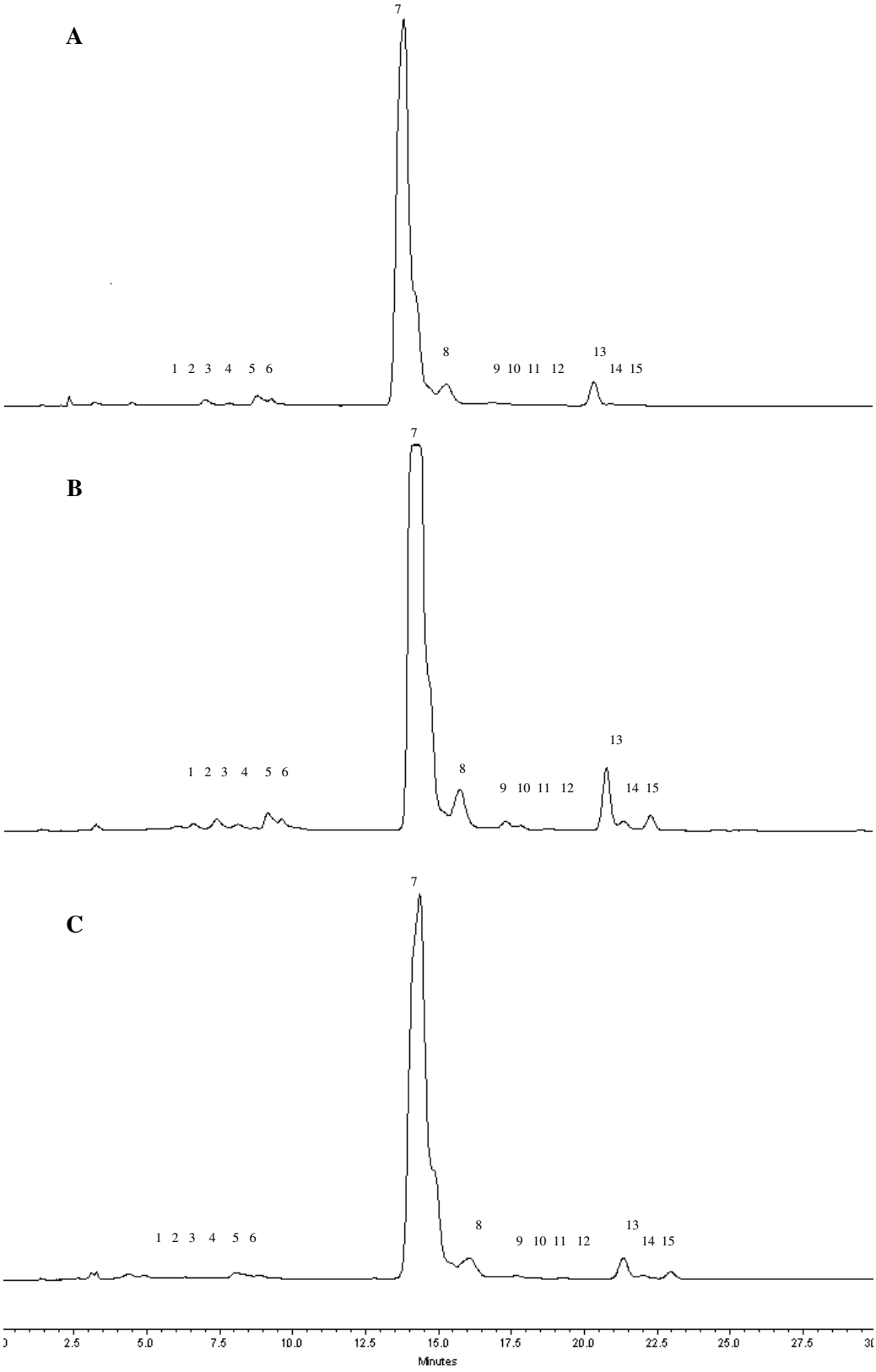

Figure 1. HPLC chromatograms of carotenoids in (A) fresh tomato, (B) tomato pulp and (C) ketchup. Chromatographic conditions described in the text. See Table 1 for peak identification.

Table 1. Chromatographic and spectral characteristics of fresh tomato, tomato pulp and ketchup carotenoids obtained by HPLC.

				Fresh tomato		Tomato pulp		Ketchup	
Peak n°	t_R (min)	λ_{max} (nm) (in line)[a]	Carotenoids	area %[d]	µg/g DW[b]	area %[d]	µg/g DW[b]	area %[d]	µg/g DW[b]
1	6.68	428 457 487	all-*trans*-anteraxanthin[c]	0.6	7.3 ± 1.0	1.2	15.7 ± 1.2	1.0	5.7 ± 0.9
2	7.53	426 447 474	all-*trans*-lutein	0.3	3.3 ± 0.5	0.9	13.1 ± 1.0	0.6	3.2 ± 0.5
3	8.21	353 423 447 475	*cis*-lutein	1.4	16.6 ± 2.4	1.5	20.0 ± 1.6	0.4	2.3 ± 0.4
4	8.83	360 (426) 447 473	*cis*-zeaxanthin	0.5	6.1 ± 0.9	0.7	9.1 ± 0.7	0.5	2.8 ± 0.5
5	9.62	369 447 468 503	*cis*-lycoxanthin[c]	1.1	13.3 ± 1.9	1.6	21.0 ± 1.7	0.9	5.3 ± 0.9
6	10.16	446 472 502	all-*trans*-lycoxanthin[c]	1.7	19.8 ± 2.8	0.6	8.2 ± 0.6	1.8	10.0 ± 1.6
7	15.11	456 482 513	all-*trans*-lycopene	82.4	1122.2 ± 26.6	73.9	974.8 ± 24.0	81.1	465.2 ± 10.5
8	16.69	361 445 466 500	*cis*-lycopene	5.7	78.8 ± 13.6	5.9	77.2 ± 6.1.4	5.4	19.4 ± 5.3
9	18.11	339 434 462 489	*cis*-γ-carotene[c]	0.8	9.5 ± 1.4	0.4	5.7 ± 0.5	0.6	6.2 ± 1.0
10	18.62	435 465 490	all-*trans*-γ-carotene[c]	0.4	4.9 ± 0.7	1.2	15.4 ± 1.2	0.4	2.5 ± 0.4
11	19.31	361 380 400 425	*cis*-ζ-carotene	0.3	3.2 ± 0.5	0.6	8.2 ± 0.6	0.5	2.6 ± 0.4
12	20.28	380 401 426	all-*trans*-ζ-carotene	0.2	2.2 ± 0.3	0.6	7.8 ± 0.6	0.3	1.5 ± 0.3
13	21.80	(428) 453 479	all-*trans*-β-carotene	4.4	52.0 ± 7.4	6.2	82.0 ± 6.5	4.2	23.5 ± 3.9
14	22.43	332 349 368	all-*trans*-phytofluene[c]	0.5	6.0 ± 0.9	1.3	17.5 ± 1.4	0.9	5.2 ± 0.9
15	23.35	342 (417) 446 472	*cis*-β-carotene	0.4	4.7 ± 0.7	2.1	27.0 ± 2.1	1.1	9.5 ± 1.6

[a]A gradient mobile phase of acetonitrile:methanol:ethyl acetate was used. [b]The values (expressed in µg/g of lycopene) are the averages of five determinations in duplicate ± standard deviation. [c]Tentative identification. [d] Expressed in percent of total peak area taken in account.

Capítulo 3

Variation in antioxidant content, antioxidant activity and color of Brazilian tomato (Lycopersicon esculentum cv. AP533).

VARIATION IN ANTIOXIDANT CONTENT, ANTIOXIDANT ACTIVITY AND COLOR OF BRAZILIAN TOMATO (*LYCOPERSICON ESCULENTUM* CV. AP533)

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Abstract

Tomatoes may have a great impact in the prevention of some types of cancer. This protective effect has been attributed to bioactive compounds naturally presented as phenolics, lycopene, and vitamin C. Brazilian tomatoes (*Lycopersicon esculentum* cv. AP533) from different date of harvest were analyzed for color [CIELAB L^*, a^* and b^*, a^*/b^* ratio, $(a^*/b^*)^2$ ratio, hue, and Chroma], antioxidant contents (ascorbic acid, total phenolics, total flavonoids, lycopene and β-carotene) and antioxidant activity. Their antioxidant activities were expressed as vitamin C equivalent antioxidant capacity (VCEAC) in mg/100 g dw using $DPPH^{\bullet}$ scavenging assay. There was significantly ($p < 0.05$) difference among tomatoes lots in terms of antioxidant constituents and antioxidant activity (VCEAC). Color analyses of fruits showed that the color differences were clearly detected among tomatoes lots. The color of tomatoes harvested in September 2006 were significantly ($p < 0.05$) more red and vivid than other. We could infer that tomatoes mentioned above were probably more ripened than others because of total phenolics, lycopene and ascorbic acid levels.

Keywords: Tomato; Lycopersicon esculentum cv. AP533; Color; Antioxidant activity; Antioxidants; HPLC

1. Introduction

Tomatoes are very popular fruits among the vegetable crops in the world and play a key role in the human diet (BRANDT et al., 2006). Tomatoes are usually consumed at their maximum organoleptic quality which takes place when they reach the full red color stage but before excessive softening (LÓPEZ CAMELO and GÓMEZ, 2004).

The color of tomatoes is a very important marketing factor that affects the buying decision of the consumer and is also a very important quality attribute for the tomato industry. Chlorophyll and carotenoids are responsible for the color of tomatoes. In the early stages of development the chlorophyll imparts a green color, and when the tomato starts the ripening process, the chlorophyll is degraded and carotenoids are synthesized (ARIAS et al., 2000) mainly, lycopene which gives the deep red color of ripe tomatoes (BRANDT et al., 2006).

According Yamaguchi (1983), six ripening stages based on the external tomatoes color were classified as: Green (100 % green), Breaker (a noticeable break in color with lesser than 10 % of other than green color), Turning (between 10 and 30 % of surface, in the aggregate, of red color), Pink (between 30 and 60 % of red color), Light red (between 60 and 90 %), and Red (more than 90 % red).

Apart from providing an interesting source of color on the plate, tomatoes (*Lycopersicon esculentum* L.) are a source of antioxidants including vitamin C, total phenolics and carotenoids, particularly, lycopene, in the diet (KERKHOFS et al., 2005). In addition, many epidemiological studies have suggested that the regular consumption of tomatoes may lead to a decreased incidence of various forms of cancer, plus heart disease (GIOVANNUCI, 1999).

Two main carotenoids are present in tomato: lycopene, which is the major carotenoid compound (~80-90%) giving the red color to the fruit (NGUYEN and SCHWARTZ, 1999), and β-carotene, which is 7-10 % of the total carotenoid content (GOULD, 1974).

Tomato contains several flavonoids of which naringenin chalcone and rutin (quercetin-3-*O*-rutinoside) are predominant. However, these compounds are found at low levels and are restricted to the peel. Only traces of rutin are found in flesh, which constitutes 95 % of the fruit (LE GALL et al., 2003). On biological system, vitamin C with carotenoids and vitamin E gave synergistic cell protection against oxidation and, vitamin C also recycles them. The vitamin C content of tomatoes was influenced by variety and harvest condition (GARDNER et al., 2000; GAHLER et al., 2003).

Genetic, environment factors (temperature, light, water availability, nutrient availability), agricultural techniques used (cultivar or variety, plant growth regulators, date harvest, etc) and post-harvest storage conditions (CANO et al., 2003; DUMAS et al., 2003; RAFFO et al., 2006) affect the antioxidant contents of tomato fruits.

From the methodological point of view, the widespread use of the stable 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical-scavenging model is recommended as fast, easy and accurate, for measuring the antioxidant activity of plant foods (ESPÍN et al., 2000; PINELO et al., 2004). The vitamin C equivalent antioxidant capacity (VCEAC) calculated on a weight or dry basis (mg/100 g or mg/100 mL) to show the total antioxidant capacity of a food is more desirable than the TEAC, TRAP, or ORAC assays based on molar units since vitamin C is a natural compound found in foods (KIM et al., 2002).

Brazil is the ninth world producer of tomatoes and its culture was responsible for 2.6 % of world production and for 1.3 % of world planted area. In this country,

almost 65 % of tomatoes production destined to fresh consumption and the rest, to industrial processing. The objective of this study was to compare the major antioxidants (total phenolic, total flavonoid, lycopene, β-carotene and L-ascorbic acid), antioxidant activity ($DPPH^{\bullet}$ radical-scavenging capacity and VCEAC), and color indexes (CIELab parameters) of Brazilian fresh tomatoes cv. AP533 harvested at different times.

2. Materials and methods

2.1. Plant material

Fresh tomatoes (*Lycopersicon esculentum* cv. AP533) were supplied by Alimentos Predilecta LTDA (São Lourenço do Turvo Matão, SP, Brazil). Harvests were carried out at five different times: August 2006, September (beginning and late) 2006, and July (beginning and late) 2007. The selected tomatoes presented a homogenous color and were separated into groups according to the maturity stages. Tomatoes were homogenized in a Waring blender to obtain a representative sample for each analysis. Analyses were carried out in triplicates. The results were expressed in fresh weight (fw) and dry weight (dw) basis.

2.2. Dry matter and total soluble solid

The total soluble solid and dry matter contents were determined according to Association of Official Analytical Chemistis (1990).

2.3. Total phenolics

Total phenolics were analyzed spectrophotometrically using the Folin-Ciocalteau colorimetric method (SINGLETON and ROSSI, 1965) modified by Scalbert et al. (1989). Each sample of fresh tomato (1.0 g) was vortexed with 10 mL of 80 % acetone. All samples were being for 1 h, in dark, at room temperature, followed by centrifugation. The supernatant (0.5 mL) was mixed with 2.5 mL of freshly diluted 0.2 N Folin-Ciocalteau reagent, and incubated at 50°C for 5 min. This reaction was neutralized by adding 2.0 mL of 7.5 % sodium carbonate. The samples were incubated at 50°C for 5 min and the absorbance of the resulting blue color was measured at 760 nm in a Beckman UV/vis spectrophotometer DU® 640. The linear reading of the standard curve was from 5 to 25 µg of gallic acid per mL.

2.4. Total flavonoids

The flavonoids were extracted using a modified method described by Yu and Dahegren (2000). Fresh tomatoes (14.0 g) were vortexed with 10 mL of 80 % acetone. All samples were being for 1 h, in dark, at room temperature, followed by centrifugation. The flavonoid content was measured using a colorimetric assay developed by Zhishen et al. (1999). A known volume (5 mL) of the supernatant was added to a tube. At zero time, 0.3 mL of 5 % sodium nitrite was added to a tube. After 5 min, 0.6 mL of 10 % $AlCl_3$ was added and, after 6 min, 2 mL of 1 M NaOH were added to the mixture, followed by addition of 2.1 mL distilled water. Sample absorbance was read at 510 nm using UV/vis spectrophotometer. The standard curve was obtained within the linear range of 100-500 µg rutin per mL.

2.5. HPLC analysis of lycopene and β-carotene

The carotenoids were extracted as modified method described by Barba et al. (2006). A sample (5.0 g) was placed in a Nalgene vessel and mixed with 25 mL of extraction solvent (hexane-acetone-ethanol, 2:1:1, v/v/v). The mixture was vortexed and centrifuged to separate the supernatant, and these operations were repeated until the residue was completely colorless. The pigment extract was partition to petroleum ether, concentrated on rotaevapory and dryness under N_2.

The analysis, separation and quantification of lycopene and β-carotene were accomplished by HPLC using a C_{18} column (4.6 x 150 mm I.D., 3.5 μm) under a gradient mobile phase of acetonitrile:methanol:ethyl acetate from 88:8:4 to 48:26:26 in 25 min, and back to the initial condition (30 min), at a flow rate of 0.8 mL/min. A calibration curves were made with standards to quantify the lycopene and β-carotene (retention times of 13.37 and 19.44 min, respectively), on the basis of the retention time of the peak and diode array spectral characteristics. The wavelength range used was 250-550 nm, and 450 and 470 nm were used to analyze the β-carotene and lycopene peaks, respectively. Average purity of the isolated carotenoids was 98 % and 99 % for β-carotene and lycopene, respectively.

2.6. Ascorbic acid

Ascorbic acid in fresh tomatoes was measured by titration with 2,6-dichlorophenolindophenol (DPI) in agreement with the method of Tillman's (A.O.A.C., 1990).

2.7. Chromatic characterization

Tomato color was measured with Hunter colorimeter (Color Quest II Sphere, CQII/UNI 1200 model) to obtained the CIELAB *L**, *a** and *b** parameters referring to the D65 illuminant and 10^{o} angle of vision. From these values we obtained the *a**/*b** ratio and $(a^*/b^*)^2$ ratio. The hue angle [$\tan^{-1}$ (*b**/*a**)] and Chroma [$(a^{*2} + b^{*2})^{1/2}$] values were calculated according by Arias et al. (2000).

2.8. DPPH$^{\bullet}$ radical-scavenging capacity

The antioxidant constituents of fresh tomato (53.0 mg) were extracted with 6 mL cold ethanol according by Vicente et al. (2006). DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was determined using a modified method of Brand-Williams et al. (1995). Briefly, 1000 μL of testing solution was mixed and react with 1575 μL of 0.5 mM freshly prepared DPPH$^{\bullet}$ methanolic solution, and left to stand for 30 min prior to being spectrophotometrically detected at 515 nm. The estimate the total DPPH$^{\bullet}$ scavenging capacity of antioxidant sample and standards, the % DPPH$^{\bullet}$ inhibition was determined according the followed equation: [1 – (sample absorbance – blank absorbance) / (control absorbance – blank absorbance)] x 100 (%).

2.8.1. Comparison of radical DPPH$^{\bullet}$ scavenging- capacity

The total free radical scavenging activity of each tomato extract was estimated and compared to gallic acid, ascorbic acid, rutin, BHT, lycopene and β-carotene according to Yu et al. (2002). The concentration of antioxidant standards was 50 mM. Lycopene and β-carotene were isolated from tomato pulp and carrot, respectively, and purified by OCC as described by Kimura and Rodriguez-Amaya (2002).

2.8.2. VCEAC of tomatoes

Vitamin C standard curve that relate the concentration of vitamin C and the amount of absorbance reduction caused by vitamin C were obtained using the $DPPH^{\bullet}$ assay. The antioxidant capacity was quantified using vitamin C standard curve (2-10 µg/mL) and expressed as vitamin C equivalent antioxidant capacity (VCEAC) calculated on fresh and dry weight basis (mg/100 g) according method developed by Kim et al. (2002).

2.9. Statistical analysis

All results were submitted to analysis of variance (ANOVA) and least significant differences (Tukey test), with significance defined as $p < 0.05$ to determine significant differences between tomatoes. The results were correlated using the Pearson product moment correlation method.

3. Results and Discussion

3.1. Quantitative analysis of antioxidant compounds

3.1.1. Dry matter and soluble total solid

Although, tomatoes dry matter can vary below 5 % until next to the double of this value, for most varieties it situates between 5 and 7.5 %. The solid matter (Table 1) was at the significantly ($p < 0.05$) highest in tomatoes harvested in Aug 2006 (6.19 %) and it was lowest in fruits from Jul (beginning) 2007 (4.63 %).

Tomatoes owns in its composition from 93 to 95 % of water. In the 5 to 7 % remaining, they meet inorganic composed, organic acids, sugars, insoluble solid in alcohol and another composed (EMBRAPA, 2008).

The soluble total solid (Table 1) was at the significantly ($p < 0.05$) highest in tomatoes from Sep (beginning) 2006 (6.49 %) and it was on those harvested in Jul (beginning and late) 2007 (4.24 %).

According to Takeoka et al. (2001), the content of soluble total solid for several tomato cultivars varied between 5 and 7 ^{o}Brix.

The content of soluble solid in the tomatoes, besides being a characteristic genetics of the cultivar, it is influenced by the mature stage, temperature and irrigation. This content in the raw material received by the industries in Brazil have been being very low (4.5 ºBrix). However, there are cultivars that own larger genetic potential, presenting, in determined terms, nearby values of 6.0 ºBrix. The cultivar AP533 presents soluble solid values between 5.0 and 5.5^{o}Brix (EMBRAPA, 2008).

The soluble total solid represent the percentage of solid that are met dissolved in the food and with the fruit maturation it tends to increase due to biosynthesis processes or by the polysaccharides degradation.

Our referring results to the soluble solid and solid matter are according to reported by Embrapa (2008), and, we could infer that tomatoes from third lot were in mature stage more advanced than other.

Table 1. Total soluble solids, dry matter, antioxidant compounds and antioxidant activities of Brazilian fresh tomatoes cv. AP533.

	Tomatoes				
	Aug 2006	**Sep 2006 (beginning)**	**Sep 2006 (late)**	**Jul 2007 (beginning)**	**Jul 2007 (late)**
Dry matter (%)	6.19 ± 0.16a	6.11 ± 0.23a	5.24 ± 0.22b	4.63 ± 0.08c	5.38 ± 0.07d
Total soluble solids (^{o}Brix)	6.47 ± 0.12a	4.38 ± 0.06b	6.49 ± 0.06c	4.24 ± 0.12d	4.24 ± 0.06d
Total phenolic (mg GAE/100 g, fw)	150.26 ± 2.03a	127.54 ± 10.52a	153.36 ± 15.77b	133.94 ± 5.89b	137.92 ± 10.75b
(mg GAE/100 g, dw)	7282.19 ± 32.77[a]	6282.54 ± 172.78b	8785.17 ± 301.13c	8685.60 ± 127.24c	7687.08 ± 199.72d
Total flavonoid (mg RE/100 g, fw)	30.56 ± 0.69[a]	29.86 ± 5.66[a]	27.15 ± 1.33[a]	35.46 ± 1.51b	25.56 ± 2.44c
(mg RE/100 g, dw)	493.66 ± 11.20[a]	490.34 ± 93.00a	518.34 ± 25.31[a]	766.57 ± 32.62b	474.76 ± 45.39c
L-ascorbic acid (mg/100 g, fw)	20.25 ± 2.98[a]	9.82 ± 0.01b	3.86 ± 0.67c	9.72 ± 0.89d	8.73 ± 0.90d
(mg/100 g, dw)	327.07 ± 48.19[a]	160.75 ± 0.31b	73.62 ± 12.75c	210.10 ± 19.30d	149.73 ± 15.37e
Lycopene (mg/100 g, fw)	7.86 ± 3.32a	10.46 ± 4.51a	20.16 ± 1.08b	17.79 ± 4.26b	17.03 ± 4.78b
(mg/100 g, dw)	127.04 ± 60.11a	171.82 ± 74.07a	384.69 ± 20.68b	384.51 ± 92.01b	316.46 ± 88.73b
β-carotene (mg/100 g, fw)	0.0002 ± 0.00a	0.0009 ± 0.00a	0.0065 ± 0.00b	0.0099 ± 0.00c	0.0104 ± 0.00c
(mg/100 g, dw)	0.003 ± 0.00a	0.015 ± 0.00a	0.125 ± 0.02b	0.214 ± 0.05c	0.193 ± 0.04c
DPPH$^{\bullet}$ scavenging (% inhibition)	63.92 ± 5.17a	47.04 ± 4.27b	52.96 ± 4.27b	44.57 ± 3.13b	44.40 ± 4.11b
VCEAC (mg/100 g, fw)	127.76 ± 11.99[a]	57.62 ± 10.87b	40.24 ± 2.94b	108.77 ± 9.53c	100.14 ± 1.10c
(mg/100 g, dw)	2065.13 ± 193.83[a]	943.30 ± 177.91b	768.39 ± 56.21b	2351.10 ± 205.98c	1860.44 ± 20.38d

Values are expressed as mean ± standard deviation to three replicates for each value. Different letter for the same line indicates significance difference ($P < 0.05$). GAE: acid gallic equivalent; RE: rutin equivalent; fw: fresh weight basis; dw: dry weight basis; DPPH$^{\bullet}$: (2,2-diphenyl-1-picrylhydrazyl); VCEAC: vitamin C equivalent antioxidant capacity.

3.1.2. Total phenolics

The total phenolics content (Table 1) in Brazilian tomatoes cv. AP533 varied from 127.5 to 153.4 mg/100 g fw (6282.5-8785.2 mg/100 g dw) to fruits harvest in 2006, and 133.9-137.9 mg/100 g fw (7687.1-8685.6 mg/100 g dw) to those from 2007.

The total phenolics content was at the significantly ($p < 0.05$) highest in tomatoes (153.4 mg/100 g fw) from Sep (late) and it was lowest in those from Sep (beginning) (127.5 mg/100 g fw). These values were higher than observed by Chassy et al. (2006) which were 35.4 (Burbank) and 34.4 mg/100 g fw (Ropreco). Odriozola-Serrano et al. (2007) reported a concentration of phenolic compounds in the range of 18.7 (cv. Rambo) and 33.6 mg/100 g fw (cv. Durinta). Martinez-Valverde et al (2002) reported a concentration of phenolic compounds between 27.3 and 49.9 mg/100 g fw to different cultivars of tomatoes.

Between tomatoes harvest in 2006, those from Sep late (8785.2 mg/100 g dw) contained significantly ($p < 0.05$) the highest phenolic content as compared to Aug (7282.2 mg/100 g dw) and Sep beginning (6282.5 mg/100 g dw). The content of two earliest lots was 17 and 29 %, respectively, lesser than those tomatoes from third. Among samples harvest in 2007, the total phenolic content was significantly ($p < 0.05$) higher in Aug 2006 (8685.6 mg/100 g dw) than other (7687.1 mg/100 g dw).

Vinson et al. (1998) found a total phenol of 36.8 mg/100 g dw in tomato which was extraordinarily lesser than content of Brazilian tomatoes analyzed.

Tomatoes total phenolics content reported by Toor and Savage (2006) ranged between 359-395, 340-446, and 476-522 mg/100 g dw to Excell, Tradiro and Flavourine cultivars, respectively. These composition were also smaller then our results.

The phenolic content of the tomatoes cuticles increased substantially during the fruit development: those from immature green and mature ripe fruit. Phenols were minor constituents (< 5 %) of the surface extracts of green fruit, whereas the surface extracts of ripe fruit ranged from 7-14 % at onset the climacteric stage to 21-26 % at the post-climacteric stage. In all the parts of the tomato, the total phenols tend to increase from the green stage to the mid-ripe stage before decreasing to the green-stage level at the ripe stage (DUMAS et al., 2003).

3.1.3. Total flavonoids

The flavonoids, which are the major components of the total phenolic content of tomatoes, were also quantified (Table 1).

No significant difference was observed in the total flavonoids content in tomatoes harvest in 2006 which was in the ranges 27.2-30.6 mg/100 g fw (490.3-518.3 mg/100 g dw). However, in fruits harvested in 2007 the total flavonoid content were significantly *($p < 0.05$)* higher on those from Jul beginning (35.5 mg/100 g fw) as compared to Jul late (25.6 mg/100 g fw). Among all samples, the tomatoes harvested in Jul beginning (35.5 mg/100 g fw, 766.6 mg/100 g dw) had the significantly ($p < 0.05$) highest total flavonoid content.

The total flavonoid content represented 20.4, 23.4, 17.7, 26.5 and 18.5 % of total phenolics presented on tomatoes from first to fifth lots, respectively.

The total flavonoid content verified with our samples were higher than those reported (210, 211 and 197 mg/100 g dw to cv. Excell, Tradiro and Flavourine, respectively) by Toor and Savage (2006). In comparison with the results of Chang et al. (2006), these content were also higher than reported by them (4.7 and 5.6 mg/100 g dw to Sheng-Neu and I-Tie-Hung varieties, respectively).

The amount of cuticular and extract flavonoids increased steadily and fairly, respectively, during the fruit development according to Dumas et al. (2003).

3.1.4. Lycopene and β-carotene

The lycopene content (Table 1) of tomatoes varied from 7.9 to 12.3 mg/100 g fw (127.0-384.7 mg/100 g dw) to fruits harvest in 2006, and 17.8-17.0 mg/100 g fw (316.5-384.5 mg/100 g dw) to those from 2007. This pigment content was at the significantly ($p < 0.05$) highest in third lot of tomatoes (Sep late 2006) and it was lowest in first lot (Aug 2006).

Our results were similar to reported by Frusciante et al. (2007) that studied the lycopene content of eighteen tomatoes lines grown in Italy in summer of 2003 which ranged from 2.3 to 16.0 mg/100 g fw, respectively.

For tomatoes, the present lycopene content disagreement with those of the Brazilian tomato cv. Santa Clara and Carmen obtained by Tavares and Rodriguez-Amaya (1994) and Niizu and Rodriguez-Amaya (2005): 3.1 and 3.5 mg/100 g fw, respectively.

Fresh tomatoes grown in Croatia had lycopene content in the range 1.8-11.2 mg/100 g fw (MARKOVIC et al., 2006). Sahlin et al. (2004) reported that lycopene levels of tomatoes cv. Aranca and Excell was 3.9 and 2.6 mg/100 g fw, respectively.

The lycopene level was very useful on evaluate of fruit maturity stage and for this reason, it was correlated with CIELAB values because of carotenoid synthesis and chlorophyll degradation throughout ripening.

Tomatoes also contained β-carotene in smaller amount. This content (Table 1) on tomatoes from 2006 ranged from 0.0002 to 0.0065 mg/100 g fw (0.003-0.125 mg/100 g dw), and in those from 2007 was in the range 0.009-0.010 mg/100 g fw

(0.21-0.19 mg/100 g dw). In tomatoes, Niizu and Rodriguez-Amaya (2005) found 0.32 mg/100 g fw of β-carotene. In 12 varieties of Hungrian tomatoes, Abushita et al. (2000) reported β-carotene levels in the range 0.28-0.62 mg/100 g fw. Barba et al. (2006) got results ranging from 0.6 to 1.2 mg/100 g fw for this pigment in Spanic fruits. The β-carotene content determined by Khachik et al. (1992) was 0.28 mg/100 of fresh tomatoes.

The carotenoid composition of foods could be affected by cultivar or variety, stage of maturity, climate or geographic site of production, handling of harvest and post-harvest and storage (RODRIGUEZ-AMAYA, 1999). Additionally, soil fertilization irrigation, light intensity and day/night temperatures could also affect lycopene formation in tomatoes (HEINONEN et al., 1989).

3.1.5. Ascorbic acid

Tomatoes harvested at different times were characterized by variable L-ascorbic acid contents (Table 1). This content varied from 3.9 to 20.3 mg/100 g fwB (73.6-327.1 mg/100 g dwB) to tomatoes harvest in 2006, and 8.7-9.7 mg/100 g fw (149.7-210.1 mg/100 g dw) to those from 2007. The ascorbic acid content was significantly ($p < 0.05$) lesser in tomatoes from Sep late (3.9 mg/100 g fw, 73.6 mg/100 g dw) as compared to other probably due the maturity stage of these fruits.

A range of seasonal variation from 7 to 23 mg/100 g was observed by Liptay et al. (1986) on greenhouse grown fruits of cv. Jumbo at the mature-green stage.

In the samples of our experiment, except first lot of tomatoes, the content of ascorbic acid was lesser than reported by Chassy et al. (2006) which was 17.5 and 16.2 mg/100 g fw to cv. Burbank and Ropreco tomatoes.

The content of tomatoes harvested in Aug was close to those observed by Toor and Savage (2006) which was 296, 310 and 247 mg ascorbic acid/100 g dw to Excell, Tradiro and Flavourine cultivars, respectively. Even though, ascorbic acid content of two last Brazilian tomatoes lots was lesser than reported by authors.

The ascorbic acid content is influence by environmental conditions, especially by direct sunlight (MARTINEZ-VALVERDE et al., 2002). Increases of 66 % in the ascorbic acid content of the ripe fruit resulted when plants were transferred from shade to sunshine at the mature-green stage. Light exposure is favorable to vitamin C accumulation in the tomato fruit (DUMAS et al., 2003).

It has been reported in several papers that the vitamin C content of tomatoes could change with fertilizers. High N (nitrogen) levels resulted in a decrease in the fruit vitamin C concentration (DUMAS et al., 2003).

Vendramini and Trugo (2000) observed that ascorbic acid was greatly reduced from the immature to mature acerola fruit with a loss of 50 % due to biochemical oxidation.

Ascorbic acid in tomato fruit increased slowly and reached a maximum and then declined. The loss of this content occurred just before the fruit reached full color intensity (YAHIA et al., 2001).

Deepa et al. (2007) verified that ascorbic acid content was found to vary significantly among sweet pepper genotypes harvested during different maturity stages which showed a declining trend with advancing maturity. Nevertheless, Kafkas et al. (2007) reported that ascorbic acid concentration increased during the ripening process and their accumulation was strongly dependent on genotypes of strawberry.

But, there was a conflicting result from other works that was reported by Yahia et al. (2001) in their paper most probably due the use of different methodology, cultivars, and the subjective determination of fruit developmental stages.

3.2. Antioxidant activity

3.2.1. Comparison of radical DPPH$^{\bullet}$ scavenging capacity

The decreased order to DPPH$^{\bullet}$ radical-scavenging capacity (Fig. 1) was verified by antioxidant standards: gallic acid (97.2 %) = ascorbic acid (97.2 %) > rutin (94.6 %) > BHT (93.4 %). This order of antioxidant activity is similar to that observed by Kim et al. (2002).

Although, lycopene was able to scavenging 85 % of DPPH$^{\bullet}$ at 30 min whose activity was 14 % lesser than gallic acid and ascorbic acid but with a test volume 40 times lesser than other. The lycopene DPPH$^{\bullet}$ radical scavenging activity was 51 % higher than β-carotene (56 %). Results in agreement with reported by Bohm et al. (2002) in whose assay lycopene was the most effective radical scavenger, followed by β-cryptoxanthin and β-carotene, with the xanthophylls showing minimal activity.

Carotenoids with nine or more conjugated double bonds are able to quench singlet oxygen with increasing activity depending on the number of conjugated double bonds (YANG and MIN, 1994). Lycopene has been shown to have a strong antioxidant activity and to exhibit the highest physical quenching rate constant with singlet oxygen (DI MASCIO et al., 1989) due to its molecular structure with 11 conjugated double bounds.

All tomatoes showed satisfactory scavenging effect on DPPH$^{\bullet}$ free radical (Table 1). Tomatoes harvested in Aug (64 %) lot gave the significantly ($p < 0.05$)

highest DPPH• radical-scavenging capciity followed by Sep late (53 %) and Sep beginning (47 %) fruits which were harvested in 2006. The fruits harvested in 2007 had lesser DPPH• radical scavenging capacity in the range 44-45 %. Thus, the percent DPPH• radical remaining obtained with tomato extracts were close to EC_{50}.

The mechanism involved in the beneficial actions of antioxidants in biological or food systems include directly quenching free radicals to terminate the radical chain reaction, for example (YU et al., 2002).

In general, our results revealed that Brazilian tomatoes cv. AP533 were scavengers by acting possibly as antioxidant probably due to their content of polyphenols (flavonoids and phenolic compounds), lycopene, and ascorbic acid.

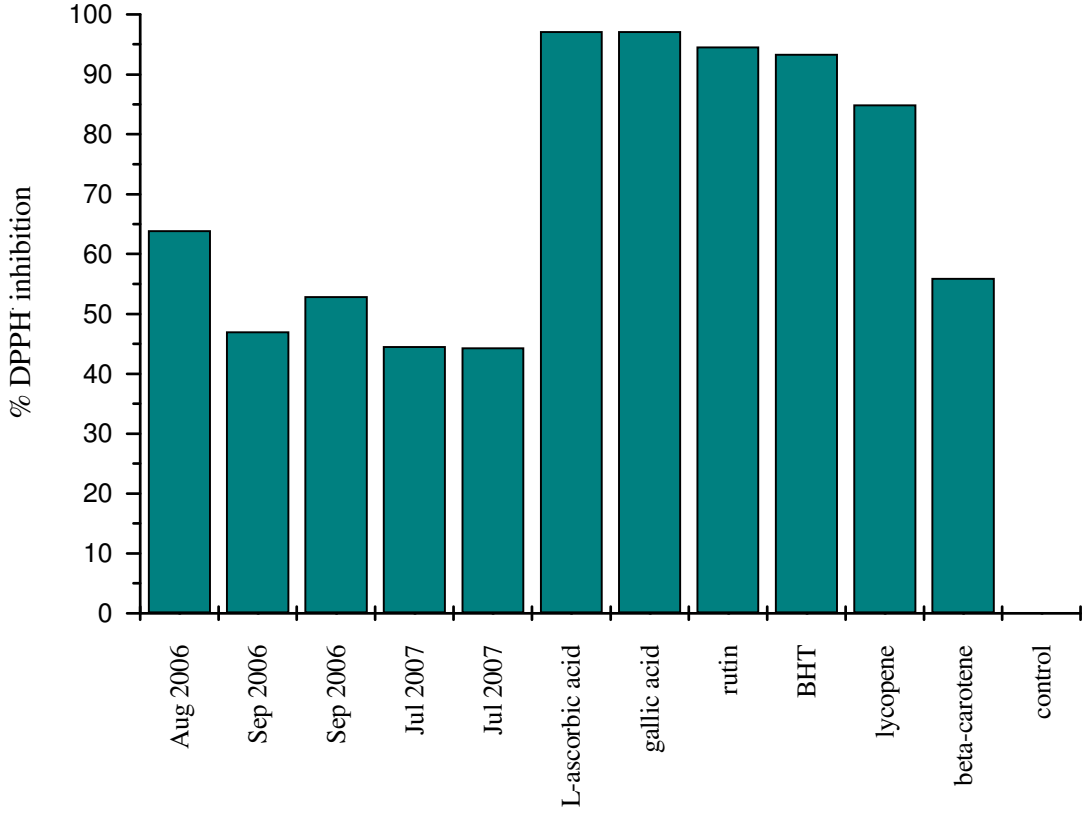

Figure 1. Comparisons of DPPH• radical-scavenging capacity (% inhibition) between tomato extracts and antioxidant standards.

3.2.2. VCEAC of tomatoes

The standard curve that relates the concentration of vitamin C and the amount of absorbance reduction caused by vitamin C was employ to calculate VCEACs (Table 1) based on the absorbance reduction of fresh tomatoes.

The overall relative antioxidant capacity of tomatoes in VCEAC (mg/100 g fw) evaluated by $DPPH^{\bullet}$ assay was in decreasing order as follows: Aug (127.8) > Jul beginning (108.8) > Jul late (100.2) > Sep beginning (57.6) > Sep late (40.2). Tomatoes harvested in Aug 2006 showed significantly ($P < 0.05$) highest VCEAC than other. Nevertheless, since VCEAC expressed in dry weight basis (mg/100 g) the antioxidant capacity of tomatoes followed this order: Jul beginning (2351.1) > Aug (2065.1) > Jul late (1860.4) > Sep beginning (943.3) > Sep late (768.4). Thus, tomatoes harvested in Jul beginning showed significantly ($p < 0.05$) highest VCEAC than other.

3.3. Chromatic characterization

CIELAB parameters (Fig. 2) were measured in tomatoes from different date of harvest (Table 2), and differences in these parameters among fruits appeared to be significant.

Tomatoes harvest in Sep beginning exhibited the highest a^* (29.70) and b^* (18.31) values, and Chroma (34.89). Those fruits harvested in Jul beginning showed the highest L^* (30.57) value, a^*/b^* (1.68) and $(a^*/b^*)^2$ (2.82) ratio, and hue (1.48). These results are in agreement with reported by Arias et al. (2000), López Camelo and Gómez (2004), and Brandt et al. (2006).

The positive values a^* (red saturation) and b^* (yellow saturation) reflected the lycopene, and yellow and orange carotenoids synthesis, respectively, and chlorophyll depletion. It well represented by red color of tomatoes which was highest on tomatoes from third lot followed by fourth. The lightness factor, L^*, reflected the darkening of the tomatoes as consequence of carotenoid synthesis and the gain of redness. The a^*/b^* ratio had similar behavior of a^* value, and it is often used as indicator of color development in tomatoes and redder hue (WORTHINGTON et al., 1969; Hall, 1964; Koskitalo and Ormond, 1972). The $(a^*/b^*)^2$ ratio is also used as a maturation index (ARIAS et al., 2000).

The Pearson correlations between the color readings and the lycopene content of tomatoes are shown in Table 3. The b^* (R^2 = - 0.18) and L^* (R^2 = 0.22) values produced a weak correlation coefficient with lycopene content. Chroma which reflects vivid of color showed a low correlation (R^2 = 0.38) with lycopene content. The a^* value showed some correlation (R^2 = 0.57) with lycopene content. We obtained a good correlation coefficient (R^2 = 0.77) between a*/b* and $(a^*/b^*)^2$ ratios and lycopene content. Similarly to Arias et al. (2000) and Brandt et al. (2006), we obtained a higher correlation between a^*/b^* and $(a^*/b^*)^2$ ratios, respectively, and lycopene content. However, the hue which is the actual color showed also good correlation (R^2 = 0.78) with lycopene content more than a^*/b^* and $(a^*/b^*)^2$ ratios.

The higher the lycopene content, the higher was hue and a^*/b^* and $(a^*/b^*)^2$ ratios. Hence it can be concluded that the color indicates the lycopene content.

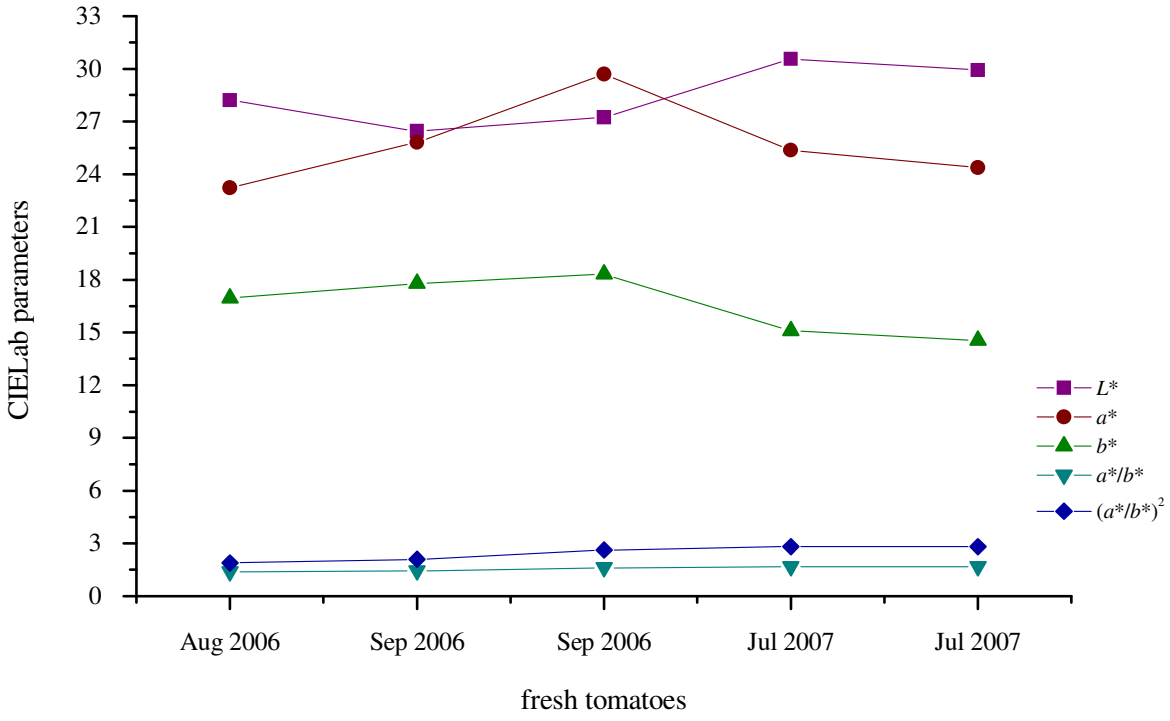

Figure 2. Color readings of tomatoes cv. AP533 in the CIELab parameters.

Table 2. Average and standard deviation of color readings of Brazilian fresh tomatoes cv. AP533.

Tomatoes	Visual Color	*L**	*a**	*b**	*a**/*b**	(*a**/*b**)^2	hue	Chroma
Aug 2006	Pink	28.23 ± 0.04a	23.23 ± 0.03a	16.95 ± 0.03a	1.37 ± 0.00a	1.88 ± 0.00a	1.12 ± 0.00a	28.75 ± 0.04a
Sep 2006 (beginning)	Pink	26.45 ± 0.02b	25.81 ± 0.09b	17.80 ± 0.02b	1.45 ± 0.00b	2.10 ± 0.01b	1.21 ± 0.00b	31.35 ± 0.08b
Sep 2006 (late)	Red	27.23 ± 0.02c	29.70 ± 0.02c	18.31 ± 0.05c	1.62 ± 0.00c	2.63 ± 0.01c	1.41 ± 0.00c	34.89 ± 0.03c
Jul 2007(beginning)	Deep red	30.57 ± 0.04d	25.37 ± 0.03d	15.10 ± 0.11d	1.68 ± 0.01d	2.82 ± 0.03d	1.48 ± 0.01d	29.53 ± 0.08d
Jul 2007 (late)	Deep red	29.92 ± 0.06e	24.38 ± 0.07e	14.55 ± 0.04e	1.67 ± 0.01d	2.81 ± 0.03d	1.47 ± 0.01d	28.39 ± 0.04e

Values are expressed as mean ± standard deviation. Different letter for the same column indicates significance difference ($p < 0.05$).

Table 3. Pearson correlations between the color readings and the lycopene content of tomatoes.

Color parameter	Correlation coefficient
*L**	0.36
*a**	0.67
*b**	- 0.22
*a**/*b**	0.93
(*a**/*b**)^2	0.92
hue	0.93
Chroma	0.44

4. Conclusions

According to the tomatoes harvest and stage of maturity, the antioxidant composition could be change. Differences on antioxidant contents and antioxidant activity were detected among tomatoes lots. Total phenolics, flavonoids, lycopene levels were higher in the tomatoes harvested in September 2006 (late), and these fruits also had the smaller amounts of β-carotene. Already, the ascorbic acid content was smaller in these fruits and it may probably reduce the $DPPH^{\bullet}$ scavenging activity as well as antioxidant capacity (VCEAC) of them. There was perceptible color change between tomatoes, and those with high lycopene level presented the biggest values for color parameters evaluated [CIELAB a^*, b^*, a^*/b^* and $(a^*/b^*)^2$ ratios, hue, and Chroma]. Among samples, the highest tomato color score (mentioned above) was found for fruits harvested in September 2006 (late) and July 2007 (beginning). Color is an indicator of lycopene content and the higher correlation with this pigment was produced by hue, and a^*/b^* and $(a^*/b^*)^2$ ratios. Therefore, we could infer that tomatoes harvested in September 2006 (late) were in more advantage stage of maturity then other and, Brazilian tomatoes cv. AP533 could be a good source of natural antioxidants as phenolic compounds, flavonoids, and lycopene.

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Capítulo 4

Optimization of extraction of flavonoids from ketchup using response surface methodology.

OPTIMIZATION OF EXTRACTION OF FLAVONOIDS FROM KETCHUP USING RESPONSE SURFACE METHODOLOGY.

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Abstract

The extraction of flavonoids from ketchup was optimized by simultaneous maximization of the yield in rutin, quercetin, kaempferol and naringenin using the response surface methodology (RSM). Separation of all compounds was carried out under reversed-phase conditions on a C_{18} column with a binary mobile phase consisting of acetonitrile and water (v/v, final pH 2.5 adjusted with acetic acid). A photodiode array detector was used and data were collected at 290 nm (to naringenin) and at 365 nm (to rutin, quercetin and kaempferol). A complete statistical delineation 23 was used, with evaluation of three parameters: methanol proportion (%), extraction time (min) and residue extraction (number of times). Individual flavonoids showed different patterns of extractability and interaction effects of the variables. The optimal conditions to extract the highest flavonoids were 80 % methanol, extraction time of 35 min and the residue extracted twice.

Keywords: Ketchup; flavonoid; optimization of extraction; response surface; HPLC.

1. Introduction

Flavonoids are widely distributed in plant food and therefore important constituents of the human diet (HERTOG et al., 1992). It has been suggested that humans consume several grams of flavonoids a day (MARKHAM, 1989). Although flavonoids generally are considered to be nonnutritive agents, interest in these substances has arisen because of possible effects on human health (HERTOG et al., 1992). Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. By virtue of their capacity to inhibit LDL oxidation, flavonoids have demonstrated unique cardio protective effects (KONDO et al., 1996; MAZUR et al., 1999). High flavonoid intake predicted lower mortality from coronary heart disease and lower incidence of myocardial infarction in older men (HERTOG et al., 1993) and reduced the risk of coronary heart disease by 38 % in postmenopausal women (YOCHUM, et al., 1999).

Flavonoids share the common skeleton of diphenylpropanes (C6-C3-C6) (HERTOG et al., 1992). Flavonoids can be further classified into anthocyanidins, flavan-3-ols, flavones, flavanones, and flavonols (TSAO and YANG, 2003). Flavonols and flavones occur in plants usually as *O*-glycosides. In vegetables, quercetin glycosides predominate, but glycosides of kaempferol, luteolin, and apigenin are also present (HERTOG et al., 1992).

Tomato is one of the most important fresh vegetables in the industrialized world. It is also important for the food industries as raw material for the production of, for example, purees and ketchup. The large tomato consumption with it is importance in public health has attracted attention of scientists (SLIMESTAD and VERHEUL, 2005).

Extraction method is critical to the recovery of antioxidant phytochemicals. The nature of both plant materials and the bioactive components should be considered in

order to achieve good extraction efficiency. Liphophilicity or hydrophilicity affects the solubility of a phytochemical in the extracting solvent, and conversely, polarity of a solvent also has an impact on the extraction efficiency. Methanol is more frequently used than ethanol due to its higher extraction efficiency. Aqueous methanol between 50 and 80 % has been used for extracting many subgroups of flavonoids (TSAO and DENG, 2004).

Classical optimization studies use the one-factor-at-a-time approach, in which only one factor is variable at a time while all others are kept constant. This approach is time-consuming and expensive. In addition, possible interaction effects between variables cannot be evaluated and misleading conclusions may be drawn. The response surface methodology (RSM) can overcome these difficulties, since it allows accounting for possible interaction effects between variables (KHURI et al., 1996; MONTGOMERY and RUNGER, 2003). If adequately used, this powerful tool can provide the optimal conditions that improve a process (BAS and BOYACI, 2007).

The objective of our work was investigated the effects of methanol proportion, extraction time and number of residue extraction on individual flavonoids concentration. Response surface methodology was applied to build a model between the flavonoids concentrations and these independent factors, and to optimize the extraction conditions for rutin, quercetin, kaempferol and naringenin.

2. Materials and Methods

2.1. Chemicals

All chemicals and solvents were of reagent HPLC. The standard of naringenin was obtained from Chromadex Inc., and rutin, quercetin, and kaempferol were from Sigma Chemical Co.

2.2. Sample

Ketchup (1.5 kg) was supplied by Alimentos Predilecta LTDA (São Lourenço do Turvo Matão, Brazil).

2.3. Extraction and hydrolysis

Approximately 1 g of sample were homogenized in 5 ml of methanol-HCl 1.5N (4:1 and 3:2, v/v) and methanol 100% for 1 min, in a vortex, and stirred for 25, 30 and 35 min, at room temperature (25-30^{o} C). The suspension was then centrifuged at 6400 rpm for 2 min. The solid residue was re-extracted twice and three times using the same amount of extraction solution. The combined fractions were concentrated in a rotary evaporator, dried under nitrogen, and the residues were dissolved in 1 ml of methanol.

2.4. Stock solutions

Stock solutions of rutin, quercetin, kaempferol and naringenin were prepared in methanol-HCl 1.5N (v/v). All stock solutions (0.1 mg/ml) were stored at -18^{o}C and protected by daylight. Prior to injection, stock solutions were appropriately diluted with methanol unless specified otherwise, before being used as working solution. Calibration curves of the standards were in the range of 0.5-36.0 µg/ml for rutin, 0.5-2.5 µg/ml for quercetin, 0.4-2.5 µg/ml for kaempferol, and 2.0-38.0 µg/ml for naringenin. All compounds had linear calibration curves (peak area versus concentration) through origin. R^2 are ranged 0.9979-0.9997.

2.5. Chromatographic conditions

Chromatograph separations were performed on a Hypersil ODS (4.6 x 250 mm I.D.; 3.5 μm) column at 25° C. The mobile phase consisted of acetonitrile:water (pH 2.5 with acetic acid) at a flow rate of 0.7 ml/min. The system was run with a gradient program: 5-95 % of acetonitrile in 30 min, and back to initial condition in 5 min. UV-vis detector set at 290 nm (to naringenin) and 365 nm (to rutin, quercetin and kaempferol). Spectra were recorded ranged 220-450 nm. Volume injection was 20 μL. Peak identity was confirmed by comparison of retention times and diode array spectral characteristics with standards. The samples and the standards solutions were filtered through a 0.22 μm membrane before injection. Results were expressed as mg/100 g dry weight (dw).

2.6. Experimental designer and the response surface analysis

A central composite design was chosen to investigate the influence of methanol proportion, extraction time and residue extraction on flavonoids (rutin, quercetin, kaempferol and naringenin). Each factor was tested at three levels, as shown in Table 1. Table 2 shows the real and coded values of experimental model. The design involved 27 experiments which were made in duplicate. All statistical analyses were carried out by using the statistical package SAS system.

Table 1. Factors and levels tested (coded values in parentheses) for the central composite design.

Factors	***Low level (-1)***	***Neutral (0)***	***High level (+1)***
MeOH concentration (X_1)	60 %	80 %	100 %
Extraction time (X_2)	25 min	30 min	35 min
Residue extraction (X_3)	01	02	03

Table 2. Real and coded values of experimental model.

Run order	Real			Coded		
	MeOH concentration (%)	**Extraction time (min)**	**Residue extraction**	X_1	X_2	X_3
1	60	25	1	-1	-1	-1
2	60	25	2	-1	-1	0
3	60	25	3	-1	-1	1
4	60	30	1	-1	0	-1
5	60	30	2	-1	0	0
6	60	30	3	-1	0	1
7	60	35	1	-1	1	-1
8	60	35	2	-1	1	0
9	60	35	3	-1	1	1
10	80	25	1	0	-1	-1
11	80	25	2	0	-1	0
12	80	25	3	0	-1	1
13	80	30	1	0	0	-1
14	80	30	2	0	0	0
15	80	30	3	0	0	1
16	80	35	1	0	1	-1
17	80	35	2	0	1	0
18	80	35	3	0	1	1
19	100	25	1	1	-1	-1
20	100	25	2	1	-1	0
21	100	25	3	1	-1	1
22	100	30	1	1	0	-1
23	100	30	2	1	0	0
24	100	30	3	1	0	1
25	100	35	1	1	1	-1
26	100	35	2	1	1	0
27	100	35	3	1	1	1

3. Results and Discussion

The extraction procedure is determined by the types of antioxidants to be extracted and whether the objectives are quantitative or qualitative. Polar antioxidants such as glycosides of many flavonoids are generally extracted using water, alcohols or a mixture of water and alcohols. For aglycones of some flavonoids, non-aqueous solvents are used (TSAO and DENG, 2004). Methanol is more frequently used than ethanol due to higher extraction efficiency. Aqueous methanol between 50 and 80 % has been used for extracting many subgroups of flavonoids (CARERI et al., 2000; HAKKINEN and AURIOLA, 1998).

Hydrolysis procedure is required to break the glycosidic bonds of flavonoids, as rutin, for determination of aglycones. Depending on the sugar, several parameters can influence the extraction recovery because degradation reactions can occur or hydrolysis can be incomplete. The major factors are the molarity of HCl, the hydrolysis time, the temperatures and the composition of the extraction solvent (CARERI et al., 2000).

In our case, we were not able to use high temperature because the pectin presence. Therefore, we studied the hydrolysis time, the composition of the extraction solvent and the number of residue extraction on individual flavonoids extraction, at room temperature.

However, since some flavonoid families are thermosensitive (ESCRIBANO-BAILON and SANTOS-BUELGA, 2003) below a certain limit. Temperature above 40^{o}C produced an extraction yield decrease due to a possible degradation of polyphenolic compounds, caused by hydrolysis, internal redox reactions and polymerizations (ALONSO-SALCES et al., 2001). Taking into accounted these facts, 25^{o} C was the temperature selected as the optimum (ABAD-GARCIA et al., 2007).

3.1. Effect of solvent proportion

The impact of solvent proportion on the extraction of flavonoids was conducted with 100, 80 and 60 % methanol, for 30 min, and the solid residue re-extracted twice. The amounts of these compounds extracted are presented in Fig. 1 for the three conditions tested. The methanol proportion affected significantly ($p < 0.05$) the quercetin and kaempferol extractions which were better extracted with 60 % methanol. Already, the methanol proportion not had statistically an effect on rutin and naringenin extractions but, they were more extracted with 100 % methanol. This different in solubility was the consequence of flavonoids polarity. In fact, the most polar flavonoid glycosides show enhanced solubility in water compared to the corresponding aglycones, which exhibit higher solubility in organic solvents such as methanol (CARERI et al., 2000).

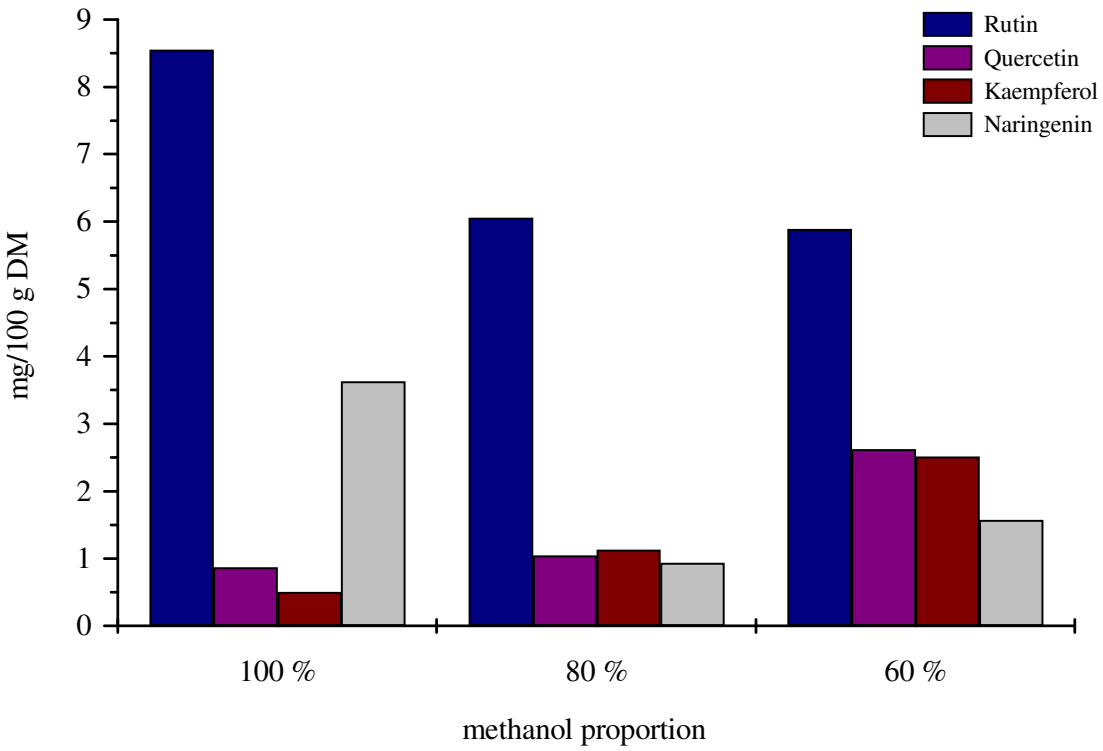

Figure 1. Effect of solvent proportion on the extraction of flavonoids from ketchup for 30 min reaction period and twice residue extraction.

3.2. Effect of extraction time

The impact of extraction time on the extraction of flavonoids was conducted for 25, 30 and 35 min, with 80 % methanol and the solid residue re-extracted twice. The amounts of these compounds extracted are presented in Fig. 2 for the three conditions tested. The extraction time affected significantly ($p < 0.05$) the quercetin and naringenin extractions; the first one was better extracted for 30 min, and the last, for 35 min. By now, the extraction time not had statistically effects on rutin and kaempferol extractions but, they were more extracted for 35 and 30 min, respectively.

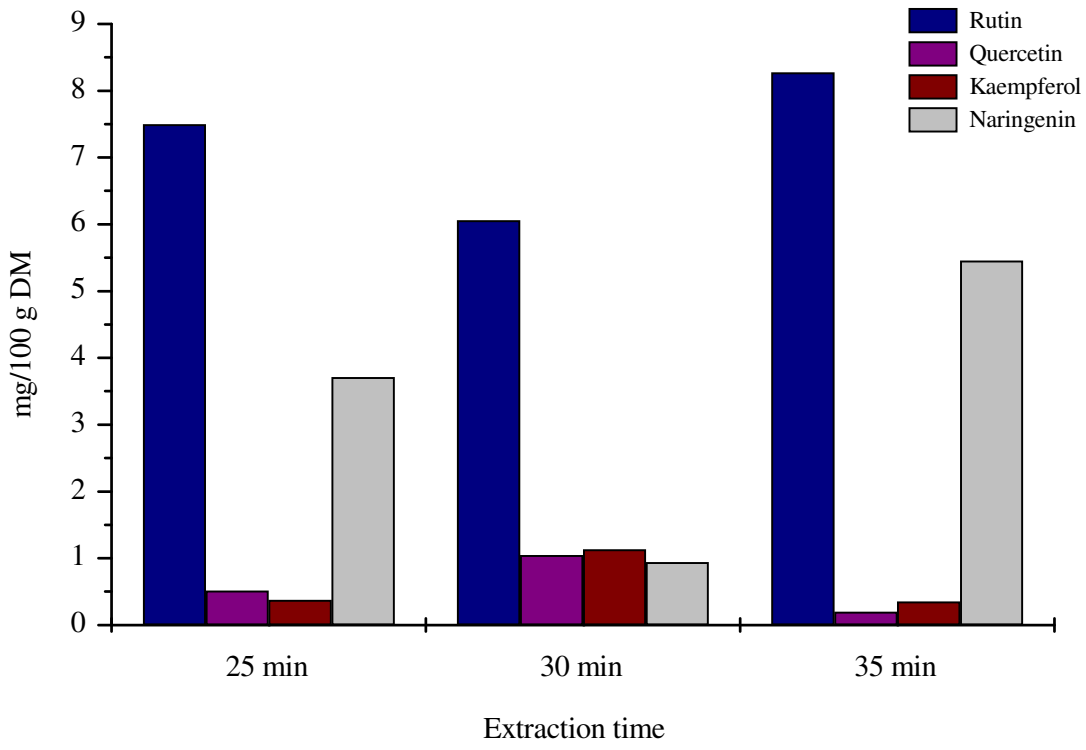

Figure 2. Effect of extraction time on the extraction of flavonoids from ketchup with twice residue extraction with 80 % methanol.

3.3. Effect of residue extraction

The impact of residue extraction on the flavonoid extractions was conducted once, twice and three times, with 80 % methanol for 30 min. The amounts of these compounds extracted are presented in Fig. 3 for the three conditions tested. The residue extraction affected significantly ($p < 0.05$) the extractions to all flavonoids. The rutin, quercetin and kaempferol extractions were enhanced when the solid residue were extracted twice. Already, naringenin extraction was improved when the solid residue was extracted once. We could note a deceleration in the extraction yield of each flavonoid from twice residue extraction which was similar to that reported by Silva et al. (2007) as Fick´s second law of diffusion predicts a final equilibrium between the solute concentrations in the solid matrix and in the bulk solution after a certain time.

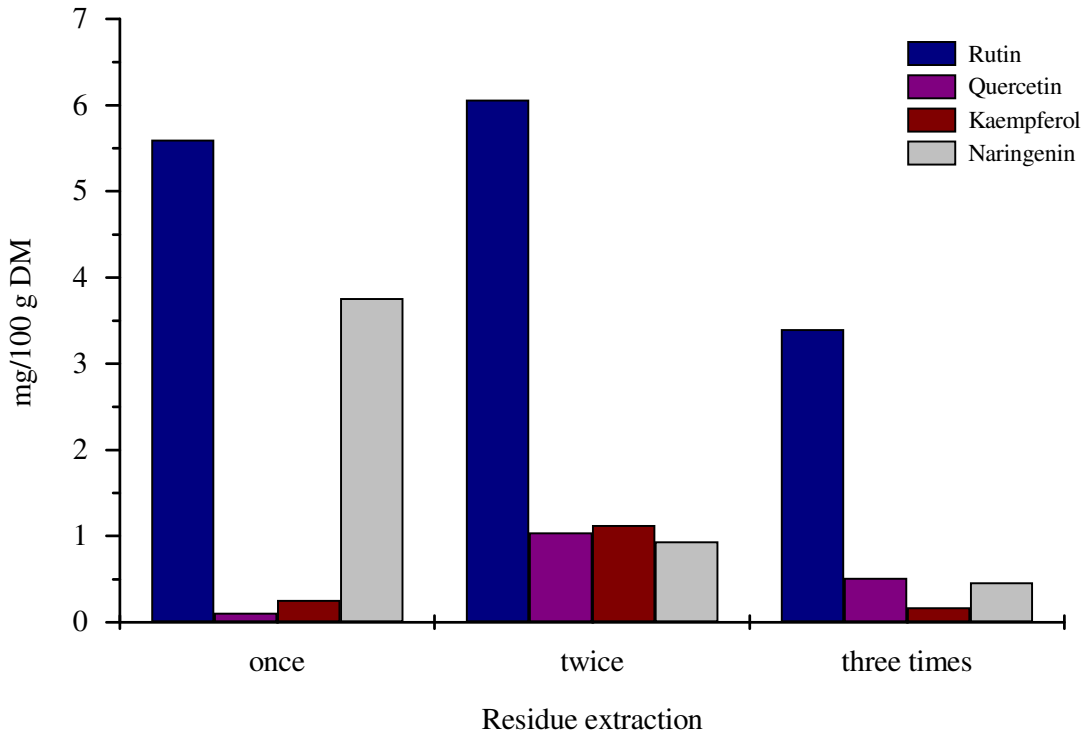

Figure 3. Effect of residue extraction times on the extraction of flavonoids from ketchup with with 80 % methanol for 30 min.

3.4. Optimization of extraction by Response Surface Methodology (RSM)

A central composite experimental design was used to investigate the effects of methanol proportion (X_1), the extraction time (X_2) and the residue extraction times (X_3) on the flavonoids (rutin, quercetin, kaempferol and naringenin) extractions from ketchup samples and to find the best extraction conditions for this compounds. The results of flavonoids for all runs are reported in Table 3.

The highest concentrations were obtained when the extraction solvent extractor was 80 % methanol, the extraction time was 35 min and the residue extracted twice (Table 3).

The surface facilitated the visualization of the statistically significant factors derived from the statistical analysis. Typical response surface methodology (RSM) graphics for individual flavonoids are shown in Figs. 4 and 5.

We could infer that the optimum conditions to maximize rutin extraction corresponded to 95-100 % methanol, extraction time between 25 and 35 min and residue extraction of 2-3 times. A positive interaction between methanol proportion and residue extraction (R^2 = 0.47) was obtained to rutin, which means that the impact of the factor residue extraction depends on the level of methanol proportion being used, and they tended generally to increase at higher levels of methanol proportion.

Quercetin showed the widest response range for methanol proportion (60-65 %), residue extraction (2-3 times) and extraction time of 35 min. This flavonoid showed a positive interaction with residue extraction (R^2 = 0.41) and quercetin yield extraction has a propensity to rise with more residue extractions.

Kaempferol revealed the highest concentration between 60 and 80 % methanol, 2-3 time extraction of residue and extraction time between 25 and 35 min.

Table 3. Flavonoid content (mg/100 g dry weight) obtained on experimental delineation.

	Flavonoid (mg/100 g dw)			
Run order	**Rutin**	**Quercetin**	**Kaempferol**	**Naringenin**
1	4.92 ± 0.24	0.32 ± 0.08	0.28 ± 0.01	3.58 ± 0.70
2	5.33 ± 0.29	0.78 ± 0.13	0.34 ± 0.02	3.12 ± 0.05
3	4.93 ± 1.62	1.49 ± 0.27	0.60 ± 0.18	0.98 ± 0.76
4	10.84 ± 6.14	0.27 ± 0.04	0.34 ± 0.04	3.70 ± 0.20
5	5.89 ± 0.43	2.62 ± 0.19	2.51 ± 0.18	1.57 ± 0.11
6	4.94 ± 0.31	0.39 ± 0.05	0.43 ± 0.01	0.15 ± 0.01
7	5.83 ± 0.44	0.31 ± 0.10	0.31 ± 0.01	0.97 ± 0.17
8	6.20 ± 0.66	0.74 ± 0.25	0.34 ± 0.19	2.30 ± 0.67
9	4.99 ± 0.08	1.52 ± 0.02	0.42 ± 0.40	1.33 ± 0.02
10	5.38 ± 0.51	0.11 ± 0.01	0.28 ± 0.03	3.77 ± 0.18
11	7.50 ± 1.35	0.51 ± 0.02	0.37 ± 0.01	3.71 ± 0.24
12	5.41 ± 0.04	1.68 ± 0.02	1.11 ± 0.42	1.06 ± 0.60
13	5.60 ± 0.20	0.11 ± 0.00	0.26 ± 0.01	3.76 ± 0.19
14	5.95 ± 0.16	1.49 ± 0.00	0.38 ± 0.06	0.94 ± 0.20
15	3.40 ± 0.71	0.50 ± 0.11	0.17 ± 0.01	0.47 ± 0.02
16	7.48 ± 0.89	0.14 ± 0.02	0.47 ± 0.01	4.94 ± 1.11
17	8.27 ± 2.51	0.20 ± 0.01	0.35 ± 0.10	5.45 ± 0.34
18	3.78 ± 0.11	1.21 ± 0.61	0.56 ± 0.33	0.66 ± 0.19
19	5.14 ± 0.16	0.13 ± 0.01	0.30 ± 0.01	4.32 ± 0.07
20	5.79 ± 0.67	0.14 ± 0.003	0.35 ± 0.01	5.47 ± 0.24
21	9.08 ± 2.37	0.15 ± 0.01	0.42 ± 0.03	6.24 ± 0.04
22	4.82 ± 0.38	0.45 ± 0.01	0.96 ± 0.05	2.61 ± 2.84
23	8.55 ± 4.35	0.87 ± 0.18	0.50 ± 0.05	3.63 ± 3.56
24	8.01 ± 1.41	0.22 ± 0.06	0.44 ± 0.01	0.13 ± 0.00
25	5.03 ± 0.21	0.14 ± 0.06	0.26 ± 0.04	4.99 ± 0.29
26	7.45 ± 0.38	0.17 ± 0.01	0.29 ± 0.01	5.73 ± 0.17
27	6.22 ± 0.32	0.48 ± 0.50	0.43 ± 0.12	3.62 ± 1.69

Naringenin had the optimum extraction conditions with 88-100 % methanol, extraction time above of 35 min and residue extraction of 1-2 times. Interestingly, this flavonoid had a negative interaction with residue extraction (R^2 = - 0.42), which may be imputed to its degradation upon longer extracted times.

Table 4 showed the comparison between the predicted value and observed value for the response variables, rutin, quercetin, kaempferol and naringenin within a 95 % confidence interval. These results confirm the predictability of the model for the flavonoids extraction from ketchup in the experimental condition used. Other conditions giving results close to those obtained for the optimum flavonoids extractions are desirable in scientific studies. From the darkness regions (Figs. 4 and 5) supplied by RSM graphics we could improved it.

A good reproducibility of the method was obtained with rutin, naringenin and quercetin (0.996, 0.967 and 0.880, respectively). To kaempferol, the method produced a reproducibility value of 0.664. A maximum relative standard deviation (RSD) was 1.66 % to naringenin, 1.47 % to rutin, 0.64 % to kaempferol, and 0.53 % to quercetin.

Table 4. Comparison between the predicted and observed values (mg/100 g dry weight) for the response variables, rutin, quercetin, kaempferol and naringenin.

Optimized conditions	
Methanol (%)	80
Extraction time (min)	35
Residue extraction	02
Predicted values	
Rutin	6.29 ± 1.97
Quercetin	0.70 ± 0.36
Kaempferol	0.41 ± 0.38
Naringenin	2.64 ± 0.09
Observed values	
Rutin	8.27 ± 2.51
Quercetin	0.20 ± 0.01
Kaempferol	0.35 ± 0.10
Naringenin	5.45 ± 0.39

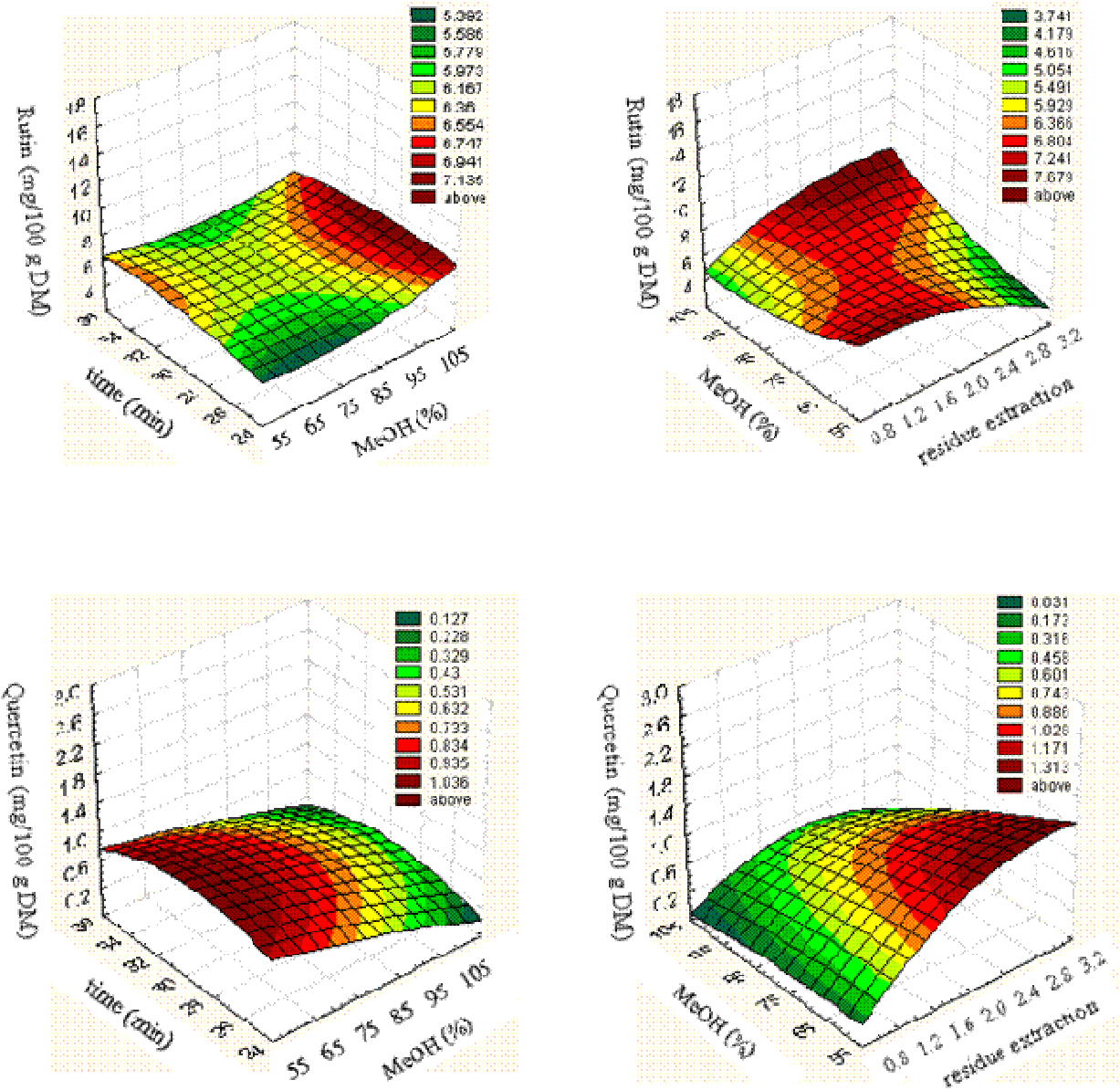

Figure 4. Response surface graphics for rutin and quercetin, in function of solvent composition, extraction time and residue extraction.

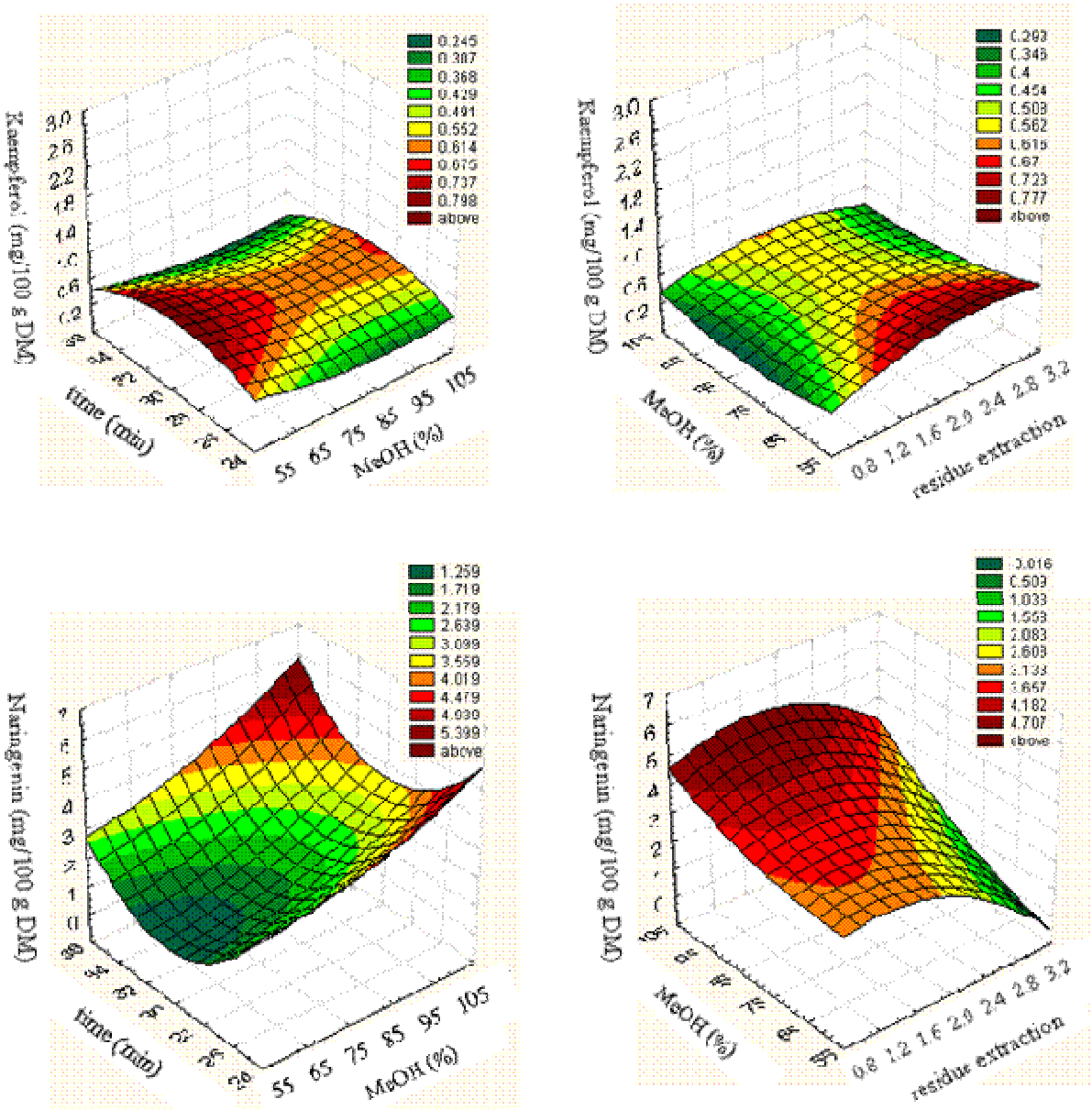

Figure 5. Response surface graphics for kaempferol and naringenin, in function of solvent composition, extraction time and residue extraction.

4. Conclusions

The response surface methodology was successfully employed to optimize the flavonoids extractions from ketchup. Methanol proportion and residue extraction were the most important factors affecting extractions. Each flavonoid showed a particular behavior in the extraction. The best conditions of methanol proportion, extraction time and residue extraction to extract all flavonoids studied were 80 % methanol, 35 min and the residue extracted twice. Hence, the optimal conditions found for ketchup could be applied to others tomato sub products in a routine analysis of rutin, quercetin, kaempferol and naringenin.

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Capítulo 5

Variation on flavonoid (rutin, quercetin, kaempferol and naringenin) content of Brazilian tomato (Lycopersicon esculentum cv. AP533).

VARIATION ON FLAVONOID (RUTIN, QUERCETIN, KAEMPFEROL AND NARINGENIN) CONTENT OF BRAZILIAN TOMATO (*LYCOPERSICON ESCULENTUM* CV. AP533)

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Abstract

The intake of dietary flavonoids is inversely associated with several diseases such as cardiovascular risk, atherosclerosis, and cancer. To evaluate seasonal variations in flavonoid levels of tomatoes, the compositional profile of Brazilian fruits (*Lycopersicon esculentum* cv. AP533) harvested at five different times, from August 2006 until July 2007, was determined by HPLC and compared. The content of flavonoids based on gram fresh weight was found to vary in following range during the season: 4.65-7.95 µg of rutin, 1.71-5.99 µg of kaempferol, 0.02-0.13 µg of quercetin, and 0.08-0.22 µg of naringenin. The major flavonoids identified were rutin and kaempferol whose levels were significantly ($p < 0.05$) higher on tomatoes harvested on late September 2006 and beginning July 2007, respectively. The content of flavonoids of ripe tomatoes were affected by the date of harvesting that possibly the fruits were in different stages of maturity.

Keywords: Tomato; Flavonoid; Rutin; Quercetin; Kaempferol; Naringenin; HPLC.

1. Introduction

There is evidence that regular tomato consumption decreases the incidence of chronic degenerative diseases such as cardiovascular diseases (PANDEY et al., 1995) and platelet aggregation in type 2 diabetes (SHERYL and LAZARUS, 2004). These beneficial effects of tomato are generally attributed to the different antioxidant molecules such as carotenoids, vitamins, and flavonoids (HIRAI et al., 2007).

Flavonoids are molecules with a phenolic benzopyran structure and occur only in plants where they are present predominantly as glycosides. The flavonoids consist of five subclasses (HARBORNE, 1994): the flavones and flavonols found in almost all plant foods; the flavanones occurring concentrated in citrus fruit; the purple colored anthocyanins found in many fruits, particularly in berries; and catechins (flavanols) predominantly occurring in tea.

Tomato is a major food crop worldwide, and its fruit contains several flavonoids of which naringenin chalcone and rutin (quercetin-3-*O*-rutinoside) are predominant. Quercetin and kaempferol are also found in tomatoes. However, these compounds are found at low levels and are restricted to the peel. Only traces of rutin are found in the flesh, which constitutes 95 % of the fruit (LE GALL et al., 2003; SLIMESTAD and VERHEUL, 2005).

Flavanone naringenin exerts antioxidant (VAN ACKHER et al., 2000), antiproliferative (SO et al., 1996), and anti-inflammatory (LYU and PARK, 2005) effects. Some chalcones have been reported to have antiallergic, antioxidative, and anti-inflammatory (LEE et al., 2006; HATZIIEREMIA et al., 2006) effects. Quercetin inhibited oxidation and cytotoxity of low-density lipoprotein in vitro (DE WHALEY et al., 1990), and can reduce risk for coronary heart disease or cancer (YOSHIDA et al., 1990).

Environmental factors (light, temperature, air composition, mineral nutrition, growth medium) and cultural practices (variety, ripening stage at harvest, training system, and irrigation system) and post-harvest storage conditions are known to affect antioxidant contents of tomatoes (CANO et al., 2003; DUMAS et al., 2003; SLIMESTAD and VERHEUL, 2005).

In many plant species the flavonol content may be enhanced response to elevated light levels, in particular to increased UV-B radiation. It has been reported that cherry tomato plants grown in greenhouse under high light accumulated an approximately two-fold greater soluble phenols content (rutin and chlorogenic acid) than low-light plant (WILKENS et al., 1996).

Brazil is the ninth largest producer of tomatoes, twelfth in the area cultivated and fourth of average productivity in accounting for 3 % of world production by 1 % of the area planted in the world. The national production of tomatoes is important economically because of the annual export of over four million tons of tomatoes and also be the result of a more vegetables consumed in the world.

The aim of this study was to estimate the variation of flavonoid contents (rutin, quercetin, kaempferol, and naringenin) in fresh tomatoes (*Lycopersicon esculentum* cv. AP533) grown in Brazil within the same geographical area and harvested at five different times of the year from August 2006 and July 2007.

2. Materials and methods

2.1. Materials

Brazilian fresh tomatoes (*Lycopersicon esculentum* cv. AP533, 1-2 kg each) were kindly supplied by *Alimentos Predilecta LTDA* (São Lourenço do Turvo Matão,

SP, Brazil) at five different times: August 2006, September 2006 (beginning and late), and July 2007 (beginning and late). Tomatoes were homogenized in a Waring blender to obtain a representative sample and kept at -18°C until further analysis which was carried out in triplicates.

2.2. Chemicals

All chemicals and solvents were of reagent HPLC. The standard of naringenin and kaempferol were obtained from Chromadex Inc., and rutin, and quercetin were from Sigma Chemical Co.

2.3. Extraction and hydrolysis

The extraction was performed according to Mauri et al. (1999) and Akissoe et al. (2004) with following modifications. One-gram fresh tomatoes was homogenized in 5 ml of methanol-HCl 1.5N (4:1, v/v) for 1 min, in a vortex, and stirred for 30 min at 35°C. The suspension was then centrifuged at 6400 rpm for 2 min. This was done twice. The filtrates were evaporated to dryness under vacuum, and the residues were dissolved in 1 ml of methanol. The resulting solution was filtered through a 0.22 µm membrane, and 20 µl was injected in a liquid chromatography.

2.4. Analytical HPLC

The HPLC system consisted of Shimadzu liquid chromatography with diode array detector SPD-M 10A VP. The column used was Hypersil ODS reversed-phase (4.6 x 250 mm I.D.; 3.5 µm) at 25°C. The eluent consisted of acetonitrile:water (adjusted to pH 2.5 with acetic acid) at flow rate of 0.7 ml/min. For the elution program, the following proportions of solvent acetonitrile were used: 5-95 % in 30 min; 95-5 %

in 2 min, and 5 % for 3 min. Acquisition was set at 365 nm (to rutin, quercetin and kaempferol), at 290 nm (to naringenin), and at 320 nm (to caffeic acid and chlorogenic acid) (spectral acquisition, 220-450 nm). The samples and the standards solutions were filtered through a 0.22 µm membrane before injection. Peak identification was performed by comparison of retention times and diode array spectral characteristics (Fig. 1) with standards and the literature spectra. Average purity was 95 %, 98 %, 99 % and 98 % for rutin, kaempferol, naringenin and quercetin, respectively. Results were expressed as µg/g of fresh (fw) and dry weight (dw), as mean ± deviation (SD).

2.5. Stock solutions

All the standards were dissolved in methanol to a concentration of 1 mg/ml and were stored in darkness at -18^{o}C and protected by daylight. Prior to injection, stock solutions were appropriately diluted with methanol unless specified otherwise, before being used as working solution. The calibration curve of each flavonoid was established by injecting 5 different concentrations of the standard mixtures consisting of three flavonols (rutin, quercetin, and kaempferol) and one flavanone (naringenin) (Table 1). Other results as retention time, linear range, and correlation coefficient were also listed in Table 1.

Table 1. Properties of the calibration curves of flavonoids obtained by liquid chromatography.

Standards	**Retention times (min)**	**Linear range (µg/mL)**	**Calibration curve**	**Correlation Coefficient (R)**
Rutin	15.50	5.0-36.0	Y = 79694.7x – 23454.7	0.9997
Quercetin	20.49	0.5-2.5	Y = 57239.2x – 12281.4	0.9979
Kaempferol	22.51	0.4-2.5	Y = 31554.1x – 8521.2	0.9996
Naringenin	21.77	2.0-38.0	Y = 113747.4x – 37226.8	0.9996

2.6. Dry matter and total soluble solid

The total soluble solid and dry matter contents were determined according to Association of Official Analytical Chemistis (1990).

2.7. Statistical analysis

Experimental data were analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Tukey test with significance defined as $p < 0.05$.

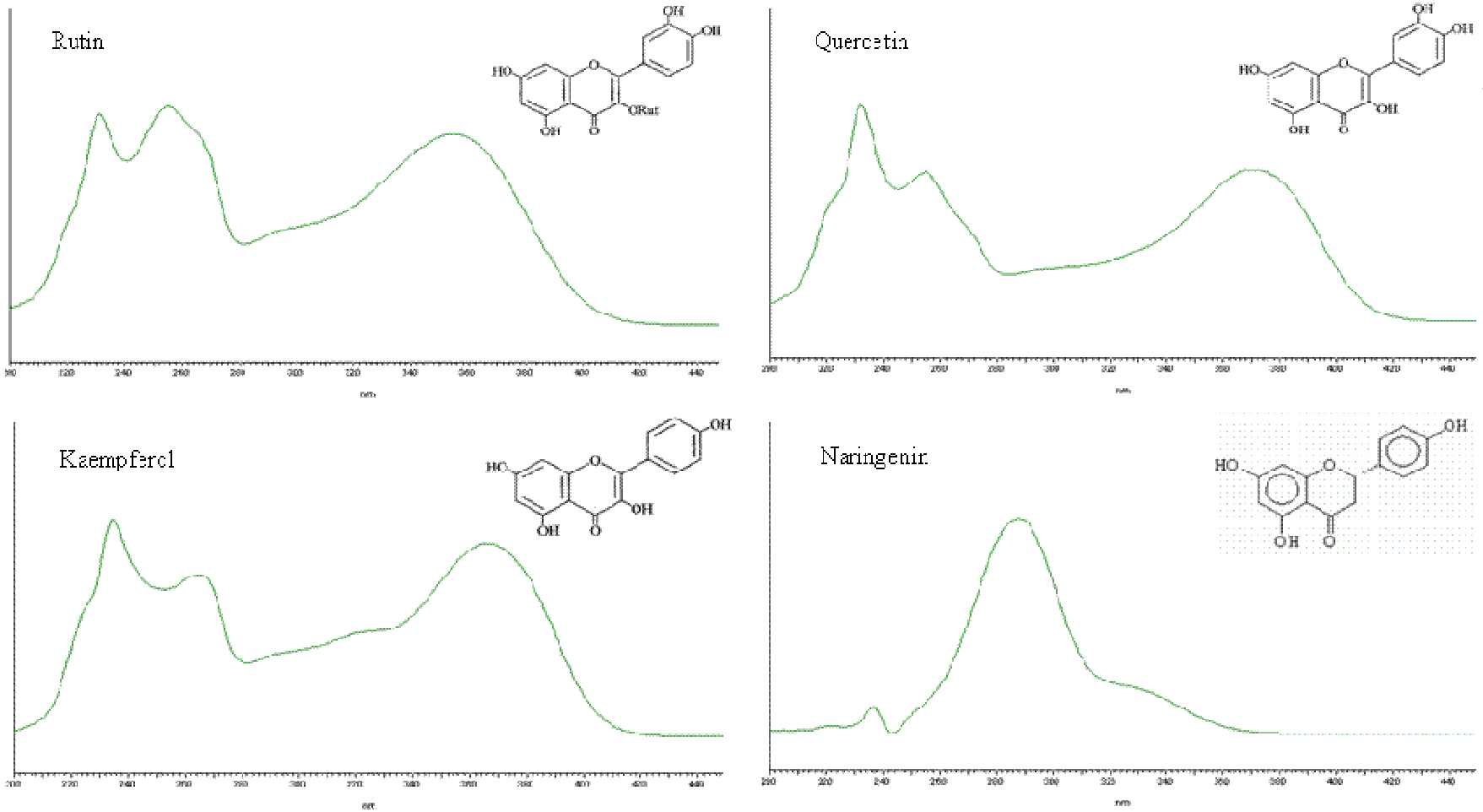

Figure 1. UV-vis spectra and molecular structures of flavonoids quantified in fresh tomatoes cv. AP533.

3. Results and Discussion

Using the method described previously, we isolated and quantified the flavonols (rutin, quercetin and kaempferol) and flavanone (naringenin) present in Brazilian fresh tomatoes cv. AP533 from different harvesting. Chlorogenic acid (t_R = 13.74 min) and caffeic acid (t_R = 14.20 min) were also identified in all samples. These results are seen in Table 2, and a typical chromatogram is shown in Fig. 2.

The major flavonoids detected on tomatoes were rutin (5.72 and 106.27 µg/g fw and dw, respectively) and kaempferol (3.76 and 71.19 µg/g fw and dw, respectively). The naringenin (0.12 and 2.22 µg/g fw and dw, respectively) and quercetin (0.07 and 1.29 µg/g fw and dw, respectively) were also found in lower concentrations.

Among tomatoes harvest in 2006, those from beginning September contained significantly ($p < 0.05$) highest levels of rutin (7.95 and 151.81 µg/g fw and dw, respectively) and kaempferol (4.99 and 95.29 µg/g fw and dw, respectively) as compared to other. Already, between tomatoes harvested in 2007, those from late July presented significantly ($p < 0.05$) highest levels of quercetin (0.13 and 2.48 µg/g fw and dw, respectively) and naringenin (0.13 and 2.41 µg/g fw and dw, respectively). In brief, the rutin, quercetin, kaempferol and naringenin contents were significantly ($p < 0.05$) higher in tomatoes harvested in late September 2006, late July 2007, beginning July 2007 and beginning September 2006, respectively.

Our results were different those reported by Arabbi et al. (2004) who observed that quercetin amounts was significant (0.5 mg/100 g fw). Most papers report, however, on naringenin as the main flavonoid (0.8-4.2 mg/100 g fw) in tomatoes (DAVIES and HOBSON, 1981; PAGANGA et al., 1999; BUGIANESI et al., 2002; MINOGGIO et al., 2003).

Slimestad and Verheul (2005) found rutin in cherry tomatoes ranged from 0.32 to 0.92 mg/100 g fw. Chassy et al. (2006) detected high levels of quercetin (2.64 and 2.18 mg/100 g fw) and kaempferol (1.35 and 1.23 mg/100 g fw) to tomatoes cv. Burbank and Ropreco, respectively, in relation to our values.

However, the kaempferol content found in our work was higher than reported in the USDA (2007) flavonoid database (0.08 mg/100 g fw) and close (4.8 µg/g fw) to that reported by Stewart et al. (2000).

Crozier et al. (1997) investigated seasonal and varietal differences on quercetin levels of tomatoes. Samples of tomatoes Dutch beef cv. Trust, Spanish cv. Assun and Daniella, and Scottish cv. Spectra contained 2.2-6.8, 2.0-8.7 and 4.6-11.2 µg of quercetin/g fw, respectively.

All these differences between the flavonoid levels reported above probably, they are due to differences in cultivars, stages of maturity and geographic or climatic location of tomatoes production.

As a matter of fact, it has been reported that cherry tomato plants grown in greenhouse under high light accumulated an approximately two-fold greater soluble phenols content (rutin and chlorogenic acid) than low-light plants (WILKENS et al., 1996).

Muir et al. (2001) verified that the levels of rutin in tomato fruit peel increased during ripening. In tomatoes, sun exposure has been demonstrated to positively correlate with increased in quercetin (LEE and KADER, 2000; DUMAS et al., 2003).

Table 2. Flavonoid contents (µg/g of sample) of Brazilian tomatoes cv. AP533.

	Fresh Tomatoes					
	Aug 2006	**Sep 2006 (beginning)**	**Sep 2006 (late)**	**Jul 2007 (beginning)**	**Jul 2007 (late)**	**-**
% H_2O	93.81 ± 0.32a	93.89 ± 0.34a	94.76 ± 0.62b	95.37 ± 0.12c	94.62 ± 0.10d	94.49 ± 0.30
Total soluble solids (^{o}Brix)	6.47 ± 0.12a	4.38 ± 0.06b	6.49 ± 0.06c	4.24 ± 0.12d	4.24 ± 0.06d	5.16 ± 0.08
Flavonlos						
Rutin (fw)	4.65 ± 0.01a	4.84 ± 0.00a	7.95 ± 0.24b	5.86 ± 0.09b	5.29 ± 0.07b	5.72 ± 0.08
(dw)	75.04 ± 2.30a	79.45 ± 0.58a	151.81 ± 46.66b	126.74 ± 18.62b	98.33 ± 12.67b	106.27 ± 16.09
Quercetin (fw)	0.08 ± 0.00a	0.09 ± 0.00a	0.03 ± 0.00b	0.02 ± 0.00b	0.13 ± 0.00c	0.07 ± 0.00
(dw)	1.27 ± 0.15a	1.45 ± 0.12a	0.68 ± 0.12b	0.59 ± 0.01b	2.48 ± 0.63c	1.29 ± 0.21
Kaempferol (fw)	2.84 ± 0.18a	3.25 ± 0.14a	4.99 ± 0.76b	5.99 ± 0.45c	1.71 ± 0.41d	3.76 ± 0.39
(dw)	45.81 ± 2.96a	53.39 ± 2.34a	95.29 ± 14.45b	129.65 ± 9.74c	31.79 ± 7.60d	71.19 ± 7.42
Flavanone						
Naringenin (fwB)	0.10 ± 0.00a	0.22 ± 0.01b	0.09 ± 0.03c	0.08 ± 0.02c	0.13 ± 0.03d	0.12 ± 0.02
(dw)	1.66 ± 0.02a	3.68 ± 0.15b	1.70 ± 0.52c	1.66 ± 0.35c	2.41 ± 0.56d	2.22 ± 0.32

Values are expressed as mean ± standard deviation to three replicates for each value. Different letter for the same line indicate significance difference ($p < 0.05$). fw: fresh weight basis. dw: dry weight basis. *av*: average value.

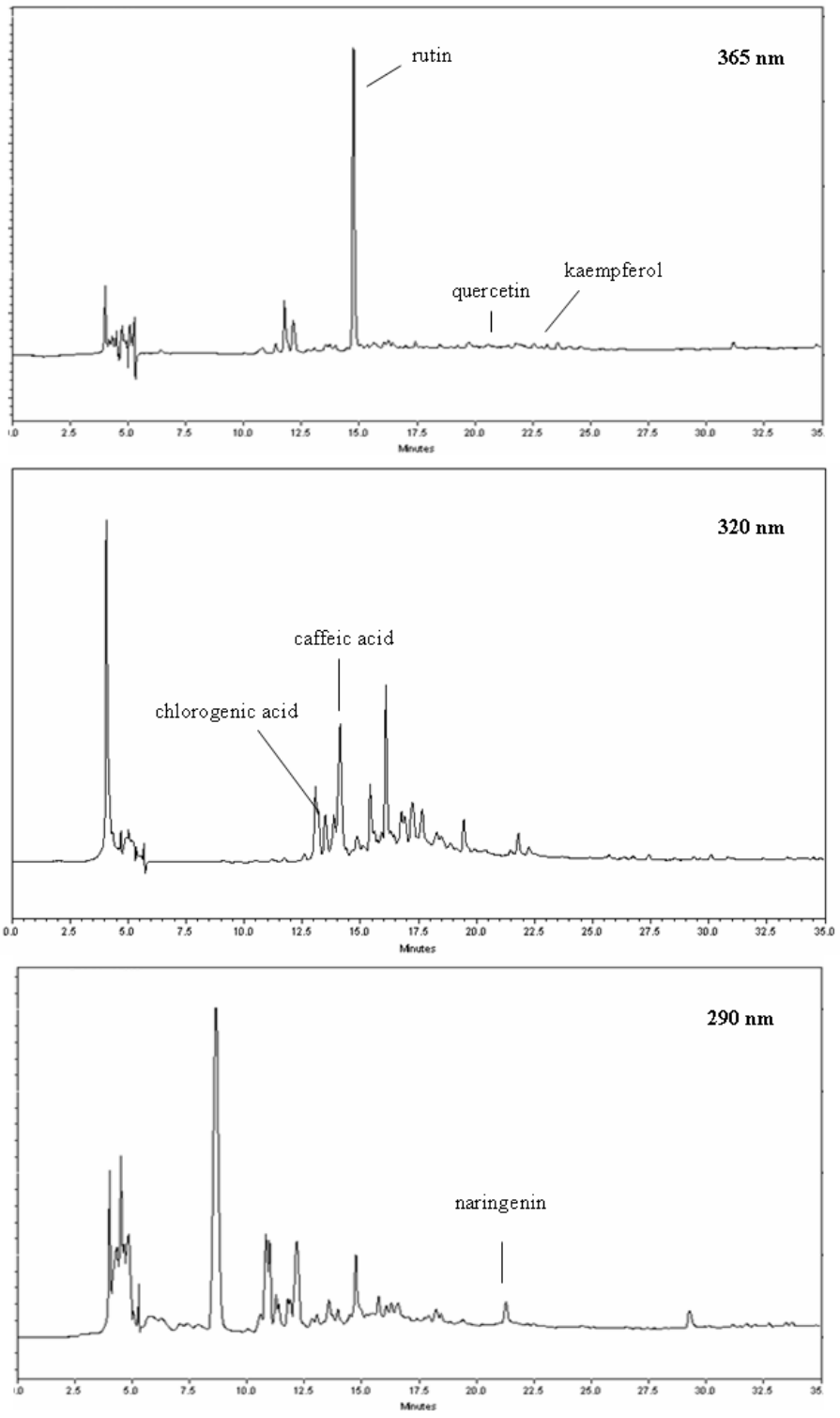

Figure 2. Chromatograms of flavonoids and phenolic acids found in Brazilian tomatoes cv. AP533. Chromatographic conditions: Hypersil ODS reversed-phase (4.6 x 250 mm I.D.; 3.5 μm); mobile phase acetonitrile:water; flow rate of 0.7 ml/min; column temperature 25°C; detection at 365 nm, 320 nm and 290 nm.

4. Conclusions

According to the tomatoes harvest and consequently stage of maturity, the flavonoid composition could be change and differences on it were detected among Brazilian tomatoes cv. AP533 harvested at different times. The major flavonoids on tomatoes were rutin and kaempferol which levels were significantly ($p < 0.05$) higher in those harvested late September 2006 and beginning July 2007, respectively. Among tomatoes harvested in 2006, those from late September had significantly ($p < 0.05$) high level of rutin. The content of flavonoids of ripe tomatoes were affected by the time of harvesting that possibly the fruits were in different stages of maturity. Once rutin levels in tomato fruit peel increased during ripening we could infer that those fruits were in an advanced maturity stage than the other.

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Capítulo 6

Evaluation of commercial ketchups: Bioactive compounds, DPPH• radical-scavenging capacity and color.

EVALUATION OF COMMERCIAL KETCHUPS: BIOACTIVE COMPOUNDS, DPPH• RADICAL-SCAVENGING CAPACITY AND COLOR

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Abstract

The influence of tomato pulp amount used in ketchup manufacture on their bioactive compounds (total phenolics, total flavonoids, lycopene, β-carotene, L-ascorbic acid, rutin, quercetin, kaempferol and naringenin), DPPH• radical-scavenging capacity (% of DPPH• inhibition and VCEAC) and color (CIELab parameters) was evaluated with two commercial Brazilian ketchups. Ketchup A was produced with 4 % more tomato pulp than other ketchup (B). The total soluble solids contents of ketchup A and B were 31.20 and 26.31 %, respectively. Significantly ($p < 0.05$) higher total phenolics, total flavonoids, lycopene, β-carotene and L-ascorbic acid contents were found in ketchup A, and consequently, the radical-scavenging capacity was also higher. In this ketchup, all bioactive compounds studied showed a higher correlation with DPPH• radical-scavenging capacity, except L-ascorbic acid whose correlation was weak. Though, in ketchup B, these compounds produced a weak correlation coefficient with antioxidant activity. The lesser tomato pulp amount of ketchup B resulted on smaller red color else. This results give an idea about that amount of tomato pulp used in ketchup manufacture influence on bioactive compounds levels, DPPH• radical-scavenging capacity and color of final product.

Keywords: Ketchup; CIELAB; Color; Bioactive compounds; DPPH• radical-scavenging capacity.

1. Introduction

Epidemiological findings that the consumption of tomatoes and tomato products is strongly correlated with a reduced risk of certain cancers (e.g., prostate, gastrointestinal, and epithelial cell) and cardiovascular disease (GIOVANNUCCI et al., 1995; CLINTON et al., 1996; GIOVANNUCCI, 1999).

Tomatoes are rich in health-related compounds as they are good sources of vitamins, carotenoids and phenolic compounds (GIOVANELLI et al., 1999). Tomatoes constitute the main available source of lycopene, a carotenoid with a high oxygen-radical scavenging and quenching capacities (BEECHER, 1998). Phenolic compounds, although present in lesser amounts, could also contribute to the beneficial effects of tomato products (ODRIOZOLA-SERRANO et al., 2007).

Consequently, tomatoes and tomato-based foods may provide a convenient matrix by which nutrients and other health-related food components can be supplied to humans (SÁNCHEZ-MORENO et al., 2006).

Tomatoes are consumed, either as fresh or as industrially processed products. Processed tomato products include canned and sun-dried tomatoes, juices, ketchup, pastes, purees, salads, sauces and soups (SHI and LE MAGUER, 2000). In contrast, knowledge about tomato products is scarce, and is generally limited to the lycopene content (SHI and LE MAGUER, 2000; TAKEOKA et al., 2001; ANESE et al., 2002; YAPING et al., 2002).

Ketchup is a condimental sauce normally used as accompaniment or complement to other foods or as an ingredient in cooking preparations, for giving flavor or enhance the flavor of other foods. Ketchup is made from tomato pulp which is usually added vinegar, salt, spices, onion, garlic, and the product usually sweetened with sucrose, syrups of glucose or mixtures of these. The tomato pulp is the basis of

the suspension biphasic that make up the ketchup, with its chemical and physical properties determine the amount of pulp in a concentration required for the production of catchup with a certain consistency and level of total solids (MARSH et al., 1979).

Brazil is the ninth world producer of tomatoes with superior crop at 3 million tons in the year 2006. About 65% of tomatoes produced are designated for industrial processing. In the country, the processed tomatoes products were estimated about US$ 380 millions.

The objective of this study was to evaluate the influence of tomato pulp amount used in ketchup manufacture on the bioactive compounds (total phenolic, total flavonoid, lycopene, β-carotene, L-ascorbic acid, rutin, quercetin, kaempferol, and naringenin), $DPPH^{\bullet}$ radical-scavenging capacity (% $DPPH^{\bullet}$ inhibition and vitamin C equivalent antioxidant capacity, VCEAC) and color (CIELab parameters) of two different ketchups commercialized in Brazil.

2. Materials and Methods

2.1. Ketchup samples

Two Brazilian commercial ketchups (3.5 kg) were supplied by *Alimentos Predilecta LTDA* (São Lourenço do Turvo Matão, SP, Brazil) differentiating itself by the tomato pulp content used in their production being the one (A) 4 % more concentrated than another (B).

2.2. Dry matter and total soluble solid

The total soluble solid and dry matter contents were determined according to Association of Official Analytical Chemists (1990).

2.3. Total phenolics

Total phenolics were determined by Folin-Ciocalteau colorimetric method (SINGLETON and ROSSI, 1965) modified by Scalbert et al. (1989). Samples of ketchup were extracted with 80 % acetone; they were being for 1 h, in dark, at room temperature, followed by centrifugation. Afterwards, 0.5 ml of the extract was mixed with 2.5 ml of freshly diluted 0.2 N Folin-Ciocalteau reagent, incubated at 50^{o}C for 5 min, it was neutralized by adding 2.0 ml of 7.5 % Na_2CO_3, and they were incubated at 50^{o}C for 5 min. The absorbance of was measured at 760 nm in a Beckman UV/vis spectrophotometer DU® 640. Results were expressed as mg of gallic acid /100 g fw (fresh weight).

2.4. Total flavonoids

The flavonoid content was measured using a colorimetric assay developed by Zhishen et al. (1999). The flavonoids were also extracted with 80 % acetone as described above. A known volume (5 ml) of the supernatant was added to a tube. At zero time, 0.3 ml of 5 % sodium nitrite was added to a tube. After 5 min, 0.6 ml of 10 % $AlCl_3$ was added and, after 6 min, 2 ml of 1 M NaOH were added to the mixture, followed by addition of 2.1 ml distilled water. Sample absorbance was read at 510 nm using UV/vis spectrophotometer. Results were expressed as mg of rutin equivalents/100 g fw.

2.5. Determination of individual flavonoids by HPLC

The extraction was performed according to Mauri et al. (1999) and Akissoe et al. (2004) with following modifications. Ketchup (1 g) was homogenized in 5 ml of methanol-HCl 1.5N (4:1, v/v) for 1 min, in a vortex, and stirred for 30 min at 35 ^{o}C.

The suspension was then centrifuged at 6400 rpm for 2 min. The solid residue was re-extracted twice using the same amount of extraction solution. The combined fractions were concentrated in a rotary evaporator, dried under N_2, and the residues were dissolved in MeOH (1 ml), filtered through a 0.22 µm membrane and injected in a Shimadzu liquid chromatography with diode array detector. The column used was Hypersil ODS (4.6 x 250 mm I.D.; 3.5 µm) at 25 ^{o}C. The mobile phase was acetonitrile:water (pH 2.5 with acetic acid) as a gradient program: from 5 to 95 % in 30, and back to initial condition in 5 min, flowing at 0.7 ml/min. Detection was at 365 nm (to rutin, quercetin and kaempferol) and 290 nm (to naringenin). Volume injection was 20 µl. The calibration curves were in the range 5.0-36.0 µg/ml to rutin, 0.5-2.5 µg/ml to quercetin, 0.4-2.5 µg/ml to kaempferol, and 2.0-38.0 µg/ml to naringenin. Peak identification was performed by comparison of retention times and diode array spectral characteristics with standards and the literature spectra. Average purity was 95 %, 98 %, 99 % and 98 % for rutin, kaempferol, naringenin and quercetin, respectively. Results were expressed as µg/g fw.

2.6. Analysis of lycopene and β-carotene by HPLC

The carotenoids were extracted as modified method described by Barba et al. (2006). The carotenoids from samples (5.0 g) were extracted with hexane-acetone-ethanol (2:1:1, v/v/v) until the residue was completely colorless. The pigment extract was partition to petroleum ether, concentrated at less than 35ºC in a rotary evaporator and dried under nitrogen. Separation of lycopene and β-carotene was performed on a C_{18} column (4.6 x 150 mm I.D., 3.5 µm particle size). The solvent system used was a gradient of acetonitrile:methanol:ethyl acetate from 88:8:4 to 48:26:26 in 25 min, and back to the initial condition (30 min). The flow rate was 0.8 ml/min and the runs were

monitored with the UV-Visible photodiode array detector at 450 and 470 nm to β-carotene and lycopene, respectively. Identification of carotenoids was done comparing the retention time and UV-Visible absorption spectra with those of the standards. The external standard method was used to quantification of pigments. Standards was isolated from carrot (β-carotene) and tomato pulp (lycopene) as described by Kimura and Rodriguez-Amaya (2002). Average purity of the isolated carotenoids was 98 % and 99 % for β-carotene and lycopene, respectively. The calibration curves were in the range 0.06-6.39 µg/ml to β-carotene and 0.15-6.16 µg/ml to lycopene. Results were expressed as mg/100 g fw.

2.7. Ascorbic acid

Ascorbic acid in fresh tomatoes was measured by titration with 2,6-dichlorophenolindophenol (DPI) in agreement with the method of Tillmans (A.O.A.C., 1990). Results were expressed as mg /100 g fw.

2.8. Color measurements

The color of ketchups was measured with Hunter colorimeter (Color Quest II Sphere, CQII/UNI 1200 model) referring to the D65 illuminant and 10^{o} angle of vision. L^* (lightness), a^* (green-red tonality) and b^* (blue-yellow tonality) values were recorded and results were expressed as: hue angle [$\tan^{-1}$ (b^*/a^*)] and Chroma [$(a^{*2} + b^{*2})^{1/2}$] (Arias et al., 2000).

2.9. DPPH$^{\bullet}$ radical- scavenging capacity

The antioxidant capacity was studied through the evaluation of free radical-scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical. This

determination was based on the method proposed by Brand-Williams et al. (1995). Samples of ketchup (53.0 mg) were extracted with cold ethanol according by Vicente et al. (2006). Aliquots of 1000 µl of the supernatant were mixed and react with 1575 µl of 0.5 mM freshly prepared $DPPH^{\bullet}$ methanolic solution, and kept in darkness for 30 min. Absorption of the samples was measured on a spectrophotometer at 515 nm. Results were expressed as percentage decrease with respect to the absorption value of a reference $DPPH^{\bullet}$ solution.

2.10. VCEAC of ketchups

The antioxidant capacity of samples was quantified using vitamin C standard curve (2-10 µg/ml) and expressed as vitamin C equivalent antioxidant capacity (VCEAC) calculated on fresh weight (mg/100 g) according method developed by Kim et al. (2002).

2.11. Statistical analysis

All data is reported as mean ± standard deviation of the mean for three replicates. Analysis of variance (ANOVA) was used and the least significant differences at $p < 0.05$ were calculated by Tukey test to determine significant differences between samples. The results were correlated using the Pearson product moment correlation method.

3. Results and Discussion

3.1. Dry matter and total soluble solid

To dry matter, values ranged from 23.20 to 37.34 %, and 28.59 to 29.05 % to ketchup A and B, respectively. The total soluble solid was 31.20 and 26.31°Brix to ketchup A and B, respectively. The dryd matter content and total soluble solids of ketchup A were 4.8 and 15.7 % higher than in other, respectively. Data obtained on solid matter and total soluble solid are shown in Table 1.

3.2. Bioactive compounds and antioxidant capacity

Table 2 shows mean values of antioxidant contents in the ketchup samples and their antioxidant capacity. We are reporting antioxidant contents based on fresh weight because they reflect relative amounts of them as they are actually delivered to the consumer.

All antioxidant contents were significantly ($p < 0.05$) higher on ketchup A except to lycopene and b-carotene contents whose highest levels were not statistically different from other ketchup. The total phenolic, total flavonoids, L-ascorbic acid, total carotenoids, lycopene and β-carotene contents were 34.2, 22.4, 71.8, 30.8, 33.6 and 7.7 % higher on first product than other, respectively.

The high bioactive compounds levels in the ketchup A resulted in a statistically higher DPPH• radical-scavenging capacity (44.85 %) than in the other type (48.65 %). Among antioxidant compounds, the ascorbic acid content was evident different between samples. Consequently, the ketchup A presented significantly ($p < 0.05$) higher vitamin C equivalent antioxidant capacity (VCEAC) value (132.93 mg/100 g) than ketchup B (68.84 mg/100 g).

Table 1. Total soluble solids, dry matter and color measurement of commercial ketchup A and B.

Ketchup	Dry matter (%)	Total soluble solids (°Brix)	Color				
			*L**	*a**	*b**	hue	Chroma
A	30.27 ± 7.07[a]	31.20 ± 4.96[a]	21.57 ± 0.49[a]	33.87 ± 7.47[a]	33.02 ± 6.43[a]	0.67 ± 0.04[a]	47.31 ± 9.83[a]
B	28.82 ± 0.23[a]	26.31 ± 2.89[a]	24.55 ± 0.25[b]	24.92 ± 0.12[a]	24.04 ± 1.69[a]	0.75 ± 0.10[a]	34.64 ± 1.25[a]

Values are expressed as mean ± standard deviation. Data followed by different letters in the same column are significantly different at 0.05 probability level.

Table 2. Antioxidant compounds and antioxidant activity of commercial ketchups A and B.

Ketchup	Antioxidants compounds (fresh weight)						Antioxidant capacity	
	Total phenolic (mg GAE/100 g)	Total flavonoids (mg rutin/100 g)	L-ascorbic acid (mg/100 g)	Total carotenoid (mg/100 g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)	% of DPPH• inhibition	VCEAC (mg/100 g fw)
A	545.59 ± 19.52[a]	204.32 ± 18.35[a]	26.38 ± 2.81[a]	13.63 ± 1.03[a]	13.64 ± 3.23[a]	0.052 ± 0.005[a]	55.15 ± 1.88[a]	132.93 ± 34.92[a]
B	358.90 ± 8.52[b]	158.64 ± 12.99[b]	7.45 ± 1.13[b]	9.44 ± 0.73[b]	9.06 ± 5.33[a]	0.048 ± 0.027[a]	51.35 ± 0.71[b]	68.84 ± 4.08[b]

Values are expressed as mean ± standard deviation to three replicates for each value. Data followed by different letters in the same column are significantly different at 0.05 probability level. GAE: acid gallic equivalent; DPPH•: (2,2-diphenyl-1-picrylhydrazyl); VCEAC: vitamin C equivalent antioxidant capacity; fw: fresh weight.

To ketchup A, the total phenolics (R^2 = -0.95), total flavonoids (R^2 = -0.92), total carotenoids (R^2 = -0.99) and β-carotene (R^2 = -0.95) contents produced a higher correlation with DPPH$^{\bullet}$ radical-scavenging capacity. Already, the lycopene (R^2 = 0.51) content produced a weak correlation with this capacity. The ascorbic acid content (R^2 = 0.23) showed a weak correlation coefficient with antioxidant activity. However, to ketchup B, all compounds analyzed showed a worst correlation between their contents and antioxidant activity.

The data obtained to individual flavonoids identified on samples and quantified were summarized in Table 3. Fig. 1 shows flavonoid chromatograms of ketchup A and B at 365 nm and 290 nm. In all samples, the major flavonoids were rutin followed by kaempferol, naringenin and quercetin. The rutin, quercetin and naringenin contents were 46.3, 84.8, and 29.5 %, respectively, higher on ketchup A than other; rutin content were significantly ($p < 0.05$) higher on this product. Already, the kaempferol level was 31.8 % higher on ketchup B.

Table 3. Flavonoids identified on commercial Brazilian ketchups A and B.

Flavonoids (μg/g fw)	t_R (min)	Ketchup	
		A	B
Rutin	15.50	16.95 ± 1.14[a]	9.10 ± 1.45[b]
Quercetin	20.49	1.25 ± 0.29[a]	0.19 ± 0.01[a]
Kaempferol	22.51	5.11 ± 3.95[a]	7.49 ± 3.15[a]
Naringenin	21.77	3.19 ± 1.27[a]	2.25 ± 0.65[a]

Values are expressed as mean ± standard deviation to three replicates for each value. Data followed by different letters in the same column are significantly different at 0.05 probability level. Fw: fresh weight; t_R: retention times.

Among these flavonoids, rutin (R^2 = -0.81) and quercetin (R^2 = 0.99) contents produced a higher correlation with DPPH• radical-scavenging capacity to ketchup A; however, kaempferol (R^2 = -0.47) and naringenin (R^2 = - 0.49) levels showed a worst correlation with antioxidant capacity. Already, to ketchup B, quercetin (R^2 = 1.0) and naringenin (R^2 = -1.0) levels produced a higher correlation with DPPH• radical-scavenging capacity, and rutin (R^2 = -0.50) and kaempferol (R^2 = 0.50) contents showed a weak correlation coefficient with antioxidant activity.

According to Amic et al. (2003) the antiradical activity (radical-scavenging activity) was verified by flavonoids: kaempferol (93.5 %) > rutin (90.9 %) > quercetin (89.9 %) > naringenin (6.3 %).

In general, the radical-scavenging activity of flavonoids depends on the molecular structure and the substitution pattern of hydroxyl groups, i.e., on the availability of phenolic hydrogens and on the possibility of stabilization of the resulting phenoxyl radical via hydrogen bonding or by expanded electron delocalization (BORS et al., 1990; RICE-EVANS et al., 1996). The structural requirement considered to be essential for effective radical scavenging by flavonoids is the presence of a 3',4'-dihydroxyl in the B ring, possessing electron donating properties and being a radical target. Also, the 3-OH moiety of the C ring is also beneficial for the antioxidant activity of flavonoids (VAN ACKER et al., 1996).

In the commercial ketchups A and B analyzed, total carotenoids content was in the range 13.63 and 9.44 mg/100 g, respectively. Lycopene was the main carotenoid found in these products: 13.64 and 9.06 mg/100 g, respectively. β-carotene content of both ketchups was minor who's ranged 0.052 and 0.048 mg/100 g, respectively. The different contents of carotenoids in the commercial tomato products could be explained in the terms of dry matter content and total soluble solids.

Markovic et al. (2006) reported that lycopene content in different Croatian ketchup types ranged from 8.10 to 24.27 mg/100 g wet weight. Samples of ketchup from 3 commercial brands of U.S. manufactures were analyzed for carotenoid content by Ishida and Chapman (2004); they reported that lycopene content ranged from 10.05 to 12.41 mg/100 g fw, and total carotenoids were in the range 10.49-16.10 mg/100 g fw, respectively.

3.3. Color measurements

CIELab parameters were measured in two types of ketchup (Table 1) and differences in these parameters among samples appeared.

Ketchup A exhibited the highest *a** (33.87), *b** (33.02) and Chroma (47.31) values. The both first values indicated the highest amount of red and yellow pigments presented. Chroma referred to vivacity of red color of sample. The *L** (21.57) value was significantly ($p < 0.05$) lower in this ketchup than in the other type (24.55). So, this ketchup presents a darker red color than ketchup B. The hue angle of ketchup A (0.67) was also lowest than in other (0.75). The hue angle of 180^{o} represents pure green and 0^{o}, pure red; hence, the closer to zero for this value will be more red the color of the food. All of these color parameters held the higher lycopene contents. In brief, the color of ketchup A was more red, vivid and dark than in ketchup B.

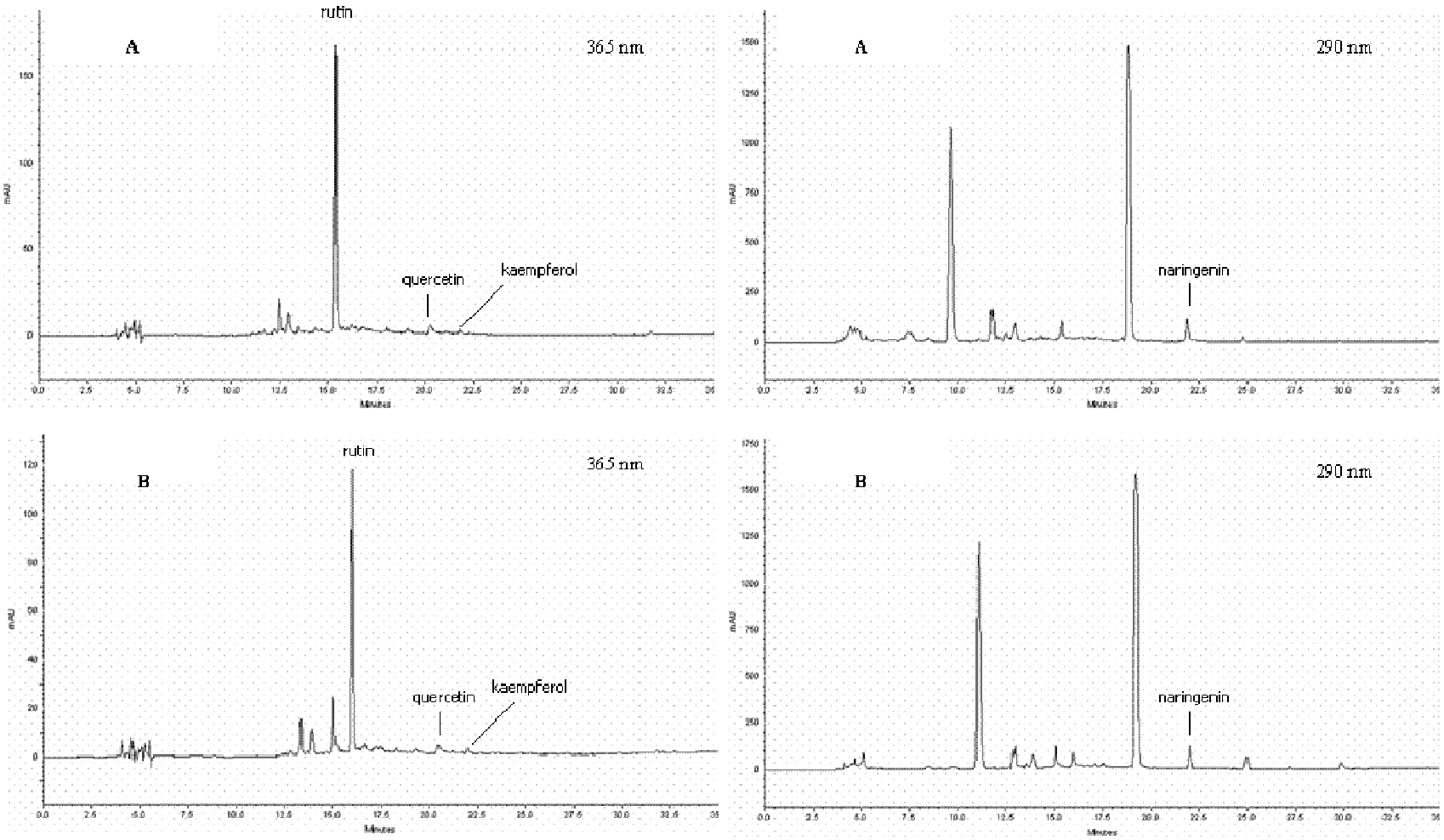

Figure 1. Chromatograms of ketchup A and B. Chromatographic conditions: Hypersil ODS reversed-phase (4.6 x 250 mm I.D.; 3.5 µm); mobile phase acetonitrile:water; flow rate of 0.7 ml/min; column temperature 25^{o}C; detection at 365 nm and 290 nm.

4. Conclusions

Ketchup is a good source of lycopene, carotenoids, and antioxidant compounds, and the lycopene content could be estimate by the dark red appearance of the product. The ketchup A had a much deeper red color than the other type. Although, the total solid content does not have been statically smaller in ketchup B, this resulted in a significantly ($p < 0.05$) minor antioxidant contents (total phenolic, total flavonoids, L-ascorbic acid, total carotenoids, and rutin), DPPH• radical-scavenging capacity (% of DPPH$^{\bullet}$ inhibition and VCEAC), as well as of the color (L^* parameter). The results reported here suggest that amount of tomato pulp used in ketchup manufacture influenced on bioactive compounds, DPPH$^{\bullet}$ radical-scavenging capacity and color of final product. Thus, it may be useful to industry of processing tomatoes in the verification of minor pulp content that can not result in lower levels in antioxidant compounds and antioxidant activity and in ketchup with color more enjoyable for consumers.

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Capítulo 7

Variations of antioxidant compounds, DPPH• radical-scavenging capacity and color parameters as affected by tomato processing into ketchup.

VARIATIONS OF ANTIOXIDANT COMPOUNDS, DPPH• RADICAL-SCAVENGING CAPACITY AND COLOR PARAMETERS AS AFFECTED BY TOMATO PROCESSING INTO KETCHUP

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Abstract

The variation in antioxidant compounds (total phenolics, total flavonoids, lycopene, β-carotene, ascorbic acid, rutin, quercetin, kaempferol and naringenin), DPPH• radical-scavenging capacity (% DPPH• inhibition and VCEAC) and color (CIELab parameters) of tomato as function of thermal processing into ketchup was evaluated in the present work. Samples of fresh tomatoes, tomato pulp and final ketchup were obtained from three different processing dates in the industry. Comparison of antioxidant compounds contents and VCEAC throughout processing indicated that were statiscally significant losses in total phenolics (63 %), total flavanoids (84 %), ascorbic acid (81 %), lycopene (92 %) and rutin (72 %) contents, and also, in VCEAC (89 %). Antioxidant acivity, measured as percentage DPPH• inhibition, of all samples was statiscally significant different and it was higher than 43.34 % of inhibition. The ketchup had a reddish-brown color which was more vivid and darker than fresh tomato color.

Keywords: Tomato; tomato pulp; ketchup; color; antioxidant compounds; DPPH• radical-scavenging capacity.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the world's major food crops (FRUSCIANTE et al., 2000). Although, tomatoes are commonly consumed fresh, over 80 % of the tomato consumption comes from processed products include canned and sun-dried tomatoes, juices, ketchup, pastes, purees, sauces and soups (GOULD, 1992; SHI and LE MAGUER, 2000; ODRIOZOLA-SERRANO et al., 2007).

In Brazil, 3.55 million tones of tomatoes are harvested annually. Of this total, about 42 % are intended to tomato processing which the appreciable form of tomato consumption was ketchup (EMBRAPA, 2008). This was available in numerous commercial brands whose market is growing every year. Ketchup (or catsup) is condiment, usually made with ripened tomatoes or tomato pulp with addition of vinegar, liquid sugar, salt, corn syrup, spice, cloves, cinnamon, onions, and garlic.

Tomatoes contain not only the nutritional antioxidants such as vitamin A, C and E, but also a great quantity of non-nutritional antioxidants, such as lycopene, flavonoids, vitamin C, and phenolic compounds (HAVSTEEN, 1983; HUDSON and LEWIS, 1983; TAKAHAMA, 1985; WANG et al., 1996; DUMAS et al., 2003).

The main carotenoid present in tomato is lycopene which provides its color (STAHL and SIES, 1996). The ability of lycopene to act as potent antioxidant is thought to protect cells against oxidative damage (RAO and AGARWAL, 1999). Studies demonstrated that a diet rich in phenolic compounds correlates with reduced risk of coronary heart diseases (AMIOT et al., 1997; LE GALL et al., 2003). On the other hand, vitamin C may prevent free radical induced damage to DNA quenching oxidants (FRAGA et al., 1991) and also, it has effects on immune system and the risk of Alzheimer diseases (SÁNCHEZ-MORENO et al., 2003). In biological systems, the

vitamin C acts in synergism with carotenoids, flavonoids and vitamin E, in promoting an effective barrier against cell oxidation and also, recycling them (YOUNG and LOWE, 2001; MIEAN and MOHAMED, 2001; FRIEDMAN, 2002; GEORGE et al., 2004).

The nutritional value of tomato products could be affect by processing conditions (SHI and LE MAGUER, 2000; ANESE et al., 2002). Generally, tomatoes sub-products have been considered to have lower nutritional value than their respective fresh commodities mainly due to the loss of compounds such as vitamin during processing (LATHROP and LEUNG, 1980; RAO et al., 1981; BURG and FRAILE, 1995; MURCIA et al., 2000). However, at the same time as bound antioxidants are enhanced by processing (STAHL and SIES, 1992; TONUCCI et al., 1995; WANG et al., 1996; STEWART et al., 2000; GAHLER et al., 2003; SAHLIN et al., 2004; CHANG et al., 2006) other, labile compounds specially, vitamin C, are being destroyed (ABUSHITA et al., 2000; GAHLER et al., 2003; TOOR and SAVAGE, 2006; CHANG et al., 2006).

Therefore the aim of the present work was to evaluate the effect of tomato processing into tomato pulp and afterwards, ketchup on bioactive compounds (total phenolics, total flavonoids, lycopene, β-carotene, L-ascorbic acid, rutin, quercetin, kaempferol, and naringenin), $DPPH^{\bullet}$ radical-scavenging capacity (% $DPPH^{\bullet}$ inhibition and vitamin C equivalent antioxidant capacity - VCEAC) and color (CIELab parameters).

2. Materials and Methods

2.1. Sampling and processing conditions

Fresh tomato, tomato pulp and ketchup were provided by *Alimentos Predilecta LTDA* (São Lourenço do Turvo Matão, SP, Brazil) and colleted in three different date of production. Whole tomatoes were homogenized in a Waring blender to obtain a representative and homogeneous sample. These, along with tomato pulp and ketchup were bottled, leaving the minimum amount of headspace volume, and wrapped in aluminum paper. Once filled, the vessel was sealed and stored at -18^{o}C until analyses which were carried out in triplicates. All results were expressed on fresh (fw) and dry weight (dw) basis.

2.1.2. Ketchup manufacture

In industry, fresh tomatoes (*Lycopersicon esculentum* cv. AP533) were received, classified, washed and screened. Subsequently, the fruits were submitted to enzyme inactivation (90^{o}C for 6 min), separation of the skin and seeds of the tomato pulp, concentration in 3 stages, until the pulp reached 32^{o}Brix to obtain tomato pulp, and sterilization (105^{o}C for 2-3 min). After cooling (until 40^{o}C), the pulp was forwarded to the ketchup production line. The tomato pulp was heated (100^{o}C for 5 min) and added the other ingredients (cinnamon, white pepper, starch from corn, onion, glucose syrup, vinegar, salt, modified starch, conservative sorbate acid, citric acid acidulant, aromatizing and glutamate monossodium). After another warming (85^{o}C for 10 min), the kecthup obtained was immediately cooled (until 50-60^{o}C), bottled, labeled, and forwarded to the dispatch. 4.0 kg of fresh tomatoes yield 1.0 kg of tomato pulp, which yield 1.939 kg of ketchup.

2.2. Dry matter and total soluble solid

The total soluble solid and dry matter contents were determined according to Association of Official Analytical Chemistis (1990).

2.3. Total phenolics

Total phenolics were analyzed spectrophotometrically using the Folin-Ciocalteau colorimetric method (SINGLETON and ROSSI, 1965) modified by Scalbert et al. (1989). Fresh tomato (1.0 g), tomato pulp (0.2 g) and ketchup (0.3 g) were vortexed with 10 ml of 80 % acetone. All samples were being for 1 h, in dark, at room temperature, followed by centrifugation. The supernatant (0.5 ml) was mixed with 2.5 ml of freshly diluted 0.2 N Folin-Ciocalteau reagent, and incubated at 50^{o}C for 5 min. This reaction was neutralized by adding 2.0 ml of 7.5 % sodium carbonate. The samples were incubated at 50^{o}C for 5 min and the absorbance of the resulting blue color was measured at 760 nm in a Beckman UV/vis spectrophotometer DU® 640. The linear reading of the standard curve was from 5 to 25 μg of gallic acid per ml. The total phenolic content was expressed as mg of gallic acid equivalents per 100 g of sample.

2.4. Total flavonoids

The flavonoids were extracted using a modified method described by Yu and Dahegren (2000). Fresh tomatoes (14.0 g), tomato pulp (1.5 g) and ketchup (4.0 g) were vortexed with 10 ml of 80 % acetone. All samples were being for 1 h, in dark, at room temperature, followed by centrifugation. The flavonoid content was measured using a colorimetric assay developed by Zhishen et al. (1999). A known volume (5 ml) of the supernatant was added to a tube. At zero time, 0.3 ml of 5 % sodium nitrite

was added to a tube. After 5 min, 0.6 ml of 10 % $AlCl_3$ was added and, after 6 min, 2 ml of 1 M NaOH were added to the mixture, followed by addition of 2.1 ml distilled water. Sample absorbance was read at 510 nm using UV/vis spectrophotometer. The standard curve was obtained within the linear range of 100-500 µg rutin per ml. The total flavonoid content was expressed as mg of rutin equivalents per 100 g of sample.

2.5. HPLC analysis of flavonoids

The extraction was performed according to Mauri et al. (1999) and Akissoe et al. (2004) with following modifications. Samples (1 g) were stirred for 30 min (35^{o}C) with in methanol-HCl 1.5N (4:1, v/v; 5 ml). The suspension was then centrifuged at 6400 rpm for 2 min. This procedure was done twice. The filtrates were evaporated to dryness under vacuum, and the residues were dissolved in 1 ml of methanol. Flavonoids of samples were separated by liquid chromatography with Hypersil ODS (4.6 x 250 mm I.D.; 3.5 µm) column. Elution was performed with a linear gradient from 95 to 5 % acetonitrile:water (adjusted pH to 2.5 with acetic acid) in 30 min, and remained on it for 5 min. Flow rate of 0.7 ml min^{-1}. Volume injection was 20 µl. A UV/vis detector set at 365 nm (to rutin, quercetin and kaempferol) and 290 nm (to naringenin) was used. Sample peaks were analyzed with the method of external standard. The calibration curves were in the range 5.0-36.0 µg/ml to rutin, 0.5-2.5 µg/ml to quercetin, 0.4-2.5 µg/ml to kaempferol, and 2.0-38.0 µg/ml to naringenin. Average purity was 95 %, 98 %, 99 % and 98 % for rutin, kaempferol, naringenin and quercetin, respectively. All extracts and standard solutions were filtered through a 0.22 µm membrane before HPLC injection. Results were expressed as mg per 100 g of sample.

2.6. Lycopene and β-carotene contents

The carotenoids were extracted as modified method described by Barba et al. (2006). The carotenoids from samples (5.0 g) were extracted with hexane-acetone-ethanol (2:1:1, v/v/v) until the residue was completely colorless. The pigment extract was partition to petroleum ether, concentrated at less than 35ºC in a rotary evaporator and dried under nitrogen. Lycopene and β-carotene contents were analyzed by injecting 20 µL aliquots into a Shimadzu liquid chromatography equipped with UV-Visible photodiode array detector. Separation of carotenoids was performed on a C_{18} column (4.6 x 150 mm I.D., 3.5 µm particle size). The solvent system used was a gradient of acetonitrile:methanol:ethyl acetate (from 88:8:4 to 48:26:26 in 25 min, and back to the initial condition, 30 min). The flow rate was 0.8 ml/min. The β-carotene and lycopene were monitored at 450 and 470 nm, respectively. Identification of carotenoids was achieved by HPLC, comparing the retention time and UV-Visible absorption spectrum with those of the standards. The standards were isolated from tomato pulp (lycopene) and carrot (β-carotene) as described by Kimura and Rodriguez-Amaya (2002). The calibration curves were in the range 0.06-6.39 µg/ml to β-carotene and 0.15-6.16 µg/ml to lycopene. Average purity of the isolated carotenoids was 98 % and 99 % for β-carotene and lycopene, respectively. Results were expressed as mg per 100 g of sample.

2.7. Ascorbic acid

Total L-ascorbic acid content of samples was determined using 2,6-dichlorophenolindophenol (DIP) titrimetric method from the *Official Methods of Analysis of the Association of Official Analytical Chemists* (A.O.A.C., 1990). Results were expressed as mg of ascorbic acid per 100 g of sample.

2.8. Color

CIELab color values of samples, expressed in L^* (lightness), a^* (green-red tonality) and b^* (blue-yellow tonality) referring to the D65 illuminant and 10^o angle of vision, were evaluated using a Hunter colorimeter (Color Quest II Sphere, CQII/UNI 1200 model). These values were recorded and results were expressed as a^*/b^* ratio, hue angle [$\tan^{-1}$ (b^*/a^*)], and Chroma [$(a^{*2} + b^{*2})^{1/2}$] (ARIAS et al., 2000).

2.9. DPPH$^\bullet$ radical-scavenging capacity

The antioxidant constituents were extracted with cold ethanol according Vicente et al. (2006). DPPH$^\bullet$ (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity was determined using a modified method of Brand-Williams et al. (1995). Briefly, 1000 μL of testing solution was mixed and react with 1575 μL of 0.5 mM freshly prepared DPPH$^\bullet$ methanolic solution, and left to stand for 30 min prior to being spectrophotometrically detected at 515 nm. The total DPPH$^\bullet$ radical-scavenging capacity was estimated from the difference in absorbance with or without antioxidants and expressed as percentage of DPPH$^\bullet$ inhibition.

2.9.1. Comparison of DPPH$^\bullet$ radical-scavenging capacity

The total free radical-scavenging capacity of each sample extract was estimated and compared to gallic acid, ascorbic acid, rutin, BHT, lycopene and β-carotene according to Yu et al. (2002). The concentration of DPPH$^\bullet$ was 0.5 mM, 50 mM for antioxidant standards, and 8 mg/ml of sample extracts. Lycopene and β-carotene were isolated from tomato pulp and carrot, respectively, and purified by OCC as described by Kimura and Rodriguez-Amaya (2002).

2.9.2. Vitamin C equivalent antioxidant capacity (VCEAC) of samples

Vitamin C standard curves that relate the concentration of vitamin C and the amount of absorbance reduction caused by vitamin C were obtained using the DPPH assay. The antioxidant capacity was quantified using vitamin C standard curve and expressed as vitamin C equivalent antioxidant capacity (VCEAC) calculated on fresh and dry weight basis (milligrams per 100 g of sample) according method developed by Kim et al. (2002).

2.10. Statistical Analysis

All results were submitted to analysis of variance (ANOVA). Differences among samples were determined using Tukey test with significance defined as $p < 0.05$. All data is reported as mean ± standard deviation of the mean for three replicates.

3. Results and Discussion

3.1. Bioactive Compounds

The bioactive compounds contents quantified in fresh and processed tomatoes (Table 1) were presenting based on fresh weight (fw) since they reflect the amounts of them as they are actually delivered to the consumer.

As expected, the contents of all compounds were higher in tomato pulp and ketchup, apart from lycopene level which were lower in the last one than in fresh tomatoes due to water evaporation and concentration of solids during thermal processing.

Table 1. Bioactive compounds contents (in fresh weight basis) and antioxidant activity of tomato, tomato pulp and ketchup.

	Fresh tomato	**Tomato pulp**	**Ketchup**
Dry matter (%)	5.40 ± 0.78a	27.44 ± 7.95b	30.14 ± 7.19bc
Total soluble solids (^{o}Brix)	4.98 ± 1.29a	26.27 ± 8.13b	31.20 ± 4.96bc
Bioactive Compounds			
Total phenolics (mg GAE/100 g fw)	140.71 ± 8.51a	953.92 ± 237.66b	545.59 ± 19.52c
Total flavonoids (mg RE/100 g fw)	30.52 ± 4.95a	913.36 ± 313.35b	204.32 ± 18.35c
L-ascorbic acid (mg/100 g fw)	12.90 ± 6.38a	88.28 ± 40.62b	26.38 ± 2.81bc
Lycopene (mg/100 g fw)	14.23 ± 5.52a	24.11 ± 5.43a	13.64 ± 3.23a
β-carotene (mg/100 g fw)	0.01 ± 0.01a	0.09 ± 0.05b	0.05 ± 0.01bc
Rutin (mg/100 g fw)	0.52 ± 0.06a	4.83 ± 3.00a	1.69 ± 0.11a
Quercetin (mg/100 g fw)	0.008 ± 0.005a	0.170 ± 0.247a	0.125 ± 0.129a
Kaempferol (mg/100 g fw)	0.35 ± 0.22a	1.57 ± 1.25a	0.51 ± 0.39a
Naringenin (mg/100 g fw)	0.010 ± 0.003a	0.306 ± 0.392a	0.257 ± 0.203a
Antioxidant activity			
$DPPH^{\bullet}$ scavenging (%)	43.34 ± 1.48a	76.78 ± 3.33b	54.38 ± 2.79c
VCEAC (mg/100 g fw)	112.22 ± 14.13a	199.51 ± 31.57b	132.93 ± 34.92b

Values are expressed as mean ± standard deviation to three replicates for each value. Different letter for the same line indicates significance difference ($p < 0.05$). fw: fresh weight; GAE: acid gallic equivalent; RE: rutin equivalent; $DPPH^{\bullet}$: (2,2-diphenyl-1-picrylhydrazyl); VCEAC: vitamin C equivalent antioxidant capacity.

The total phenolics content in fresh tomato (140.71 mg/100 g) was significantly ($p < 0.05$) lower than in tomato pulp (953.92 mg/100 g) and ketchup (545.59 mg/100 g). Likewise, the total flavonoids content of fresh tomatoes (30.52 mg/100 g) was significantly ($p < 0.05$) lower than in tomato pulp (913.36 mg/100 g) and ketchup (204.32 mg/100 g). The flavonoids accounted 22, 96 and 37 % of total phenolics presented in tomatoes, ketchup and pulp, respectively.

Rutin, quercetin, kaempferol and naringenin contents of tomato (0.52, 0.008, 0.35 and 0.01 mg/100 g, respectively) were not statistically lower than in ketchup

(1.69, 0.125, 0.51 and 0.257 mg/100 g, respectively) and pulp (4.83, 0.17, 1.57 and 0.306 mg/100 g, respectively).

The β-carotene content was significantly ($p < 0.05$) lesser in tomatoes (0.01 mg/100 g) when compared to pulp (0.09 mg/100 g) and ketchup (0.05 mg/100 g). The lycopene content of tomato (14.23 mg/100 g) was not statistically lower than in the pulp (24.11 mg/100 g) but higher than in ketchup (13.64 mg/100 g).

The ascorbic acid level of tomato (12.90 mg/100 g) was significantly ($p < 0.05$) lower than in pulp (88.28 mg/100 g) and ketchup (26.38 mg/100 g).

3.2. Antioxidant Activity

Antioxidant activity of natural compounds has been shown to be involved in termination of free radical reactions (SHIMADA et al., 1992). The scavenging effect on $DPPH^{\bullet}$ radical measurement can estimate the capacity of the most reactive compounds against a reference radical (ANESE et al., 2002).

The antioxidant activity (Table 1) of all sample were determined as $DPPH^{\bullet}$ radical-scavenging capacity expressed as % $DPPH^{\bullet}$ inhibition and vitamin C equivalent antioxidant capacity (VCEAC).

Fresh tomatoes, tomato pulp and ketchup showed scavenging effect on $DPPH^{\bullet}$ radicals, and they were in the range of 41.9-44.8, 73.4-80.1 and 51.6-57.2 %, respectively. The percentage $DPPH^{\bullet}$ inhibition of all samples was significantly ($p < 0.05$) different. The tomato pulp gave the highest antioxidant capacity followed by ketchup and fresh tomato. The total antioxidant activity expressed as $DPPH^{\bullet}$ radical-scavenging capacity increased with industrial processing of tomatoes. Odriozola-Serrano et al. (2007) observed no changes in antioxidant capacity ($DPPH^{\bullet}$ radical-scavenging) after cutting and packaging of tomatoes.

The VCEAC value based on fresh weight was significantly ($p < 0.05$) lower in tomato (112.22 mg/100 g) than in pulp and ketchup (199.51 and 132.93 mg/100 g, respectively) as consequence of lower ascorbic acid content in the first one.

The total free radical scavenging-capacity of fresh tomato, tomato pulp and ketchup mentioned above was also compared to antioxidant standards (Figure 1). The decreased order in DPPH$^{\bullet}$ radical-scavenging capacity was verified: gallic acid (97.2 ± 0.4 %) = ascorbic acid (97.2 ± 0.3 %) > rutin (94.6 ± 0.1 %) > BHT (93.4 ± 0.4 %) > lycopene (84.9 ± 0.8 %) > β-carotene (56.5 ± 0.9 %). These results were similar to that observed by Kim et al. (2002) and Bohm et al. (2002).

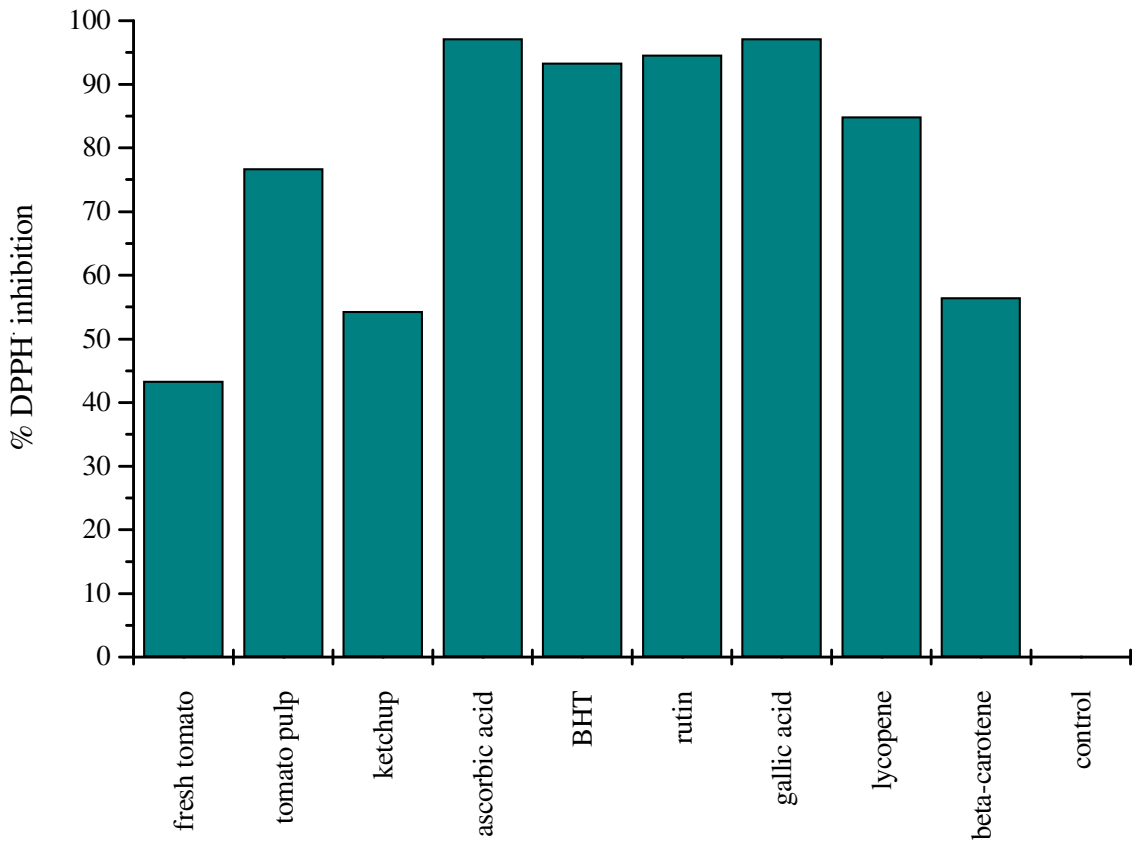

Figure 1. Comparison of DPPH• radical-scavenging capacity expressed as percentage radical inhibition of samples and antioxidant standards.

3.3. Color Measurements

CIELab parameters measured in all samples were presented in Table 2.

Fresh tomatoes had the lower red color (*a** value) than pulp (32.10) and ketchup (33.79). The *a** value of tomatoes (24.33) increased after thermal processing as consequence of lycopene amounts reduction. The *b** value of tomatoes (15.54) also increased with pulp (30.00) and ketchup (31.88) manufactures. The *a**/*b** ratio of tomatoes (1.58) also decreased after pulp (1.13) and ketchup (1.07) manufactures; this value was significantly ($p < 0.05$) higher on tomatoes than ketchup. The intensification of *a** and *b** parameters values could represent an orange to brown color due to the heat treatment.

Table 2. Color parameters evaluated on tomato and their sub-products.

Color parameters	Fresh tomato	Tomato pulp	Ketchup
*L**	29.57 ± 1.22a	23.64 ± 2.70a	25.64 ± 4.80a
*a**	24.33 ± 1.07a	32.10 ± 6.55a	33.79 ± 7.61a
*b**	15.54 ± 1.26a	30.00 ± 10.93a	31.88 ± 8.40a
*a**/*b**	1.58 ± 0.18a	1.13 ± 0.25a	1.07 ± 0.05b
hue	1.36 ± 0.21a	0.80 ± 0.32a	0.73 ± 0.06b
Chroma	28.89 ± 0.58a	44.06 ± 12.08a	46.54 ± 11.16a

Values are expressed as mean ± standard deviation. Different letter for the same line indicates significance difference ($p < 0.05$). *L**: lightness; *a**: redness; *b**: yellowness (the asterisk is a part of each color measurement).

After tomatoes processing, an increase in the darkness (a decrease in the *L** value) was observed in tomato pulp (23.64) and ketchup (25.64) as compared to fresh fruits (29.57). The amounts of sugar, acids (pH), and amino acids, as well as time of processing have been reported to affect the color of processed tomato products by causing formation of brown pigments (Gould, 1992). Olorunda et al. (1990) report

that an increase in drying time and temperature result in tissue darkening whereas other studied report an increase in darkness (L^*) and decrease in redness (a^*/b^* value) of tomatoes after air drying (SHI et al., 1999). Typically, the red color of tomatoes turns reddish-brown during drying, and later on turns brown. This color change is probably due to combination of non-enzymatic browning (Maillard reaction) and lycopene degradation (KERKHOFS et al., 2005).

The hue, which is the actual color and when close to zero represents pure red color, was higher in tomato (1.36) than in pulp (0.80) and ketchup (0.73). This parameter was significantly ($p < 0.05$) lowest in ketchup as compared to tomato. Chroma represents the vivid of color; when this value is close to zero, the color was grayish, and up to 40, it is vivid. The processing of tomatoes resulted in increase of Chroma values of pulp (44.06) and ketchup (46.54) as compared to first (28.89).

Thus, the concentration process of tomatoes resulted in slight decrease in red color due to the appearance of reddish-brown color in ketchup which was more vivid and darker.

3.4. Effects of Tomato Processing into Ketchup

Under the conditions of the industry, the samples were taken at three stages of processing: fresh tomato, tomato pulp and ketchup.

To evaluate consequences of fresh tomato processing into ketchup and to avoid the effect of water evaporation and concentration of solids taking place during thermal processing on the quantification, bioactive compounds contents and VCEAC values were corrected with mass balance and they were (Table 3) were expressed as dry weight basis (dw).

Table 3. Effects of tomato processing in tomato pulp and, afterwards, into ketchup in bioactive compounds contents and antioxidant activity (in dry weight basis).

	Processing Steps		
	Fresh tomato	**Tomato pulp**	**Ketchup**
Bioactive Compounds			
Total phenolics (mg GAE/100 g dw)	10513.28 ± 963.10a	3518.61 ± 287.46b	3933.42 ± 1042.08bc
Total flavonoids (mg RE/100 g dw)	9253.28 ± 2612.71a	3285.85 ± 242.45b	1454.20 ± 300.30cd
L-ascorbic acid (mg/100 g dw)	915.87 ± 360.65a	308.13 ± 70.73b	178.19 ± 53.16bc
Lycopene (mg/100 g dw)	1104.02 ± 533.68a	89.48 ± 9.61b	93.75 ± 37.89bc
β-carotene (mg/100 g dw)	0.55 ± 0.47a	0.32 ± 0.11a	0.34 ± 0.06a
Rutin (mg/100 g dw)	40.01 ± 10.36a	16.85 ± 7.44b	11.19 ± 1.81bc
Quercetin (mg/100 g dw)	0.58 ± 0.38a	0.53 ± 0.08a	0.88 ± 0.17a
Kaempferol (mg/100 g dw)	27.64 ± 10.17a	6.71 ± 1.12b	3.61 ± 1.20bc
Naringenin (mg/100 g dw)	0.76 ± 0.17a	1.05 ± 0.16a	1.54 ± 0.98a
Antioxidant activity			
VCEAC (mg/100 g dw)	8368.89 ± 985.80a	752.18 ± 133.01b	917.14± 389.79bc

Values are expressed as mean ± standard deviation to three replicates for each value. Different letter for the same line indicates significance difference ($p < 0.05$). dw: dry weight; GAE: acid gallic equivalent; RE: rutin equivalent; $DPPH^{\bullet}$: (2,2-diphenyl-1-picrylhydrazyl); VCEAC: vitamin C equivalent antioxidant capacity.

Tomato pulp manufacture resulted in a significant ($p < 0.05$) losses of total phenolics (67 %), total flavonoids (65 %), ascorbic acid (66 %), lycopene (92 %), rutin (58 %), and kaempferol (76 %) contents. However, no significant changes in β-carotene, quercetin, and naringenin contents were observed as the fresh tomatoes were processed into tomato pulp and ketchup.

Significant ($p < 0.05$) decreases in total phenolics (63 %), total flavonoids (84 %), ascorbic acid (81 %), lycopene (92 %), rutin (72 %), and kaempferol (87 %) contents occurred as the tomatoes were processed into ketchup. Nevertheless, we could verify gains of 52 and 103 % in quercetin and naringenin levels, respectively, probably due to ingredients addition specially, onion, in ketchup manufacture. Once

industrial ketchup manufacture was carried out at high temperature over an extended period and also in the presence of oxygen, these losses were expected for ascorbic acid and in particular to lycopene for their susceptibility to thermal degradation.

Toor and Savage (2006) reported losses of 30 % in phenolics levels on semi-dried tomatoes. The major losses of phenolics during processing are brought about by the action of oxidative enzymes such as polyphenoloxidases and peroxidases (SHAHIDI and NACZK, 1995).

Considerable losses of ascorbic acid have been reported during the production of dried tomato halves and tomato pulp using high temperatures (ZANONI et al., 1999; DEWANTO et al., 2002; GIOVANELLI et al., 2002). Lavelli et al. (1999) observed an 88 % loss in ascorbic acid content when tomatoes were dehydrated at 80^{o}C for 7 h to 10 % moisture content. Zanoni et al. (1999) showed that the loss of ascorbic acid was largely dependent on temperature, and reported significant losses of ascorbic acid (40 and 80 % at temperatures of 80^{o} and 110^{o}C, respectively) at 80 % moisture content. Toor and Savage (2006) observed that ascorbic acid content in fresh tomatoes decreased 27 % after drying. Vitamin C is heat instable vitamin; thus, high temperatures led to a loss of vitamin C. With increasing time or processing steps of production of tomato juice, the vitamin C contents decreased (GAHLER et al., 2003).

As expected, the decrease in lycopene level was higher than that of β-carotene due to its extensive conjugated double bonds which was more reactive and consequently, more susceptible to degradation. Oxygen permeability, light exposure, and presence of some metals in the processing system favor the isomerization and oxidation of lycopene during dehydration (SHI et al., 1999). Takeoka et al. (2001) reported significant losses (9-28 %) in the lycopene content during production of tomato paste from fresh tomatoes using high temperatures. Shi et al. (1999) observed

a 3 % loss in lycopene in vacuum-dried tomatoes (55°C for 4-8 h) and 4 % loss in air-dried tomatoes (95°C for 10 h). The presence of both light and oxygen could lead to a significant loss of lycopene during processing tomatoes (COLE and KAPUR, 1957; ZANONI et al., 1999).

When we expressed the antioxidant capacity as vitamin C equivalent antioxidant capacity (VCEAC) in dry weight basis, we could take in that the thermal processing of the tomatoes resulted in a significant ($p < 0.05$) loss of this activity in pulp (91 %) and ketchup (89 %). These results are in agreement with the ascorbic acid levels of tomato, tomato pulp and ketchup; how much larger this content, larger was vitamin C equivalent antioxidant capacity. Consequently, the VCEAC was higher on tomato followed by ketchup and pulp.

Antioxidant capacity of vegetables is known to depend on a wide number of compounds (ODRIOZOLA-SERRANO et al., 2007). Eberhardt et al. (2000) indicated that most of the antioxidant capacity comes from the natural combination of different phytochemicals. In tomato products, vitamin C and polyphenols (flavonoids and hydroxycinnamic acid) are reported to be the major antioxidant hydrophilic components, and vitamin E and carotenoids mainly constitute the hydrophobic fraction (TAKEOKA et al., 2001; MARTINÉZ-VALVERDE et al., 2002). According to Toor and Savage (2006), declines in both total phenolic and ascorbic acid during processing are likely to be responsible for decreased in antioxidant activity in the semi-dried tomatoes.

4. Conclusions

Thermal processing of tomatoes into ketchup resulted in statiscally significant decrease in total phenolics (63 %), total flavanoids (84 %), ascorbic acid (81 %),

lycopene (92 %) and rutin (72 %) contents, and also, in antioxidant activity expressed as VCEAC (89 %). The decrease of VCEAC was closely related to the decrease of ascorbic acid content in ketchup. Longer processing times of heating, required to achieve the desired final solids levels, may be associated with losses. The percentage $DPPH^{\bullet}$ inhibition of all samples was statiscally significant different and tomato pulp (76.8 %) gave the highest antioxidant capacity followed by ketchup (54.4 %) and fresh tomato (43.3 %). The ketchup had a vivid (higher Chroma value) and darker (lower L^* value) reddish-brown color than fresh tomato which could be related to non-enzymatic browning and lycopene degradation.

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CONCLUSÕES GERAIS

- Nos tomates frescos (*Lycopersicon esculentum* cv. AP533) e em seus produtos processados (polpa de tomate e cacthup), os carotenóides e os flavonóides separados por CLAE e identificados foram: (i) os carotenóides licopeno (79-88 %), β-caroteno (5-8 %), fitoeno, fitoflueno, neurosporeno, neoxantina, violaxantina, anteraxantina, luteína, licoxantina α-criptoxantina, β-criptoxantina, α-caroteno, δ-caroteno, ζ-caroteno, e (ii) os flavonóides rutina (majoritária), naringenina, quercetina e kaempferol, respectivamente.

- A data da colheita dos tomates influenciou nos teores dos compostos antioxidantes, atividade antioxidante e cor. Os frutos colhidos em Setembro 2006 (final) apresentaram teores significativamente ($p < 0,05$) maiores em fenólicos totais, licopeno e rutina, e menor teor em ácido L-ascórbico. Conseqüentemente, a atividade antioxidante equivalente em vitamina C (VCEAC) foi também significativamente ($p < 0,05$) menor. Uma vez que, os teores em rutina e licopeno são indicativos do estádio de maturação de tomates pudemos inferir que esses frutos provavelmente estavam em um estádio mais avançado de maturação do que os demais. Os mesmos frutos apresentaram a coloração vermelha significativamente ($p < 0,05$) mais intensa e viva do que a dos demais.

- O processo de obtenção do catchup resultou na significativa ($p < 0,05$) diminuição dos teores em flavonóides totais, fenólicos totais, licopeno, ácido L-ascórbico e rutina, e da atividade antioxidante expressa em VCEAC. O catchup apresentou uma cor vermelho-amarronzada mais viva e escura do que a do tomate provavelmente devido ao escurecimento não-enzimático e degradação do licopeno.

- O teor de polpa de tomate utilizado na produção dos dois tipos comerciais de catchup (A e B) influenciou nos teores de seus compostos antioxidantes, atividade antioxidante e cor. O catchup B (produzido com 4 % a menos de polpa de tomate) apresentou teores significativamente ($p < 0,05$) menores em fenólicos totais, flavonóides totais, ácido ascórbico e rutina, e conseqüentemente uma menor ($p < 0,05$) capacidade seqüestrante do radical $DPPH^{\bullet}$ e VCEAC. A cor vermelha do catchup B foi menos intensa, mais apagada e clara do que a do outro tipo.

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