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COMPOSIÇÃO E BIOACESSIBILIDADE *IN VITRO* DE CAROTENOIDES EM ALIMENTOS PARA BEBÊS

COMPOSITION AND *IN VITRO* BIOACCESSIBILITY OF CAROTENOIDS IN BABY FOOD

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Alimentos e Nutrição

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RESUMO GERAL

O consumo de papinhas industrializadas é alto em todo o mundo, chegando a representar a única fonte alimentar de muitos bebês a partir dos 6 meses de idade. Dessa forma, devem conter todos os nutrientes essenciais para a saúde dos bebês. Os carotenoides, como o β-caroteno e o α-caroteno (provitamina A), a luteína e a zeaxantina são alguns desses compostos bioativos que contribuem para a saúde dos olhos, do cérebro, na resposta imune, além do seu papel anticâncer. Nas crianças, a deficiência de vitamina A é particularmente importante, pois pode levar a cegueira noturna, atraso no crescimento e desenvolvimento cognitivo. Os bebês estão em um momento vulnerável da vida e a alimentação nessa fase irá refletir na saúde do indivíduo na vida adulta. Sendo assim, o objetivo do presente estudo é determinar a composição e a bioacessibilidade *in vitro* dos carotenoides presentes em alimentos para bebês (papinhas) a base de frutas. Foram analisadas 5 papinhas industrializadas a base de frutas; 5 papinhas caseiras a base de frutas e os 15 ingredientes utilizados na preparação das papinhas caseiras (frutas, legumes e cereal). Foram realizadas análises de composição centesimal, minerais (ferro, cálcio, zinco, magnésio, manganês, cobre e potássio), composição de carotenoides e a bioacessibilidade *in vitro* dos carotenoides. Além disso, foram calculados o teor de vitamina A em RAE (Retinol Activity Equivalent, na sigla em inglês) para os carotenoides provitamina A e a adequação à IDR (ingestão dietética recomendada) para os bebês. Os resultados demonstraram que os lipídios e os minerais apresentaram correlação positiva com a bioacessibilidade dos carotenoides das papinhas industrializadas, porém, o mesmo não foi observado com as papinhas caseiras e os vegetais. As frutas e legumes apresentaram as maiores bioacessibilidade de all-*E*-β-caroteno, variando de 5% a 98% em manga e abacaxi, respectivamente. Porém, a concentração de carotenoides foi mais elevada nas papinhas caseiras do que nos vegetais (com exceção da cenoura) e nas papinhas industrializadas. Por possuírem maior quantidade de carotenoides provitamina A, como o all-*E*-β-caroteno, as papinhas caseiras também apresentaram maior valor de RAE e, consequentemente, maior porcentagem de adequação a IDR. Entretanto, mesmo a papinha com maior valor de RAE fornece baixa ingestão de vitamina A (5% da IDR). Desta forma, o aleitamento materno ou uso de fórmulas infantis deve ser continuado após a introdução das frutas na alimentação a fim de garantir o adequado suporte de vitamina A.

ABSTRACT

Consumption of industrialized baby food is high worldwide, representing the only food source for many babies from 6 months of age. In this way, they must contain all the essential nutrients for the health of babies. Carotenoids, such as β-carotene and α-carotene (provitamin A), lutein, and zeaxanthin are some of these bioactive compounds that contribute to the health of the eyes, brain, and immune response, besides their anticancer role. In children, vitamin A deficiency is critical as it can lead to night blindness, delayed growth, and cognitive development. Babies are vulnerable, and the food at this stage will reflect the individual's health in adulthood. Therefore, the present study aims to determine the composition and *in vitro* bioaccessibility of carotenoids present in fruit-based baby food. Five industrialized fruit-based baby food were analyzed; 5 homemade fruit-based baby food, and the 15 ingredients used in preparing homemade baby food (fruits, vegetables, and cereal). Analysis of proximate composition, minerals (iron, calcium, zinc, magnesium, manganese, copper, and potassium), the composition of carotenoids, and the *in vitro* bioaccessibility of carotenoids were carried out. In addition, vitamin A content in RAE (Retinol Activity Equivalent) for provitamin A carotenoids and adequacy to RDI (Recommended Dietary Intake) for infants were calculated. The results showed that lipids and minerals positively correlated with the bioaccessibility of carotenoids in industrialized baby food; however, the same was not observed with homemade baby food, fruits, and vegetables. Fruits and vegetables showed the highest bioaccessibility of all-*E*-β-carotene, ranging from 5% to 98% in mango and pineapple. However, the concentration of carotenoids was higher in homemade baby food than in vegetables (except carrots) and industrialized baby food. Because they have a higher amount of provitamin A carotenoids, such as all-*E*-β-carotene, homemade baby food also had a higher RAE value and, consequently, a higher percentage of adequacy to RDI. However, even the food with the highest RAE value provides a low intake of vitamin A (5% RDI). Thus, breastfeeding or the use of infant formulas should be continued after the introduction of fruits in the diet to ensure adequate vitamin A support.

SUMÁRIO

INTRODUÇÃO GERAL

Segundo a Organização Mundial da Saúde (OMS), o aleitamento materno deve ser a única fonte alimentar até os 6 meses de vida. A partir dos 6 meses, deve-se iniciar a alimentação complementar e manter o aleitamento até os 24 meses (WHO, 2002).

Os alimentos para esta fase de transição devem respeitar a consistência aceita pelo bebê e compreendem sopinhas, papinhas e purês, que podem ser industrializados ou preparados em casa (OKESLI et al., 2011; SBP, 2012; WHO, 2002).

A alimentação complementar é essencial para que a criança obtenha os nutrientes necessários para seu adequado crescimento e desenvolvimento, o que inclui o correto fornecimento de macro e micronutrientes, principalmente ferro e zinco (OKESLI et al., 2011). Para tanto, os produtos de origem animal devem ser incluídos na alimentação dos bebês a partir dos 6 meses de idade (ESPGHAN, 2007; SBP, 2012).

A alimentação no início da vida pode modular a resposta metabólica e predispor a doenças na idade adulta, como diabetes, hipertensão e hipercolesterolemia, as quais estão associadas a ingestão excessiva de açúcares e lipídios (HANLEY et al., 2010; UAUY; CASTILLO, 2003).

Na Europa e nos Estados Unidos, as papinhas industrializadas representam a maior parte dos alimentos fornecidos para bebês a partir dos 6 meses (BRIEFEL et al., 2004; HURLEY; BLACK, 2010; MESCH et al., 2014; OKESLI et al., 2011). A apresentação é feita na forma de conservas prontas para comer que podem ou não necessitar de aquecimento. O elevado consumo se deve principalmente à praticidade de manuseio e transporte (CZAJKOWSKA-MYSŁEK; LESZCZYŃSKA, 2017).

Apesar da praticidade das papinhas industrializadas, suas formulações podem ser responsáveis por elevada ingestão de energia, sódio e gordura saturada se considerarmos a IDR (Ingestão Diária Recomendada) para a idade (OKESLI et al., 2011).

Muita preocupação tem surgido com relação aos alimentos infantis industrializados e sua qualidade nutricional, especialmente em relação ao conteúdo

de açúcar e sódio (COGSWELL et al., 2015; ELLIOTT, 2011), perfil de minerais (MIR-MARQUÉS et al., 2015) e biodisponibilidade de micronutrientes (MELØ et al., 2008).

Entre os micronutrientes e compostos bioativos estudados em alimentos para bebês, estão os carotenoides, com destaque aos alimentos fontes de carotenoides provitamina A, como α-caroteno e β - caroteno, além de outros carotenoides como luteína, zeaxantina e licopeno que também possuem papel fundamental na saúde dos bebês (JIWAN et al., 2010).

Os carotenoides provitamina A, são precursores da vitamina A, que por sua vez está ligada as funções visuais normais, crescimento, desenvolvimento e funcionamento correto do sistema imune em crianças (JIWAN et al., 2010). A deficiência de vitamina A na infância, pode levar ao desenvolvimento da cegueira e ao aumento na frequência e gravidade de infecções (BAILEY; WEST; BLACK, 2015).

Além destes carotenoides, os alimentos para bebês podem conter também luteína e zeaxantina, que atuam como filtro de luz azul na retina, por se tratar de pigmentos maculares (JOHNSON, 2014). Além disso estas xantofilas podem ser encontradas no cérebro, onde estão relacionadas a manutenção das funções cognitivas (BRITTON, 2020).

Para verificar a potencial absorção dos carotenoides, podem ser realizados estudos de bioacessibilidade destes compostos, ou seja, a determinação da fração que estará disponível para absorção após a digestão (FERNÁNDEZ-GARCÍA; CARVAJAL-LÉRIDA; PÉREZ-GÁLVEZ, 2009). Os carotenoides, por se tratarem de compostos lipofílicos, precisam ser incorporados a micelas para serem absorvidos pelo intestino, sendo que este processo pode ser afetado pela presença de outros componentes da matriz alimentar, como as fibras e os lipídios (GUYTON; HALL, 2006; HAMDAN et al., 2018; OSTLUND, 2004).

Geralmente, o estudo de bioacessibilidade de uma única refeição em adultos não consegue predizer a qualidade nutricional da alimentação como um todo, porém, em bebês a composição nutricional de uma refeição, que geralmente compreende uma papinha, pode nos dar uma referência da qualidade de sua alimentação (OKESLI et al., 2011).

OBJETIVOS

1. Objetivo Geral

O objetivo do presente estudo é determinar a bioacessibilidade *in vitro* dos carotenoides presentes em papinhas industrializadas a base de frutas e papinhas caseiras desenvolvidas com os mesmos ingredientes das industrializadas.

2. Objetivos Específicos

a) Determinar o teor de carotenoides totais e individuais em papinhas industrializadas e caseiras.

b) Determinar o teor de carotenoides nas frutas, legumes e cereais utilizados na formulação das papinhas caseiras.

c) Determinar a composição de minerais nas papinhas industrializadas, caseiras e nas frutas, legumes e cereais.

d) Determinar a bioacessibilidade *in vitro* de carotenoides em frutas, legumes e cereais utilizados na formulação das papinhas caseiras.

e) Determinar a composição centesimal das papinhas industrializadas e caseiras.

f) Verificar a correlação entre a composição centesimal e os minerais com a bioacessibilidade dos carotenoides

g) Identificar os carotenoides provitamina A presentes nas papinhas industrializadas e caseiras e determinar o teor de equivalente de atividade de retinol.

CAPÍTULO I

REVISÃO DE LITERATURA

1. Carotenoides

A estrutura básica dos carotenoides consiste em um esqueleto isoprenóide composto por 40 carbonos (C_{40}). A presença de um longo cromóforo de ligações duplas conjugadas (cadeia poliênica) é responsável pela absorção de luz na região do visível pelos carotenoides. Essa característica química favorece a observação das cores amarela, laranja e vermelha em organismo que contém carotenoides.

Além dos carotenoides com 40 átomos de carbono (mais abundantes na natureza), podemos encontrar carotenoides com 30 (C_{30}), 45 (C_{45}) ou 50 (C_{50}) átomos de carbono, e ainda os apocarotenoides com 20 átomos de carbono (C_{20}) (RODRIGUEZ-CONCEPCION et al., 2018).

Os carotenoides podem ser classificados em cíclicos ou acíclicos dependendo da presença de um anel terminal na estrutura. Além disso são classificados em carotenos, formados exclusivamente por átomos de carbono e hidrogênio, ou xantofilas, quando contém também átomos de oxigênio.

Outra possibilidade de classificação dos carotenoides é conforme sua isomerização em *trans/cis* ou *E/Z.* Os isômeros *trans* ocorrem com maior frequência na natureza, devido a sua maior estabilidade. Porém isômeros *cis* também ocorrem de forma natural e são encontrados em plantas, animais, algas e bactérias, além de estarem presentes no sangue e tecidos humanos. O processo de isomerização pode ocorrer tanto por meio de catalise enzimática; quanto pela ação térmica e/ou fotoquímica, ou ainda na presença de ácidos (LIAAEN-JENSEN; LUTNAES, 2008; SCHIEBER; CARLE, 2005).

2. Biossíntese dos carotenoides

A figura 1 apresenta de forma esquemática a biossíntese dos carotenoides. A biossíntese dos carotenoides se inicia a partir do isopentenil difosfato (IPP) e ocorre nos plastídios. A união de 4 IPPs dá origem ao geranilgeranil pirofosfato (GGPP) com 20 carbonos. O GGPP é o precursor imediato dos carotenoides e a partir da condensação de duas moléculas do GGPP, pela ação da enzima fitoeno sintase, ocorre a formação do carotenoide incolor fitoeno. Reações de desidratação e isomerização enzimática levam a formação consecutiva do fitoflueno, ζ-caroteno e licopeno. O licopeno pode sofrer ciclização de um ou dos dois grupos-ψ terminais, essa é uma etapa crítica na síntese dos carotenoides, pois nesse ponto a via de

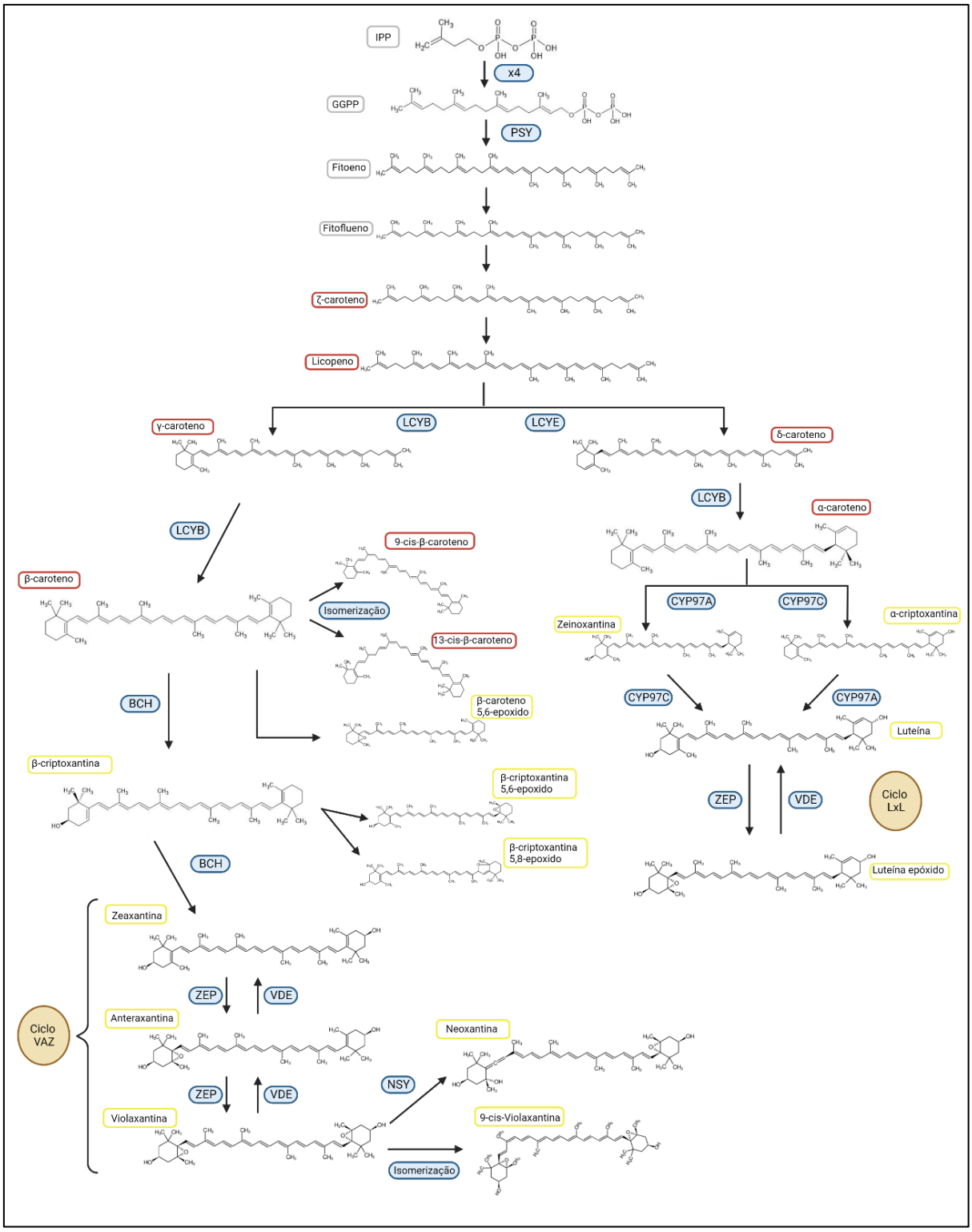

Figura 1 Esquema representativo da biossíntese dos carotenoides. Criada pelo autor em Biorender.com, baseado em (RODRIGUEZ-CONCEPCION et al., 2018; SCHWEIGGERT; CARLE, 2017; DRAPAL; FRASER, 2019)
IPP: isopentenil difosfato; GGPP: geranilgeranil pirofosfato; PSY: fitoeno sintase; LCYB: licopeno β-ciclase; LCYE: licopeno ε-ciclase; CYP97A: citocormo P450 caroteno β-hidroxilase; CYP97C: caroteno ε-hidroxilase; BCH: não-heme di-ferro caroteno β-hidroxilase; ZEP: zeaxantina epoxidase; VDE: violaxantina de-epoxidase; ciclo LxL: ciclo luteína epóxido; ciclo VAZ: ciclo violaxantina-anteroxantina-zeaxantina; NSY: neoxantina sintase.

síntese se ramifica. Caso essa ciclização ocorra nos dois grupos terminais pela ação da enzima licopeno β-ciclase, a síntese segue o caminho do β-caroteno, passando antes pelo γ-caroteno. Já se em um dos grupos terminais ocorrer ciclização pela ação da enzima licopeno ε-ciclase, o carotenoide resultante será o δ-caroteno, antecedendo a síntese do α-caroteno (RODRIGUEZ-CONCEPCION et al., 2018; SCHWEIGGERT; CARLE, 2017).

O β-caroteno por sua vez pode sofrer isomerização dando origem ao 9-*cis* ou 13-*cis*-β-caroteno; epoxidação formando β-caroteno 5,6-epóxido ou pode sofrer uma hidroxilação formando a β-criptoxantina. Esta, por sua vez, pode passar por uma epoxidação resultando nos carotenoides β-criptoxantina 5,6-epóxido ou β-criptoxantina 5,8-epóxido; ou pode ocorrer uma hidroxilação dando origem a zeaxantina (DRAPAL; FRASER, 2019; RODRIGUEZ-CONCEPCION et al., 2018).

A zeaxantina faz parte do ciclo das xantofilas, conhecido como ciclo VAZ (Violaxantina-Anteraxantina-Zeaxantina). Esse ciclo é uma via de mão dupla em que a zeaxantina pode sofrer epoxidações consecutivas pela enzima zeaxantina epoxidase (ZEP), resultando em anteraxantina e, em seguida, em violaxantina. O caminho inverso se dá com a enzima violaxantina de-epoxidase (VDE), levando a formação da zeaxantina (RODRIGUEZ-CONCEPCION et al., 2018).

Caso a violaxantina não entre no ciclo das xantofilas, ela pode sofrer a ação da enzima neoxantina sintase, formando a neoxantina ou sofrer isomerização em 9-*cis*-violaxantina (RODRIGUEZ-CONCEPCION et al., 2018).

Já pela segunda via formada a partir do licopeno, o α-caroteno pode sofrer ação de duas enzimas diferentes, a citocromo P450 caroteno β-hidroxilase (CYP97A) formando a zeinoxantina, ou a citocromo P450 caroteno ε-hidroxilase (CYP97C) formando a α-criptoxantina. Ambos os produtos da ação enzimática podem ser convertidos em luteína, a zeinoxantina pela ação da CYP97C e a α-criptoxantina pela CYP97A (RODRIGUEZ-CONCEPCION et al., 2018).

A luteína entra no ciclo das xantofilas, o ciclo luteína epóxido (ciclo LxL), onde a enzima zeaxantina epoxidase leva a formação da luteína epóxido, que por sua vez sofre ação da enzima violaxantina de-epoxidase voltando a luteína (RODRIGUEZ-CONCEPCION et al., 2018).

Nas plantas, os carotenoides são sintetizados em estruturas chamadas plastídios. Em vegetais com coloração verde os carotenoides são encontrados nos cloroplastos, onde fazem parte do complexo proteína-pigmento dos fotossistemas I e

II nos estromas- e grana-ticalóides. Porém, a quantidade de clorofila nos cloroplastos mascara a presença dos carotenoides. Conforme a planta amadurece ocorre uma diferenciação dos cloroplastos em cromoplastos, com degradação da membrana ticalóide e formação de estruturas cristaloides e/ou tubulares contendo os carotenoides. Há também degradação da clorofila tornando os carotenoides presentes na planta mais visíveis (RODRIGUEZ-CONCEPCION et al., 2018; VÁSQUEZ-CAICEDO et al., 2006).

Nas frutas e vegetais com coloração amarela, laranja ou vermelha os carotenoides são sintetizados e depositados nos cromoplastos. Os cromoplastos podem ser classificados em globulares, tubulares, cristaloides, membranosos ou uma junção de duas formas (Figura 2). Nos cromoplastos os carotenoides podem estar em diferentes formas, sendo: dissolvidos em lipídios; sólido-cristalina ou líquido-cristalina (SCHWEIGGERT; CARLE, 2017).

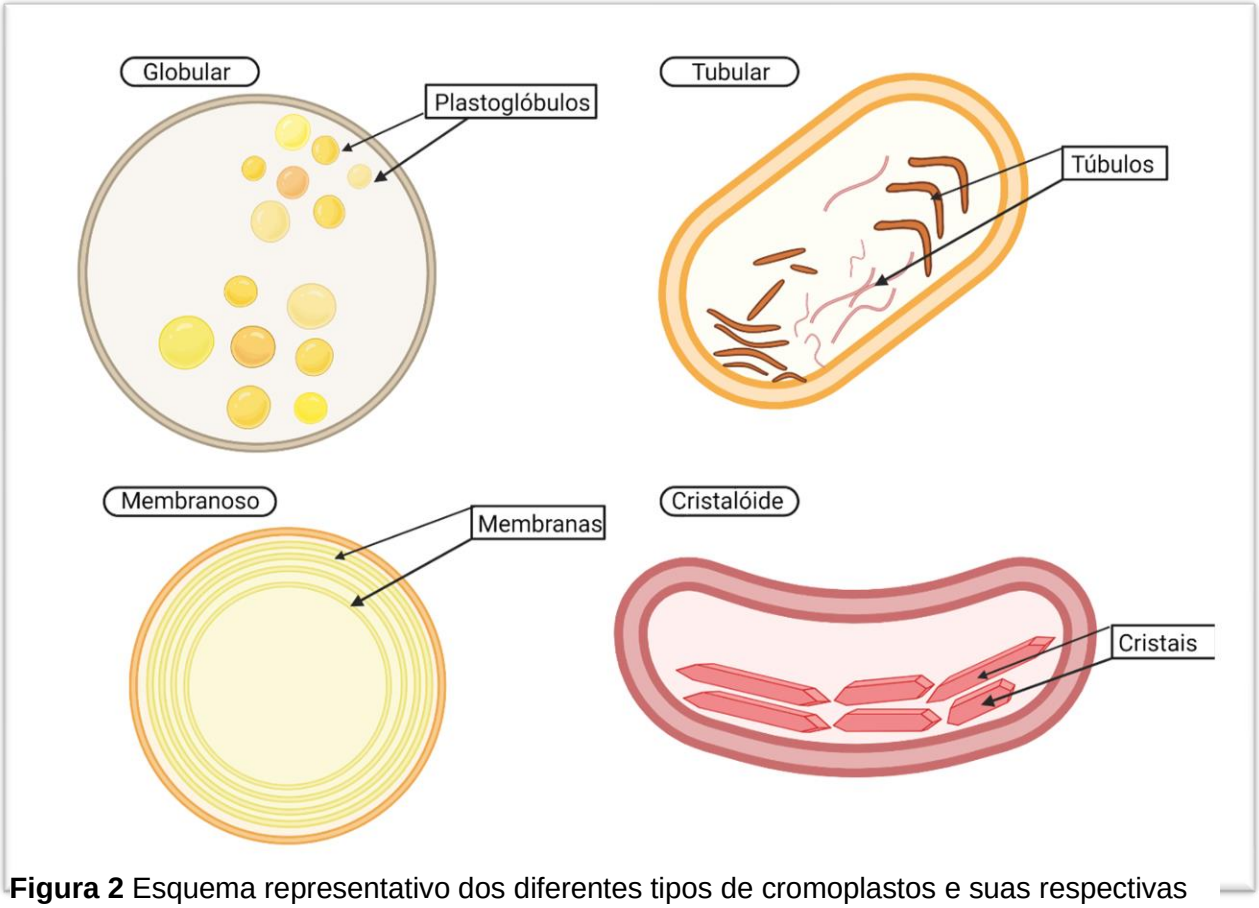

Figura 2 Esquema representativo dos diferentes tipos de cromoplastos e suas respectivas formas de armazenamento dos carotenoides, presentes nas estruturas vegetais. Criada pelo autor em Biorender.com, baseado em (SCHWEIGGERT; CARLE, 2017)

Os cromoplastos globulares contem, predominantemente, em seu interior, pequenos glóbulos de gordura chamados plastoglóbulos. Os plastoglóbulos possuem um interior formado por triglicerídeos e outros lipídios neutros e uma monocamada polar composta de glico e fosfolipídios e proteínas (BRÉHÉLIN; KESSLER, 2008) . Dessa forma, os carotenoides apolares como o β-caroteno são encontrados dissolvidos no interior dos plastoglóbulos enquanto os carotenoides mais polares ficam próximos a monocamada ou ligados a ela (BOREL et al., 1996). Cromoplastos exclusivamente globulares não são comuns em plantas comestíveis, sendo encontrados em pétalas de flores de diferentes espécies (SCHWEIGGERT; CARLE, 2017).

Cromoplastos tubulares possuem em seu interior tubos alongados, chamados túbulos. Os carotenoides estão presentes nesse tipo de cromoplasto na forma líquido-cristalina no interior dos túbulos (KNOTH; HANSMANN; SITTE, 1986). Porém, a maioria dos cromoplastos tubulares são encontrados na forma tubular-globular, em que os túbulos estão associados aos plastoglóbulos, formando uma estrutura intermediária (SCHWEIGGERT; CARLE, 2017). O β-caroteno presente na manga e no mamão, por exemplo, está depositado em cromoplastos tubulares-globulares e o mesmo foi observado na β-criptoxantina presente no mamão. Nesses cromoplastos os carotenoides estão na forma líquido-cristalina ou dissolvidos nos lipídios (SCHWEIGGERT et al., 2011).

Os cromoplastos cristalóides, contém cristais em seu interior. Esses cristais alteram a forma do cromoplastos, principalmente quando há uma grande deposição de carotenoides. Os carotenoides presentes neste tipo de cromoplasto encontram-se na forma sólido-cristalina (SCHWEIGGERT; CARLE, 2017). Esse tipo de estrutura pode ser identificada na cenoura (β-caroteno), no tomate (β-caroteno e licopeno) (VÁSQUEZ-CAICEDO et al., 2006) e no mamão (licopeno) (SCHWEIGGERT et al., 2011).

Cromoplastos membranosos são compostos por diversas membranas internas e são considerados raros na natureza, sendo encontrados em apenas algumas espécies de flores, pimentas e tomate de polpa amarela (SCHWEIGGERT; CARLE, 2017).

3. Alimentação de bebês de 6 a 12 meses de vida

O aleitamento materno deve ser a única fonte alimentar até os seis meses de vida, podendo ser continuado até os 24 meses, em concomitância com a alimentação complementar (WHO, 2002).

A alimentação complementar é definida como a introdução de alimentos sólidos ou líquidos, que não o leite materno ou fórmula para lactentes. O início da alimentação complementar é necessário para atender as necessidades de macro e micronutrientes que não são mais supridas exclusivamente pelo leite materno (SBP, 2012; WHO, 2002).

Um dos motivos de se iniciar a introdução alimentar na dieta dos bebês aos 6 meses, deve-se a maturação das funções renal e gastrointestinal, que passam a metabolizar de forma adequada os nutrientes provenientes da alimentação. Nessa idade os dentes também estão próximos da gengiva e auxiliam a triturar os alimentos (ESPGHAN, 2007; SBP, 2012).

A maturação gastrointestinal, por exemplo, envolve o aumento na produção de insulina a fim de dar conta do novo aporte de carboidratos provenientes da alimentação. Da mesma forma a maturação das glândulas adrenais aumenta a produção de hormônios adrenais responsáveis pelo metabolismo de alguns nutrientes, como por exemplo a vitamina D (ESPGHAN, 2007).

Durante os primeiros anos de vida, o desenvolvimento do sistema neurológico e fisiológico permite que a criança ingira texturas cada vez mais complexas (BOULANGER; VERNET, 2017). Segundo a Sociedade Brasileira de Pediatria (SBP, 2012), a partir dos 6 meses a criança deve consumir alimentos na forma de papas, passando a pedaços pequenos entre os 9 e 11 meses, e atingindo a mesma consistência do restante da família aos 12 meses.

Os alimentos consumidos neste período geralmente compreendem as sopinhas, papinhas e purês (com aumento gradual na textura até atingir a alimentação da família), podendo ser tanto industrializados quanto feitos em casa (OKESLI et al., 2011). O alimento complementar é qualquer alimento que venha a ser introduzido na alimentação de lactentes e crianças na primeira infância. Esses podem ser utilizados de forma direta ou no preparado de alimentos, que complemente o aleitamento (materno ou com uso de fórmulas) e que exercem a função de adaptação progressiva aos alimentos comuns (consumidos pela família), e que respeitem a maturidade fisiológica e o desenvolvimento neuropsicomotor do bebê (ANVISA, 2002).

Alimentos complementares prontos para consumo destinados a bebês são amplamente utilizados devido a disponibilidade comercial e facilidade para transporte e consumo. Porém, há poucos estudos sobre a composição e as consequências do consumo destes alimentos pelos bebês (CZAJKOWSKA-MYSŁEK; LESZCZYŃSKA, 2017). O estudo FITS (*Feeding Infants and Toddlers Study)* de 2002 identificou que 87% a 95% dos americanos entrevistados alimentavam os bebês entre 6 e 12 meses com papinhas industrializadas (BRIEFEL et al., 2004). Outro estudo também realizado nos Estados Unidos, encontrou valores semelhantes com 81% dos bebês alimentados com papinhas industrializadas (HURLEY; BLACK, 2010). Em países da Europa, como a Alemanha, o consumo de papinhas industrializadas fica entre 55% e 60% (DAY et al., 2010). No Brasil, não há dados sobre o consumo de papinhas industrializadas.

4. Digestão em bebês e modelos de digestão *in vitro*

Estudos envolvendo recém-nascidos alimentados com leite materno ou fórmula infantil, demonstram as diferenças entre a digestão destes e de adultos. Além do tamanho menor dos órgãos, a digestão dos lipídios depende de enzimas distintas da lipase pancreática, com ênfase para a lipase gástrica (LG), lipase dependente de sal biliar (LDSB) e lipase pancreática relacionada a proteína 2 (LPRP2). As enzimas digestivas pancreáticas encontradas nos lactentes são: α-amilase, tripsina, quimiotripsina e elastase, além das lipolíticas (ABRAHAMSE et al., 2012).

Ao se iniciar a introdução de alimentos sólidos, a secreção de sais biliares aumenta devido a maturação da função hepática e as enzimas pancreáticas lipolíticas (LDSB e LPRP2) se tornam menos importantes para a digestão de triglicerídeos (ABRAHAMSE et al., 2012).

Em adultos, o pH do estômago em jejum fica entre 1 e 3,5. Ao nascimento, os recém-nascidos apresentam um pH estomacal de 6 a 8, sendo que em algumas horas, o pH reduz para 1,5 a 3. Entre o primeiro e décimo dia de vida, o pH volta a se elevar ficando na faixa de 6 a 8, reduzindo novamente após esse período e ficando em torno de 1,4 até os 2 anos de idade. Já o pH intestinal é o mesmo em crianças e adultos. O esvaziamento gástrico também é diferente em bebês até os 6 meses de idade, após esse período passa a ser semelhante ao de adultos, entre 12 e 50 minutos dependendo do alimento ingerido, também deve-se levar em consideração o reduzido volume gástrico, de 90 a 500ml em crianças de 6 a 24 meses (KAYE, 2011).

Em geral, estudos com alimentos para bebês a partir de seis meses utilizam as mesmas metodologias de digestão *in vitro* empregadas em estudos que simulam a digestão em adultos (DA SILVA; DE FARIAS; CADORE, 2018; JIWAN et al., 2010).

5. Digestão dos carotenoides

Os carotenoides, antes de sua absorção intestinal, devem ser liberados da matriz do alimento e incorporados a micelas, que contém também lipídios provenientes da dieta, sais biliares e outros compostos lipofílicos (BARBA et al., 2017; CILLA et al., 2012; ERDMAN; BIERER; GUGGER, 1993).

Estes compostos têm sua digestão iniciada no estômago com ação da lipase gástrica, porém, a maior parte da digestão dos compostos lipofílicos ocorre no intestino delgado. Na fase intestinal ocorre a emulsificação dos lipídios pelos sais biliares, os glóbulos de gorduras resultantes são fragmentados pela movimentação intestinal para que as lipases possam agir sobre a sua superfície. Os sais biliares agem formando as micelas, que carregam triglicerídeos e demais compostos lipofílicos, como os carotenoides, para a superfície do enterócito onde ocorre a absorção (GUYTON; HALL, 2006; LEMMENS et al., 2014).

A bioacessibilidade é definida como a fração do nutriente ou não nutriente, que após ser liberado de sua matriz no sistema digestório, está disponível para absorção efetiva pelo intestino. Desta forma, para se determinar a bioacessibilidade deve-se considerar todos os processos que ocorrem para transformar o alimento em uma substância que possa ser absorvida (FERNÁNDEZ-GARCÍA; CARVAJAL-LÉRIDA; PÉREZ-GÁLVEZ, 2009). A fração bioacessível, que contém os compostos que poderão ser absorvidos pelas células da borda em escova do intestino, pode ser determinada por técnicas *in vitro* que simulam a digestão gastrointestinal (VAGHINI et al., 2016).

O protocolo Infogest 2.0 é um método estático de digestão *in vitro* que utiliza proporções de alimentos, fluidos e pH constantes em cada etapa. Esse método é uma atualização do método Infogest desenvolvido para que houvesse um padrão nas análises de bioacessibilidade, possibilitando comparação entre os resultados de diferentes estudos. O método utiliza equipamentos comuns de laboratório, possibilitando o amplo uso em diversos estudos pelo mundo (BRODKORB et al., 2019).

No método Infogest 2.0, o alimento é submetido a digestão sucessiva em fases, sendo essas salivar, gástrica e intestinal. As condições (quantidade de enzima e eletrólitos, pH e temperatura) permanecem constantes em cada fase (método estático). (BRODKORB et al., 2019).

Os estudos de bioacessibilidade *in vitro* são bons modelos preditivos da absorção dos nutrientes, porém, não nos dão a informação completa visto que não consideram as variáveis relativas ao hospedeiro, neste caso o ser humano, como hormônios, regulação da liberação de sais biliares e enzimas conforme a matriz alimentar, uso de medicamentos, estresse, entre outros que podem afetar a digestão (BIEHLER et al., 2011; RODRIGUEZ-CONCEPCION et al., 2018).

A bioacessibilidade dos carotenoides pode ser afetada por diversos fatores, entre eles a matriz alimentar (presença de fibras, proteínas, lipídios, minerais); tipo dos carotenoides encontrados no alimento e a interação entre os mesmos; tipo de cromoplasto e forma de armazenamento dos carotenoides nos mesmos; quantidade de alimento ingerido (RODRIGUEZ-CONCEPCION et al., 2018; SCHWEIGGERT et al., 2012).

6. Fatores que afetam a bioacessibilidade dos carotenoides

3. 6.1. Matriz alimentícia

Os diferentes tipos de cromoplastos e o estado dos carotenoides armazenados nestes influenciam a bioacessibilidade dos carotenoides (KOPEC; FAILLA, 2018).

Carotenoides em cromoplastos cristaloides estão no estado sólido-cristalino, como o β-caroteno na cenoura e o licopeno no mamão. Nessa forma, os carotenoides apresentam menor bioacessibilidade visto que há maior dificuldade na quebra dos cristais durante a digestão e menor liberação dos carotenoides. Já cromoplastos com a forma tubular-globular e os carotenoides no estado líquido-cristalino ou dissolvidos em lipídios (por exemplo, β-caroteno na manga e no mamão), apresentam maior bioacessibilidade, pois ocorre maior liberação dos carotenoides do cromoplasto e o estado destes carotenoides (solubilizados nos lipídios) é mais

susceptível à digestão (SCHWEIGGERT et al., 2012; VÁSQUEZ-CAICEDO et al., 2006).

O tamanho da partícula do alimento também possui papel fundamental na bioacessibilidade dos carotenoides, visto que quanto menor a partícula maior a bioacessibilidade. A redução do tamanho da partícula pode acontecer tanto por trituração do alimento quanto pela mastigação. O aumento na bioacessibilidade pode ser explicado pela maior superfície de contato com as enzimas ou ainda pelo menor diâmetro das gotículas de gordura que facilitam a transferência dos carotenoides para a micela mista (BOHN et al., 2019).

Para serem absorvidos, os carotenoides necessitam de uma certa quantidade de lipídios na refeição que auxiliam sua solubilização e emulsificação (CORTE-REAL et al., 2016). Por este motivo, os lipídios são amplamente estudados em relação a sua contribuição para a bioacessibilidade e absorção dos carotenoides. Diversos fatores podem estar relacionados ao aumento da bioacessibilidade dos carotenoides causado pelos lipídios, dentre eles: solubilização dos carotenoides liberados da matriz alimentar nos lipídios; aumento da secreção de enzimas digestivas e sais biliares; redução do trânsito intestinal aumentando o tempo para liberação, e promoção da formação de micelas levando ao aumento na micelarização dos compostos lipofílicos (KOPEC; FAILLA, 2018; PRIYADARSHANI, 2017).

O consumo de saladas juntamente com molhos a base de óleo de canola está associado ao aumento nos níveis séricos de carotenoides (BROWN et al., 2004). O mesmo foi observado na adição de abacate, uma fruta rica em lipídios, a saladas e molhos (UNLU et al., 2005).

Em um estudo *in vivo*, foi observado que a quantidade de lipídio na refeição pode ser mais importante no aumento da absorção dos carotenoides do que o tipo de ácidos graxos presente (GOLTZ et al., 2012). Porém, os estudos *in vitro* mostraram que a micelarização do β-caroteno e do licopeno é maior na presença de óleos com ácidos graxos insaturados, como por exemplo, os óleos vegetais, do que em gorduras saturadas como a manteiga (FAILLA et al., 2014). Considerando apenas os ácidos graxos insaturados, a biodisponibilidade dos carotenoides é maior na presença de ácidos graxos poli-insaturados (PUFA) do que na presença dos monoinsaturados (MUFA) (YAO; TAN; KIM, 2022).

Uma recente meta-análise (YAO; TAN; KIM, 2022), verificou os efeitos dos lipídios na bioacessibilidade e biodisponibilidade dos carotenoides, por meio da

análise de 27 estudos *in vitro* e 12 estudos controlados randomizados. Os resultados demonstraram uma associação dose-dependente entre a concentração de lipídios e a bioacessibilidade dos carotenoides, sendo que os lipídios devem estar em um determinado intervalo de concentração: 0% a 10 % para luteína, zeaxantina e α-caroteno; 0% a 20% para β-caroteno; 0% a 6% para β-criptoxantina e 0% a 8% para carotenoides totais. Acima destas concentrações, os lipídios não possuem mais efeito sobre a bioacessibilidade dos carotenoides. Para o licopeno não foi encontrada associação entre a concentração de lipídios e a sua bioacessibilidade.

Outro fator importante que influencia a bioacessibilidade dos carotenoides são as fibras. Fibras solúveis como pectina e goma guar podem reduzir a bioacessibilidade do β-caroteno, já o licopeno e a luteína podem ter a bioacessibilidade negativamente afetada por todos os tipos de fibra. A ação das fibras pode ocorrer possivelmente por reduzir a micelarização, inibir a atividade da lipase e aumentar a viscosidade do meio (RIEDL et al., 1999).

Amyooni *et al* (2017) demonstraram em um estudo *in vitro* uma correlação negativa entre a bioacessibilidade do β-caroteno e a quantidade de goma guar, principalmente pelo potencial desta em alterar a emulsificação dos lipídios, reduzindo a micelarização e posterior absorção.

A pectina afeta a viscosidade do meio gástrico conforme sua concentração, ou seja, quanto maior a concentração de pectina, maior a viscosidade do meio. A maior viscosidade pode afetar a micelarização dos carotenoides por dificultar a difusão dos carotenoides das gotículas de lipídio para as micelas, assim como a difusão das enzimas lipolíticas do meio para o sítio de atuação nas gotículas lipídicas, dificultando a lipólise (CERVANTES-PAZ et al., 2017).

As fibras também podem afetar o tamanho das gotículas de lipídio, porém, esse efeito só foi observado em altas concentrações de pectina que aumentam o tamanho da partícula, sendo que em baixas concentrações não foi observado nenhum efeito. A alteração no tamanho das gotículas pode ser explicada pela redução na floculação causada pela presença de pectina na fase aquosa, isso pode levar a dissociação dos grupos carboxila da pectina e consequente exclusão da pectina presente na superfície da gotícula de gordura, aumentando a pressão osmótica e favorecendo a agregação das gotículas, formando partículas maiores (CERVANTES-PAZ et al., 2017). Partículas maiores apresentam maior dificuldade para ação das lipases devido a menor superfície de contado com os lipídios contidos no interior das

gotículas, dessa forma a digestão destes compostos é prejudicada, o que reduz a bioacessibilidade dos compostos lipofílicos.

Apesar dos achados acima, pequenas concentrações de pectina e celulose estão relacionadas a manutenção ou aumento da micelarização dos carotenoides. Já altas concentrações de pectina foram associadas a redução na micelarização, enquanto altas concentrações de celulose tiveram o efeito contrário, aumentando ainda mais a micelarização (CERVANTES-PAZ et al., 2017; LIU et al., 2020). A micelarização do β-caroteno, por exemplo, apresenta redução de 35 a 43% conforme o aumento da concentração de pectina, principalmente na fruta madura (ORNELAS-PAZ et al., 2008). Na fruta madura, a despolimerização da pectina é responsável pelo aumento da maciez da polpa durante o amadurecimento, esse fator reduz o peso molecular da pectina de 1300 para 21 kDa. Como o efeito de aumento da viscosidade dos fluidos durante a digestão está relacionado ao peso molecular da pectina, a redução deste peso durante o amadurecimento, além da maciez da polpa madura, pode explicar o aumento da micelarização dos carotenoides nas frutas totalmente maduras (ORNELAS-PAZ et al., 2008).

6.1.1. Processamento

Frutas e verduras *in natura* apresentam bioacessibilidade menor do que as que passam por algum tratamento térmico. Este é responsável por romper as membranas e paredes celulares, tornando a polpa mais macia e mais susceptível a ação das enzimas durante a digestão (PRIYADARSHANI, 2017). Além disso, o tratamento térmico pode levar a alterações químicas nos carotenoides, como a isomerização e perda de carotenoides presentes no alimento cru (KOPEC; FAILLA, 2018).

O efeito do processamento térmico depende tanto do tipo de processamento, quando do carotenoide analisado, sua localização na matriz alimentar e possível ligação a outros compostos como as proteínas (CILLA et al., 2018).

Ryan et al (2008), analisaram tomate, pimenta vermelha e abobrinha, submetidos aos tratamentos térmicos (cocção em água; grelhado; micro-ondas e no vapor). Os autores observaram redução nos níveis de β-caroteno, porém, foi observada maior micelarização durante a digestão. A cocção em água foi o método que apresentou maior micelarização do β-caroteno em todas as amostras analisadas,

sendo de 79,4%, 76,7% e 47,2% (abobrinha, pimenta vermelha e tomate, respectivamente). Já o licopeno variou conforme a amostra analisada, no tomate e na pimenta vermelha apresentou redução na micelarização quando cozido no vapor (1,7% e 8,2%, respectivamente) e a cocção em água não apresentou variação significativa quando comparada com a amostra crua. Por outro lado, na abobrinha, o licopeno apresentou maior micelarização quando aquecido em micro-ondas ou grelhado (49,9% e 50,4%, respectivamente), já quando cozido em água não houve micelarização.

Outro estudo conduzido por Courraud et al (2013) apresentou resultados similares ao analisar os efeitos da cocção em espinafre, com redução na quantidade de carotenoides após a cocção, mas aumento na micelarização em 15 vezes para o β-caroteno e 72 vezes para a luteína.

Erikssen et al (2016), estudaram a influência de diferentes métodos de cocção caseira (cozido no vapor e frito) na bioacessibilidade de luteína e β-caroteno de espinafre em diferentes formas (folha inteira, picado e na forma de purê). Verificaram que após a fritura houve redução na bioacessibilidade da luteína em todas as formas de apresentação do espinafre, sendo que no purê frito a bioacessibilidade foi de “zero”, enquanto no purê sem tratamento térmico foi de 30%.

Esses estudos demonstram que o processamento térmico reduz a quantidade de carotenoides no alimento, porém, aumenta a porcentagem de micelarização dos mesmos. Esse aumento ocorre provavelmente devido à quebra na parede celular e dissociação dos carotenoides da matriz. Além disso, mostram o efeito que os diferentes métodos de cocção podem apresentar sobre os diferentes carotenoides, sendo responsáveis tanto por aumento na micelarização quando redução.

6.2. Estrutura do carotenoide

O tipo de carotenoide presente na amostra também pode influenciar uma maior ou menor bioacessibilidade. Os isômeros *cis* do β-caroteno, por exemplo, apresentam micelarização maior (2 a 3 vezes) do que o all-*trans*-β-caroteno. Apesar de percentualmente o all-*trans*-β-caroteno apresentar micelarização menor, ele está presente em maior quantidade do que os isômeros *cis* (FERRUZZI et al., 2006).

Isso ocorre, provavelmente, devido ao fato de os isômeros *cis* possuírem maior solubilidade em solventes orgânicos, o que pode facilitar a incorporação dos isômeros *cis* nas micelas (FERRUZZI et al., 2006; LIAAEN-JENSEN; LUTNAES, 2008).

A micelarização do β-caroteno também pode ser afetada pela presença de luteína juntamente com zeaxantina, sendo que quanto maior a concentração dessas xantofilas menor a micelarização do β-caroteno. A polaridade maior das xantofilas está relacionada a maior incorporação destas nas micelas, isso se deve ao fato de elas serem incorporadas nas partes mais externas da gotícula ao invés do centro (DUBE et al., 2018; PRIYADARSHANI, 2017). Em milho, onde a luteína e a zeaxantina estão presentes em maior quantidade do que o β-caroteno, a concentração das xantofilas também foi associada a maior micelarização das mesmas em detrimento do β-caroteno (DUBE et al., 2018).

O licopeno também apresenta maior absorção (estudos *in vitro*) entre os isômeros *cis* do que na forma all-*trans*-licopeno, provavelmente devido a maior solubilidade dos isômeros *cis*. Os isômeros *trans* do licopeno são pouco solúveis e tendem a formar cristais quando dissolvidos em lipídios, o que dificulta a incorporação destes as micelas (FERRUZZI et al., 2006; PRIYADARSHANI, 2017).

7. Composição e bioacessibilidade de carotenoides em papinhas

Papinhas industrializadas são fonte de carotenoides provitamina A (α-caroteno, β-caroteno e β-criptoxantina) e de carotenoides não provitamina A, mas que possuem importantes funções no organismo humano como luteína, licopeno e zeaxantina.

Apesar da importância dos carotenoides para a saúde dos bebês, existem poucos estudos que avaliaram a composição de carotenoides e a bioacessibilidade destes em alimentos para bebês (papinhas/purês).

Majchrzak et al (2000) analisaram o perfil de carotenoides de 65 papinhas divididas em 2 grupos: a base de vegetais e a base de frutas e cereais. Identificaram β-caroteno em todas as amostras analisadas com variação de 0,03 mg/100 g, na amostra 4 cereais e frutas, a 9,2 mg/100 g, na amostra de cenoura. O α-caroteno foi identificado em 97% das amostras, estando presente em maior quantidade nas papinhas a base de cenoura (3,4 mg/100 g na amostra de cenoura). A β-criptoxantina

foi identificada em 74% das amostras, sendo que as amostras que continham maior quantidade foram as que apresentavam mandarina (0,056 mg/100 g) ou pêssego (0,057 mg/100 g) como principal ingrediente. A luteína também foi identificada em 100% das amostras, porém, as amostras que continham espinafre como ingrediente principal foram as que apresentaram maior teor deste carotenoide (1,15 mg/100 g). A zeaxantina apresentou os maiores teores nas amostras de milho doce (0,113 mg/100 g) e foi detectada em 89% do total de amostras. Por fim, o licopeno foi detectado em apenas 18% das amostras e somente nas amostras que continham tomate ou polpa de tomate (variação de 0,17 a 2,52 mg/100 g).

Jiwan, et al (2010) analisaram 9 amostras de papinhas industrializadas sendo 4 orgânicas e 5 convencionais, em 2 sabores diferentes: frango e vegetais e a base de frutas. Os teores de β-caroteno variaram de 6,61 µg/100 g a 535 µg/100 g nas amostras de frutas convencionais e frango e vegetais orgânica, respectivamente. Para a β-criptoxantina houve variação de 0,35 µg/100 g a 1,79 µg/100 g para as amostras de frango e vegetais orgânico e frutas convencional, respectivamente. Para luteína, a variação foi de 3,94 µg/100 g (frutas orgânicas) a 221 µg/100 g (sabor frango e vegetais convencional). A zeaxantina seguiu o mesmo padrão da luteína com 0,32 µg/100 g na amostra de frutas orgânicas e 23,2 µg/100 g na amostra de frango e vegetais convencional. Por fim, o licopeno variou de 1,07 µg/100 g a 719 µg/100 g na amostra de frutas convencional e na de frango e vegetais orgânica, respectivamente.

Para a bioacessibilidade, os autores encontraram que os carotenoides provitamina A apresentaram porcentagem bioacessível maior nas papinhas de frango e vegetais do que nas de frutas. O β-caroteno, especificamente, apresentou maior micelarização nas amostras orgânicas, já a luteína, zeaxantina e licopeno, nas amostras convencionais (JIWAN et al., 2010).

Quando avaliado o processamento das papinhas, tanto industrial quanto caseiro, percebe-se que as papinhas caseiras apresentam bioacessibilidade menor (0,5%) do que as esterilizadas (6,34%) e as industrializadas (5,32 %). Isso ocorre tanto pelo tamanho da partícula resultante, que pode impedir a liberação dos carotenoides da matriz no caso da papinha caseira, quanto pelo tratamento térmico ao qual as papinhas foram submetidas. As papinhas industrializadas passam por processo de branqueamento seguido de esterilização em autoclave nas próprias embalagens em que serão comercializadas, reduzindo o contato com o ar e possível oxidação (DHUIQUE-MAYER et al., 2018). Esse estudo também demonstrou maior

bioacessibilidade do 13-*cis*-β-caroteno do que do all-*trans*-β-caroteno, sendo mais de 10 vezes maior, tanto para amostras caseiras (6,44%) quanto industrializadas (59,7%).

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CAPÍTULO II

CAROTENOIDS

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Chapter 5
Carotenoids

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5.1 Introduction

Since 1870 life expectancy has been increasing exponentially (Roser et al., 2013), supposedly because of all the improvements in healthcare, medical diagnosis, and treatment. However, as a side effect, aging is associated with increases in noncommunicable diseases rate that currently represents more than 73% of the global death (Ritchie & Roser, 2018). In this context, the nutrition and lifestyle are key factors to improve health and enhance the life quality, especially in elderly.

Carotenoids are natural pigments present mainly in fruits and vegetables and responsible for colors from yellow to red that also have a role as nonnutrients. Apart from the well-known pro-vitamin A activity, their intake has been positively associated with low rates of many noncommunicable diseases.

The molecular structure of carotenoids is constituted of eight isoprenoid units, configuring a linear chain possessing several conjugated double bonds and an inverse symmetry in the center of the molecule. This basic structure of 40 carbon atoms can be modified by cyclization of terminal groups, chain extension or shortening, isomerization, hydrogenation or dehydrogenation, and the introduction of substituents, generating more than 800 free carotenoids known in nature so far.

According to the atoms in their structure, carotenoids can be divided into carotenes, which are hydrocarbon molecules formed only by carbon and hydrogen atoms, and xanthophylls, when oxygenated functional groups such as ketone, carbonyl, epoxy, and hydroxyl groups are present in the molecule (Fig. 5.1). Xanthophylls with hydroxyl groups in the molecule can also be found as free carotenoids or esterified with fatty acids, in this case, forming the carotenoid esters (Fig. 5.1) (Mariutti & Mercadante, 2018).

Carotenoids are not synthesized by humans and can be consumed through the diet as food or supplements (Chaco´nOrdo´n˜ez et al., 2017; Hempel et al., 2017; Pe´rez-Ga´lvez et al., 2003). Since they are lipophilic molecules, it is important to

understand their fate during digestion, absorption, and delivery to the target tissues, metabolization, and accumulation, so they can exert their bioactive role.

Digestion and absorption of carotenoids are quite similar to those of other dietary lipids (Fig. 5.2). The carotenoids must be firstly released from the food matrix, solubilized in lipid droplets, and incorporated into the bile salt micelles. The carotenoids in the micelles are considered bioaccessible, that is, they are available for uptake by the small intestinal epithelial cells through their brush border membrane. After the carotenoids enter the enterocytes, they have to be incorporated into the chylomicrons to be secreted into the lymph so they can be delivered to the target tissues (Failla et al.,2019).

This process is quite well-known for free carotenoids, and so far, only free carotenoids were detected in human plasma. More studies are required for clarifying the carotenoid esters' fate, and the pieces of evidence suggest that they undergo complete hydrolysis to free carotenoids and free fatty acids, mediated by carboxyl ester lipase, before being incorporated into the mixed micelles. Hydrolysis of carotenoid esters probably occurs during digestion in the lumen or the enterocytes brush borders in the gut before absorption (Failla et al., 2019).

This chapter will present scientific evidence about the beneficial effects of carotenoid intake as well as their mechanisms of action based on experimental protocols using in vitro assays, animal models, and clinical trials (Fig. 5.3).

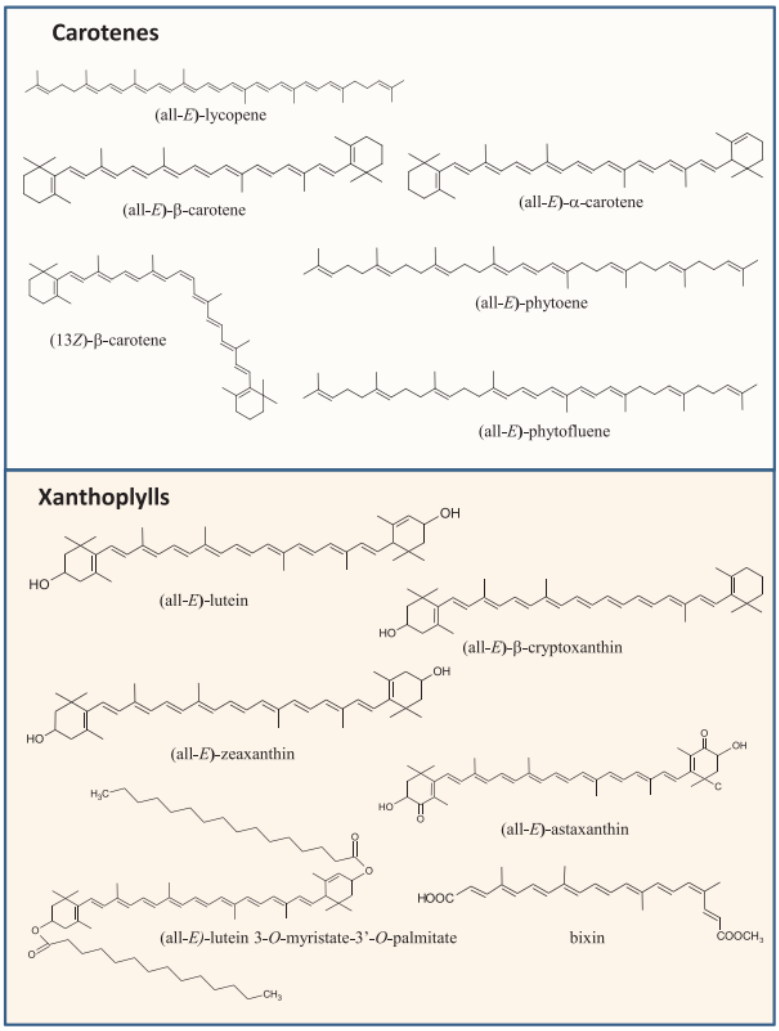

FIGURE 5.1 Molecular structures of carotenoids. *Own authorship created by ACD/ChemSketch Freeware.*

5.2 Animal studies

The uses of experimental models are indispensable in understanding how these compounds act and the mechanisms involved in regulating the biological pathways. These data are essential to guide new treatments or even to elucidate the protective effects against chronic noncommunicable diseases observed in populations with high consumption of fruits and vegetables.

In this way, there are evidence in the literature about the carotenoids' anticarcinogenic effects. Different cancer models have been used to evaluate the carotenoid effect and protective mechanism. The main mechanisms involved with this protection are related to carotenoids' ability to inhibit the cancer cell cycle progression, inducing apoptosis, decrease or control the metastasis, and decrease the angiogenesis, a fundamental mechanism to promote tumor growth, invasion, and metastasis (Niranjana et al., 2015). Pradeep and Kuttan (2003) observed the antimetastatic activity of β-carotene in lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice. In this study, the animals received 106 cells via lateral tail vein and 200 μmol/kg of β-carotene for 10 days. The treatment promoted a twofold increase in animals' survival compared to the control group, probably

because of the decrease in 71.36% of tumor formation (metastatic colonies) and consequently decreased lung fibrosis, which is a condition that impacts pulmonary function.

FIGURE 5.2 Digestion, absorption, delivery, and metabolization of carotenoids. Own authorship created by smart.servier.com

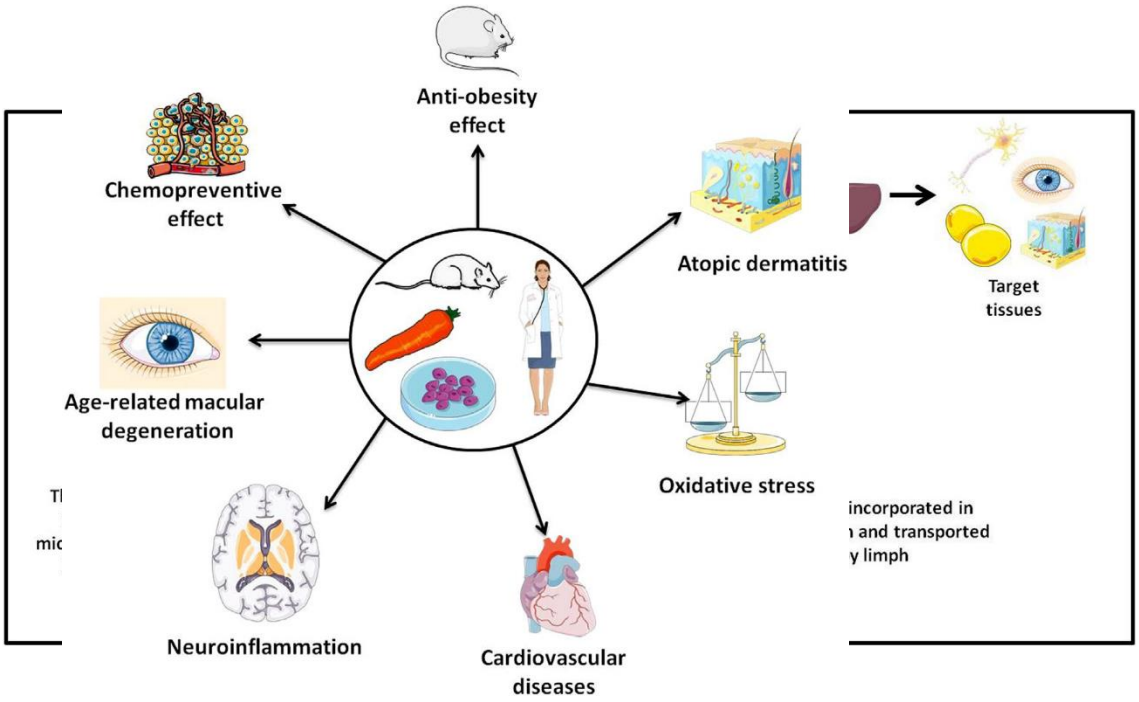

FIGURE 5.3 Protective effects associated with carotenoid intake. Own authorship created by smart.servier.com

Lycopene is another carotenoid that showed a chemopreventive effect against esophageal cancer induced by N-nitrosomethylbenzylamine in F344 rats. Cui et al. (2020) showed that 25 and 50 mg/kg (b.w.) intake during 25 days reduced the tumor incidence by 13.3%, increased antioxidant enzymes' level (glutathione peroxidase and superoxide dismutase), and decreased the malondialdehyde level, a marker of lipid peroxidation. The authors also observed a decrease in protein expression of NF-κB and COX-2, indicating an antiinflammatory action and an increase in PPAR protein expression, a marker associated with inhibiting cell proliferation and induced apoptosis (Cui et al., 2020). The chemopreventive effect of carotenoids was also tested in an experimental model of osteosarcoma. This malignant bone tumor metastasizes rapidly, and it shows the lung as the more frequent site of metastasis. The current treatment for osteosarcoma includes chemotherapy combined with surgery; however, respiratory failure is usually the death cause of this kind of cancer. In this way, looking for alternatives to control the tumor's development and metastasis is necessary to improve the life expectancy of the individuals affected by this disease.

Rokkaku et al. (2013) observed a decrease in the primary lesion and lung metastasis volume in C3H mice injected subcutaneously into the back murine osteosarcoma cell line (5 3 106 cells) 35 days of fucoxanthin (200 mg/kg b.w.) treatment. The authors also demonstrated an increase in survival rate in the fucoxanthin group compared to the control group.

Carotenoids are also related to eye health, mainly protecting the macula against age-related macular degeneration (AMD), which is a major cause of irreversible blindness. Oxidative stress and cellular accumulation of lipofuscin are the leading causes of macular degeneration, and carotenoids have been associated with a protective effect by quenching reactive oxygen species (ROS) or by the increased level of lipofuscin, a metabolic product. Bhosale et al. (2009) studied the effect of lutein and zeaxanthin supplementation (0.5 mL of a microbial extract rich in these two carotenoids, representing 0.2 mg of carotenoids/day) in Japanese quail (Coturnix japonica). The animals fed for 16 weeks with a low carotenoids' content diet and supplemented with the extract rich in lutein and zeaxanthin showed a decrease A2E level in the retinal pigment epithelium (RPE), a major fluorophore of ocular lipofuscin, and an increment in carotenoids content in the retina compared to the control group. Since A2E acts as a generator of ROS, the authors concluded that the intake of lutein and zeaxanthin could protect the eyes by decreasing the oxidative stress and the formation of this metabolite.

The carotenoids' antioxidant capacity and their protective effect against oxidative stress could be one of the key biological effects associated with their intake to combat many noncommunicable diseases development. Obesity is one noncommunicable disease considered a pandemic that contributes to the increase in the rates of diabetes, dyslipidemia, cardiovascular diseases, cancer, among other noncommunicable diseases worldwide. Mice fed a high-fat (HF) diet supplemented with 0.05% or 0.1% lycopene showed improvement in intraperitoneal glucose tolerance testing (IPGTT) and insulin tolerance testing (IPITT) in a dose-dependent manner compared to the normocaloric control group (Zeng et al. 2017). Insulin resistance is a metabolic consequence of increased fat deposits and, consequently, increased circulating levels of pro-inflammatory cytokines that impair the signaling of muscle and liver insulin, characterizing the low-grade inflammation seen in obesity. Serum levels and the RNA expression of pro-inflammatory cytokines [interleukin (IL)-1 and TNF-α] in the liver decreased in the two groups supplemented with lycopene compared to the

HF group evidencing a possible antiinflammatory effect associated with their intake (Zeng et al., 2017). Additionally, the hepatic glycogen content in animals supplemented with lycopene increased, corroborating the insulin sensitivity observed in IPGTT and IPITT since insulin signaling activates glycogen synthesis (de Luca & Olefsky, 2008; Zeng et al., 2017). The authors also observed that the consumption of an HF diet for 12 weeks increased serum triglyceride, total cholesterol, LDLcholesterol (low density lipoprotein-cholesterol), and decreased HDL-cholesterol (high density lipoprotein-cholesterol) compared to the normocaloric diet (Zeng et al., 2017). Increases in triglycerides and total cholesterol were also observed in the liver of the animals. However, the diet supplemented with lycopene improved the serum and liver lipid profiles, probably through modulating the sterol regulatory element-binding protein (SREBP-1c) pathway. The RNA expression of SREBP-1c, an isoform expressed in the liver responsible for cholesterol and unsaturated fatty acids synthesis, decreased in the animals supplemented with lycopene, as well as acetyl coenzyme-A carboxylase 1, and fatty acids synthase compared to the HF control group (DeBose-Boyd & Ye, 2018; Zeng et al., 2017). These data suggest that lycopene can act as modulating glucose and lipid metabolism.

Kilany et al. (2020) also observed the protective effects of lycopene in HF diet-induced obesity. Sprague Dawley rats were fed with a normocaloric or hypercaloric diet for 8 weeks and orally supplemented with lycopene (20 mg/kg). The carotenoid supplementation in a normocaloric group promoted only changes in the lipid profile, decreasing the serum levels of total cholesterol and LDL-cholesterol compared to the control group. However, the lycopene supplementation in animals fed a HF diet promoted lower body weight gain, a better lipid profile, improved antioxidant status, and decreases the level of pro-inflammatory cytokines, leptin, and resistin. Dyslipidemia is commonly observed in obese individuals and is a risk factor for cardiovascular disease development. However, lycopene consumption (20 mg/ kg) seemed to modulate the lipid metabolism by decreased triglyceride levels, total cholesterol, LDL-cholesterol, and nonesterified fatty acids in the HF group. The animals also showed improvement in serum endogenous antioxidant enzymes and total antioxidant capacity, which may have protected them from the lipid peroxidation translate by lower levels of malondialdehyde in the serum. Moreover, lower levels of pro-inflammatory cytokines, leptin, and resistin could be associated with lower body weight gain, decreased fat depots, and regulating lipid metabolism (Kilany et al., 2020).

To better understand how carotenoids could protect the body from disorders associated with obesity, El-Baz et al. (2020) developed a study of Wistar rats fed HF diet supplemented with a carotenoid-rich extract of microalgae Dunaliella saline (150 mg/kg) for 6 weeks. The authors observed an increase in plasma adiponectin protein in the group fed HF diet supplemented with microalgae extract compared to the control group HF. Adiponectin is a protein responsible for signaling important pathways involved in fatty acid oxidation, cellular glucose uptake, vasodilation, and other biological responses (Achari & Jain, 2017). Also, the consumption of microalgae extracts rich in carotenoids decreased the levels of PAI-1 (plasminogen activator inhibitor-1), C-reactive protein, troponin I, vascular cell adhesion molecule, and intercellular adhesion molecule, indicating a cardiovascular protective effect of this extract. In a systematic review and meta-analysis, including 5,961 manuscripts with 11,557 patients, Jung et al. (2018) concluded that elevated levels of PAI-1 are correlated with adverse cardiovascular events. All these finds suggesting that the consumption of the carotenoid-rich fraction of the microalgae *Dunaliella salina* could protect individuals against some cardiovascular disorders associated with obesity (El-Baz et al., 2020).

Likewise, Wang et al. (2019) observed a reduction in body weight gain, triglycerides, and total cholesterol in the serum and liver of C57BL/6J mice fed for 8 weeks on a HF diet supplemented with 0.01% astaxanthin. The authors also observed that astaxanthin's addition to the HF diet controlled the microbiota changes due to the HF diet's ingestion. The ratio *Firmicutes* to *Bacteroides* increased in the HF group contributing to calorie absorption, and *Verrocomicrobia* and *Akkarmansia* decreased. The intake of the HF diet supplemented with 0.01% of astaxanthin could reduce the ratio of *Firmicutes* to *Bacteroidetes* and increase the abundance of *Akkermansia*. These microbiota alterations could be associated with the lower body weight gain observed in the group supplemented with astaxanthin by decreasing the animals' calorie absorption (Wang et al., 2019).

The antiinflammatory effect of lycopene was also investigated in neuroinflammation by Li et al. (2020), which used an experimental posttraumatic stress disorder model. This experimental model causes the animals' anxiety-like behavior, a symptom observed in individuals after traumatic experiences, using an experimental model of a single prolonged stress test. The intraperitoneal administration of lycopene (20 mg/kg) decreased the neuroinflammation and anxiety-like behavior in C57BL6/J

mice compared to sertraline (15 mg/kg), an antidepressive drug. In this way, the authors speculate that lycopene supplementation or lycopene-containing plants' consumption could be an alternative to treat anxietylike behaviors observed in mood-associated disorders (Li et al., 2020). Finally, dermatology is another clinical area where carotenoids have been showing protective effects. Atopic dermatitis is a chronic relapsing disease characterized by itchy, inflamed skin that affects more frequently children with a history of other allergic diseases (Nutten, 2015). The consumption of a diet low in zinc and magnesium by HR-hairless mice for 8 weeks was the experimental model used to study atopic dermatitis-like symptoms in animals (Makiura et al., 2004). Hiragun et al. (2016) showed that this diet supplemented with carotene or lycopene (1 mg/g of diet) decreased the skin thickness and the infiltration of inflammatory cells in this murine model, indicating a protective effect by suppressing T-helper 2 chemokines. The authors also suggest that carotenoids oral intake could prevent or alleviate atopic dermatitis symptoms

5.3 Human studies

The effects of carotenoids on human health are mostly known because of their action as precursors of vitamin A (especially α-carotene, β-carotene, and β-cryptoxanthin), antioxidant capacity by distinctive mechanisms (see next section), and also for being photo-protectors, acting as a blue light filter on the retina (Johnson, 2014; Olson, 1993; Schulze & Christian, 2013).

The beneficial effects of carotenoids depend on their adequate intake, mainly through fruits and vegetables. The intake of fruits and vegetables is directly related to the plasma levels of total carotenoids (Al-Delaimy et al., 2005), and serum concentrations of carotenoids are inversely associated with several diseases, such as type 2 diabetes and insulin resistance (A¨ rnlo¨v et al., 2009; Coyne et al., 2005; Hozawa et al., 2006), obesity, metabolic syndrome (Harari et al., 2020; Liu et al., 2016), and cardiovascular diseases (Howard & Thurnham, 2017; Riccioni et al., 2010).

Several clinical trials have studied the association between ingestion of carotenoids alone or in combinations, with some diseases such as cancer, cardiovascular diseases, and AMD, among other health conditions (Table 5.1). There are also some systematic reviews and/or meta-analyses in the literature that analyze in-depth the health benefits allegations of carotenoids (Table 5.2).

TABLE 5.1 Human studies relating to carotenoid intake and health benefits.

Carotenoid	Objective	Population (*n*)	Experimental design	Results	Country	References
TNC[a] and lutein	Protection against UVA, UVB, and UVA1 at the molecular level	Health adults (52 men and 13 women)	The randomized study, double-blind. Groups: Active lycopene (TNC—5 mg lycopene + phytoene, phytofluene, tocopherols, and phytosterols)—Placebo lycopene (soybean oil)—Active lutein (10 mg lutein + 10% carnosic acid)—Placebo lutein (soybean oil) Dose: two capsules of TNC twice a day; one lutein capsule twice a day. Duration: 2-week washout phase followed by two 12-week treatment phases separated by another 2 weeks of washout	TNC completely inhibited radiation's action on the genes related to oxidative stress, photoaging, and photo-dermatosis. Lutein showed complete protection during the first phase of the study.	Germany	Grether-Beck et al. (2017)
TNC	Erythema induced by UVB radiation; positive regulation of molecular markers of inflammation and immunosuppression	Health adults (149)	The randomized study, double-blind. Groups: Experimental group (capsules -15 mg lycopene, 5.8 mg phytoene, and phytofluene, 0.8 mg β-carotene, 5.6 mg tocopherol, and 4 mg carnosic acid from rosemary) Placebo group (capsules—medium-chain triglycerides). Duration: 5-week washout phase, followed by a 12-week treatment phase	TNC protects against UVB-induced erythema and positive regulation caused by UVB in IL-6 and TNF-α.	Germany	Groten et al. (2019)
Vitamin A from food or synthetic source	Night adaptation of pregnant with night blindness	Pregnant with night blindness (397)	Groups: 850 μg retinol equivalent in the form of retinyl palmitate—Rice fortified with vitamin A—Goat liver—Amaranth leaves—Carrots—2,000 μg retinol equivalent/d in the form of retinyl palmitate. Duration: 6 days/week for 6 weeks	Significant reduction of night blindness in all groups; however, no significant difference among the groups.	Nepal	Haskell et al. (2005)
Mixture of carotenoids[b]	Effects on adipokines and abdominal fat accumulation	Children from 8 to 11 years old with BMI[c] in the 90 percentile or above (20)	The randomized study, double-blind. Groups: mixture of carotenoids (2,000 IU β-carotene, 500 mg α-carotene, 10 mg lutein, 2 mg zeaxanthin, 10 mg lycopene, 500 mg astaxanthin, and 10 mg γ-tocopherol)—placebo group. Duration: twice a day for 6 months	Higher reduction in the *z* score of BMI, waist to height ratio, and subcutaneous adipose tissue.	United States	Canas et al. (2017)
Paprika xanthophylls	Reduction of abdominal fat in overweight people	Health volunteers with BMI 25 to <30 kg/m^2 (100)	The randomized study, double-blind. Groups: capsule containing paprika xanthophylls (9 mg)—placebo—capsule without xanthophylls. Duration: 12 weeks	Ingestion reduced the abdominal fat area and BMI without causing adverse effects.	Japan	Kakutani et al. (2018)
Lutein and zeaxanthin	Brain function	Older adults between 64 and 84 years old (44)	The randomized study, double-blind. Groups: capsules containing 10 mg lutein and 2 mg zeaxanthin—placebo: inert capsules. Duration: 1 year	Carotenoid supplementation slightly helped the maintenance of cognitive functions due to the increase of blood perfusion.	United States	Lindbergh et al. (2018)
Pro-vitamin A from biofortified orange maize	Adaptation to darkness	Children from 4 to 8 years old (1,024)	The randomized controlled trial. Groups: 200 g biofortified orange maize (about 15 mg β-carotene/g)—control: white maize. Duration: twice a day, six times/week for 6 months	Regular consumption of biofortified orange maize showed functional impact in the adaptation to darkness in children with marginal status or vitamin A deficiency.	Zambia	Palmer et al. (2016)
Carotenoids (lutein, zeaxanthin, and astaxanthin)	Progression of intermediary AMD[f] according to AREDS[g] classification	Patients between 55 and 80 years old, diagnosed with intermediary AMD (74)	GOAL group[h] a prospective study, double-blind. Groups: carotenoids (10 mg lutein, 4 mg astaxanthin, and 2 mg zeaxanthin), antioxidants (90 mg vitamin C, 30 mg vitamin E, 22.5 mg zinc, and 1 mg cupper), and omega-3 (500 mg fish oil)—control: placebo tablet. Duration: 24 months	Significant stability of macular degeneration during treatment.	Italy	Piatti et al. (2020)

Macular xanthophylls (lutein, zeaxanthin, and meso-zeaxanthin)	Oxidative and inflammatory status and systemic brain-derived neurotrophic factor (BDNF)	Young adults between 18 and 25 years old (59)	The randomized study, double-blind. Groups—13 mg/day xanthophylls—27 mg/day xanthophylls—control: placebo. Duration: 6 months	Significant decrease in the levels of inflammatory interleukins, and an increase in the antioxidant capacity, BDNF in cognitive performance parameters.	United States	Stringham et al. (2019)
TNC and synthetic lycopene	Determine the TNC effective dose to maintain normal systolic blood pressure	Hypertensive individuals without treatment (61)	The randomized study, double-blind. Groups: 5 mg lycopene TNC—15 mg lycopene TNC—30 mg lycopene TNC—15 mg synthetic lycopene—placebo. Duration: 8 weeks	15 and 30 mg lycopene TNC were associated with a significant reduction in systolic blood pressure. Lower doses and synthetic lycopene did not show significant results.	Israel	Wolak et al. (2019)
Lutein	Effects on serum cytokines, apoE and lipoprotein	Individuals with early atherosclerosis (65)	The randomized study, double-blind. Groups: 20 mg lutein/day—control: placebo. Duration: 3 months	Lutein supplementation reduced inflammatory cytokines and regulated serum lipids.	China	Xu et al. (2013)
AREDS[c]	Age-related lens opacities and loss of visual acuity	Individuals between 55 and 80 years old, with at least one eye free from any disease (4757)	AREDS an experimental and observational study. Groups: antioxidants (500 mg vitamin C, 400 IU vitamin E, and 15 mg β-carotene)—zinc (80 mg zinc and 2 mg copper)—antioxidants and zinc—placebo. Duration: 6 years	No significant difference in the risk of progression or development of different types of opacities or loss of visual acuity.	United States	Age-Related Eye Disease Study Research (2001)
AREDS 2[d]	Late AMD progression	Individuals between 50 and 80 years old, at risk of late AMD development (4,203)	AREDS 2 the randomized study, double-blind. Groups: placebo lutein (10 mg) and zeaxanthin (2 mg)—DHA[i] (350 mg) and EPA[j] (650 mg)—lutein/zeaxanthin + EPA/DHA. Duration: 5 years	Supplementation with lutein/zeaxanthin decreased AMD progression compared to β-carotene (AREDS formulation).	United States	Age-Related Eye Disease Study 2 Research et al. (2014)
β-carotene	Cancer and cardiovascular diseases	Male medical doctors between 40 and 84 years old (22,071)	PHS[k] the randomized study, double-blind. Groups: 50 mg β-carotene on alternate days for 12 years—control: placebo.	β-Carotene supplementation did not result in any benefits for cancer, cardiovascular diseases, or death.	United States	Hennekens et al. (1996)
β-carotene	Cognitive performance	Male medical doctors older than 55 years (5,956)	PHS II the randomized study, double-blind. Groups: treatment group following PHS: active β-carotene, on alternate days for 18 years—new treatment group: active β-carotene, on alternate days for 12 months—control groups following PHS and new: placebo.	Long term supplementation can bring beneficial effects on cognitive functions.	United States	Grodstein et al. (2007)

[a]TNC (Tomato Nutrients Complex—rich in lycopene).
[b]Mix of carotenoids (β-carotene, α-carotene, lutein, zeaxanthin, lycopene, astaxanthin, and γ-tocopherol).
[c]AREDS formulation (oral total daily supplementation of antioxidants (500 mg vitamin C, 400 IU vitamin E, and 15 mg β-carotene) or zinc (80 mg of zinc as zinc oxide, 2 mg of copper as a cupric oxide to prevent potential anemia), or the combination of antioxidants and zinc).
[d]AREDS 2 formulation—β-carotene substituted with lutein/zeaxanthin in the original AREDS formulation, with or without the addition of omega-3 fatty acids or without zinc.
[e]BMI (body mass index).
[f]AMD (age-related macular degeneration).
[g]AREDS (Age-Related Eye Disease Study).
[h]GOAL group (Gruppo Oculisti Ambulatoriali Liberi—Scientific Association of Italian Ophthalmologists operating in Eye Primary Care).
[i]DHA (docosahexaenoic acid).
[j]EPA (eicosapentaenoic acid).
[k]PHS (Physicians' Health Study).

One of the main roles of carotenoids in the body is pro-vitamin A activity. This vitamin is an essential nutrient responsible mainly for normal vision (Mariutti et al., 2018). Vitamin A deficiency is already well related to xerophthalmia, but it can also lead to an increased risk of infectious diseases (Sherwin et al., 2012). In fact, vitamin A supplementation (source nonspecified) has shown an improvement in the immune response by increasing the production and proliferation of T cells and some cytokines, such as IL-5 (Ahmad et al., 2009).

Vitamin A can have different functions throughout life, and studies regarding the effects of vitamin A from conception to aging can be found in the literature. Pregnant women with night blindness received vitamin A supplementation through capsules, fortified foods, or foods that are a source of this vitamin. They showed

TABLE 5.2 *Meta*-analysis and systematic reviews on carotenoid intake and health benefits.

Carotenoid	Health issue	Number of studies or population (*n*)	Results	References
Carotenoids, retinyl ester, and retinol	Metabolic syndrome	The relation between vitamin A and metabolic syndrome (41)	Individual carotenoids were inversely related to metabolic syndrome. No association between retinol and metabolic syndrome.	Beydoun et al. (2019)
β-Carotene, α-carotene, lutein, zeaxanthin, lycopene, and β-cryptoxanthin	Esophageal cancer	Patients with esophageal cancer (1,958) and control with no cancer (4,529)	Higher ingestion of carotenoids reduces the risk of esophageal cancer.	Ge et al. (2013)
Lutein (ingestion or serum content)	Cardiometabolic health	Different age groups (387,569)	High lutein ingestion and high serum concentration are associated with cardiometabolic health.	Leermakers et al. (2016)
Lycopene	Metabolic syndrome	Transversal studies (8) and intervention studies (3)	Protective effect of lycopene on metabolic syndrome.	Senkus et al. (2019)
Tomato	Prostate cancer	Case-control studies (11) and cohort studies (10)	Tomato products can have a role in preventing prostate cancer; however, the effect is minimal.	Etminan et al. (2004)
Carotenoids	Head and neck cancer	Epidemiological studies. Case-control (15) and cohort (1)	High carotenoid ingestion through the diet can reduce the risk of head and neck cancer.	Leoncini et al. (2015)
Carotenoids	Colorectal cancer	Epidemiological studies. Case-control (16) and cohort (6)	No significant association between carotenoid ingestion and colorectal cancer.	Panic et al. (2017)
Lycopene	Prostate cancer	Studies investigating the association between lycopene and prostate cancer (42)	Inverse association between lycopene ingestion and serum lycopene with prostate cancer.	Rowles et al. (2017)
Carotenes and Lycopene	Prostate cancer	Case-control (13), cohort (10), and nested control case (11) studies	α-carotene and lycopene were inversely associated with the risk of prostate cancer. No effects were observed in the risk of advanced cancer.	Wang et al. (2015)
Lycopene	Low-degree chronic inflammation	Relationship between lycopene and inflammation (35)	No relationship was established between circulant lycopene and a reduction in low-degree inflammation.	van Steenwijk et al. (2020)
Carotenoids	Breast cancer	Carotenoids associated with breast cancer (33)	No association.	Hu et al. (2012)

improved adaptation to the dark, with better results for women who consumed goat liver compared to those who consumed rice fortified with retinol, possibly due to the other nutrients present in the liver such as iron, zinc, and proteins. However, studies in pregnant women are limited, as vitamin A is known to be teratogenic and, therefore, supplementation doses should not exceed 7 mg RE per week (Haskell et al., 2005).

Newborns with very low birth weight usually require respiratory support. In a study conducted with this group, vitamin A (aqueous retinol solution) reduced mortality from all causes, as well as the requirement and time of noninvasive oxygen support (Basu et al., 2019). Children aged 4 to 8 years old with vitamin A deficiency showed improved visual adaptation to the dark after consumption of pro-vitamin A-biofortified orange maize (Palmer et al., 2016).

Many health problems related to chronic noncommunicable diseases, such as obesity and cardiovascular diseases, are common among adults. Metabolic

syndrome is characterized by a set of cardiometabolic diseases, such as hyperglycemia, hypertension, and hypertriglyceridemia, associated with obesity. Elevated serum concentrations of total carotenoids, β-carotene, and retinoic acid were associated with a lower incidence of metabolic syndrome, showing that a diet rich in carotenoids may exert cardioprotection (Beydoun et al., 2019; Liu et al., 2016). In patients with atherosclerosis, vitamin A (25,000 IU retinyl palmitate per day) has been associated with reduced disease progression, as it decreases the expression of genes linked to inflammation (Mottaghi et al., 2014).

The Uppsala Longitudinal Study of Adult Men evaluated the concentration of β-carotene in the serum and type 2 diabetes incidence. Patients were followed for 27 years, and a high serum β-carotene concentration was associated with a lower incidence of type 2 diabetes (nlöv et al., 2009). Similar results were found in other studies, demonstrating an inverse relationship between serum β-carotene concentrations and the risk of developing type 2 diabetes (Coyne et al., 2005; Hozawa et al., 2006; She et al., 2017).

The ingestion of a mixture of carotenoids by overweight children showed an effect on the reduction of BMI (bodymass index) and waist-to-height ratio, as well as a reduction in visceral fat (Canas et al., 2017). The xanthophylls present in paprika (capsanthin, capsorubin, and cryptocapsin) have been associated with reduced abdominal fat in obese individuals, as well as reduced subcutaneous fat area, BMI, and LDL levels (Kakutani et al., 2018). Another xanthophyll associated with the reduction of LDL-cholesterol was lutein, which also reduced pro-inflammatory cytokines, thus decreasing the risk of atherosclerosis (Xu et al., 2013).

Lutein is beneficial for cardiometabolic health due to its protection against oxidative stress. The high intake and serum lutein concentration have been associated with reduced risk of coronary heart disease, heart attack, and metabolic syndrome, but there was no difference in the risk of developing type 2 diabetes. These effects of lutein have been associated with vascular changes, side effects, antioxidants, immune response, and inflammation, showing the role of lutein both in specific organs and in general health (Leermakers et al., 2016).

Lycopene (in the form of a nutrient complex of lycopene-rich tomatoes, that is, tomato nutrient complex—TNC) in doses of 15 and 30 mg was associated with a reduction in systolic blood pressure, an effect not observed in lower doses or the intake of synthetic lycopene (Wolak et al., 2019). Intake of lycopene, mainly as a supplement,

reduced serum proteins linked to inflammation such as serum amyloid A, a predictor of cardiovascular disease, which is released by adipocytes and related to chronic low-grade inflammation characteristic of obesity. Thus, lycopene supplementation may be related to reducing the risk of cardiovascular disease in overweight individuals (McEneny et al., 2013). However, a recent meta-analysis failed to find this same relationship between serum lycopene and a reduction in factors linked to low-grade inflammation; reduced serum lycopene can be considered one of the first signs of inflammation (van Steenwijk et al., 2020). When ingested in beverages, lycopene had a protective effect considering different factors linked to metabolic syndrome. Despite being a favorable result, it was not possible to identify which factors lycopene showed the best results, impairing the association with the reduction of metabolic syndrome (Senkus et al., 2019).

The xanthophylls lutein, zeaxanthin, and meso-zeaxanthin are preferentially accumulated in the retina's macularregion and are associated with a reduced risk of AMD (Arunkumar et al., 2020). They protect eye tissues by acting as a blue light filter and preventing light damage to photoreceptor cells, in addition to acting as antioxidants (Johnson et al., 2013; Schulze & Christian, 2013; Arunkumar et al., 2020; Krinsky et al., 2003).

The Age-Related Eye Disease Study (AREDS) evaluated the supplementation of β-carotene and vitamins C and E in the development and progression of cataracts, as well as their effect and high doses of zinc in AMD. No significant difference was observed among the treated groups and the placebo group in the risk of developing or progressing cataracts or AMD (Age-Related Eye Disease Study Research, 2001). Smoking patients withdrew from the AREDS due to the results of the CARET study (Beta-carotene and Retinol Efficacy Trial) in which supplementation with β-carotene (30 mg) and retinyl palmitate (25,000 IU) increased the risk of lung cancer by 28% and mortality by 17% in smoking patients (Omenn et al., 1996).

AREDS 2 replaced β-carotene with lutein and zeaxanthin in the original formulation of AREDS to verify the effects of these xanthophylls on AMD progression. The substitution by lutein and zeaxanthin showed better results in reducing the risk of late AMD compared to β-carotene, demonstrating that xanthophylls would be more suitable for the formulation of AREDS (Age-Related Eye Disease Study 2 Research Group et al., 2014). More recently, the Gruppo Oculisti Ambulatoriali Liberi reported

that individuals who received a mixture containing carotenoids (lutein, astaxanthin, and zeaxanthin), antioxidants (vitamin C, vitamin E, zinc, and copper), and omega-3 fatty acids presented a lower risk of progression of AMD (Piatti et al., 2020)

Lutein and zeaxanthin also accumulate in brain tissue, where they affect cognitive aspects. Xanthophylls are linked to improvement in neurocognitive functions by increasing blood perfusion in the brain, especially in regions related to cognition. In addition to the expression of factors linked to neuroplasticity, the supplementation with lutein and zeaxanthin improved learning and memory, not directly, but associated with improved cognitive processes such as attention. They also reduce serum levels of pro-inflammatory interleukins, which also contributes to improving cognition in the elderly (Lindbergh, 2018; Stringham et al., 2019). Lutein and zeaxanthin also act on brain functions from the macular region and are associated with improvement in visual memory and visual processing speed (Ceravolo et al., 2019). The Georgia Centennial Study linked serum concentrations of lutein, zeaxanthin, and β-carotene with better cognition in the entire population. However, only serum lutein content was significantly related to better cognition in octogenarians. While in the brain, lutein and β-carotene were related to cognition (Johnson et al., 2013).

The Physicians' Health Study II evaluated β-carotene supplementation in the short (50 mg on alternate days for 12 months) and long term (50 mg on alternate days for 18 years) in relation to cognitive functions. The men who received the supplementation had slightly better cognition than the placebo group, and this supplementation must be at least for 15 years (Grodstein et al., 2007). TNC, together with lutein, has been associated with photoprotection of the skin against short UVA and UVB rays, as well as against long UVA-1 rays. UVA-1 radiation is known to cause photoaging and photocarcinogenesis in the skin. Lycopene was responsible for inhibiting UV rays' action on genes related to oxidative stress, photoaging, and photodermatoses; that is, it protected the organism from damage caused by UV radiation (Grether-Beck et al., 2017). The TNC also demonstrated protection against erythema caused by UVB rays in addition to reducing serum proinflammatory cytokines (IL-6 and TNF-α) (Groten et al., 2019).

Lycopene has also been associated with bone health, as it reduces the risk of hip fracture and nonvertebral osteoporotic fracture, mainly for its action in inhibiting osteoclast synthesis (Sahni et al., 2009). Analyzing serum levels of lycopene in

postmenopausal women, lycopene was associated with a reduction in oxidative stress and bone turnover markers, reducing the osteoporosis risk (Rao et al., 2007). Carotenoids' effect in reducing the risk of different types of cancer is mainly related to their action as antioxidants by scavenging free radicals, inhibiting the damage caused by ROS and lipid peroxidation (Leoncini et al., 2015).

High lycopene intake and elevated lycopene serum levels were associated with a reduced risk of prostate cancer (Rowles et al., 2017). Intake of β-carotene, and serum levels of α-carotene and lycopene were also associated with decreased risk of developing this type of cancer (Wang et al., 2015). However, none of the studies found significant results for reducing advanced prostate cancer (Rowles et al., 2017; Wang et al., 2015). A meta-analysis demonstrated an inverse relationship between the ingestion of tomato products and prostate cancer risk; however, in a discreet way. The highest correlation was identified in the consumption of high amounts of products that use cooked tomatoes, such as sauces, which can be explained by lycopene's greater bioaccessibility in such products (Etminan et al., 2004).

Regarding esophageal cancer, the ingestion of different carotenoids (β-carotene, α-carotene, lutein, zeaxanthin, β-cryptoxanthin, and lycopene) was associated with a reduced risk of this type of cancer, with β-carotene being associated with a reduction in the risk of adenocarcinoma and the others with a reduction in squamous cell cancer (Ge et al., 2013). α-Carotene but no other carotenoids was associated with reduced risk of breast cancer (Hu et al., 2012).

The intake of carotenoids from natural sources protected against the development of head and neck cancer compared to the ingestion of the carotenoids individually. β-Carotene was more linked to reducing cancer risk in the oral cavity and larynx, lycopene and β-cryptoxanthin in the reduction of laryngeal cancer, and together with α-carotene in the reduction of the risk of oral and pharyngeal cancer (Leoncini et al., 2015).

Consumption of carotenoids from natural sources did not show any association with colorectal cancer, suggesting that carotenoids have no protective effect on this type of cancer (Panic et al., 2017).

5.4 Mechanistic studies

Driven by the prospect of positive epidemiological data, mechanistic studies have been carried out and supported that carotenoids may promote health potentially

via different mechanisms and acting in multiple molecular targets (Fig. 5.4). Selective examples of in vitro studies evidencing carotenoid activities in the context of noncommunicable diseases are presented in this section.

Lutein, zeaxanthin, and meso-zeaxanthin selectively accumulate in the retina macula, where they constitute the macular pigment, and are presumed to protect the eye from photo-induced oxidative damage associated with the development and progression of AMD. Carotenoids present a system of conjugated double bonds responsible for their characteristic pattern of light absorption in the electromagnetic spectrum's visible region (Britton, 1995). Macular carotenoids absorb maximally around 460 nm, and thus, these pigments can act as filters of the blue light, attenuating the intensity with which this potentially harmful light reaches photoreceptors and retinal cells (Bone et al., 1992; Junghans et al., 2001). Efficient filtering properties of lutein and zeaxanthin against blue light wavelength range were demonstrated in vitro in lipophilic membrane models (Junghans et al., 2001; Sujak et al., 1999). Likewise, the electron-rich conjugated structure is also responsible for the ability of these carotenoids to either scavenge ROS or physically quench photo-induced singlet oxygen and triplet state of photosensitizers, which may limit the oxidative damage in the retina (Britton, 1995; Krinsky et al., 2003; Trevithick-Sutton et al., 2006). Carotenoids' capacity to act as effective antioxidants in vitro has been extensively reported but whether this corresponds to the in vivo situation is still a question (Krinsky et al., 2003). Khachik et al. (1997) analyzed ocular tissues from tissue banks and first reported the presence of oxidation products of lutein and zeaxanthin in the human retina, suggesting that these xanthophylls may offer protection to retinal structures by functioning as an antioxidant (Bernstein et al., 2001). Macular xanthophylls have also been shown to quench light-induced singlet oxygen in human RPE and choroid in vitro (Li et al., 2010). In the cultured retinal cell model (ARPE-19), inhibited photodynamic killing of cells accompanied by accumulation of singlet oxygen-specific hydroperoxides was reported upon zeaxanthin pretreatment, alone or in combination with either α-tocopherol or ascorbic acid, when compared to control cells (Wrona et al., 2004). Of particular interest, systematic in vitro studies by Gruszecki group evidenced the specific transmembrane localization of both lutein and zeaxanthin in liposome model, reported as a determinant aspect for their efficiency in protecting ocular biomembranes against oxidative damage (Sujak et al., 1999; Gruszecki & Strzałkab, 2005; Grudzinski et al., 2017).

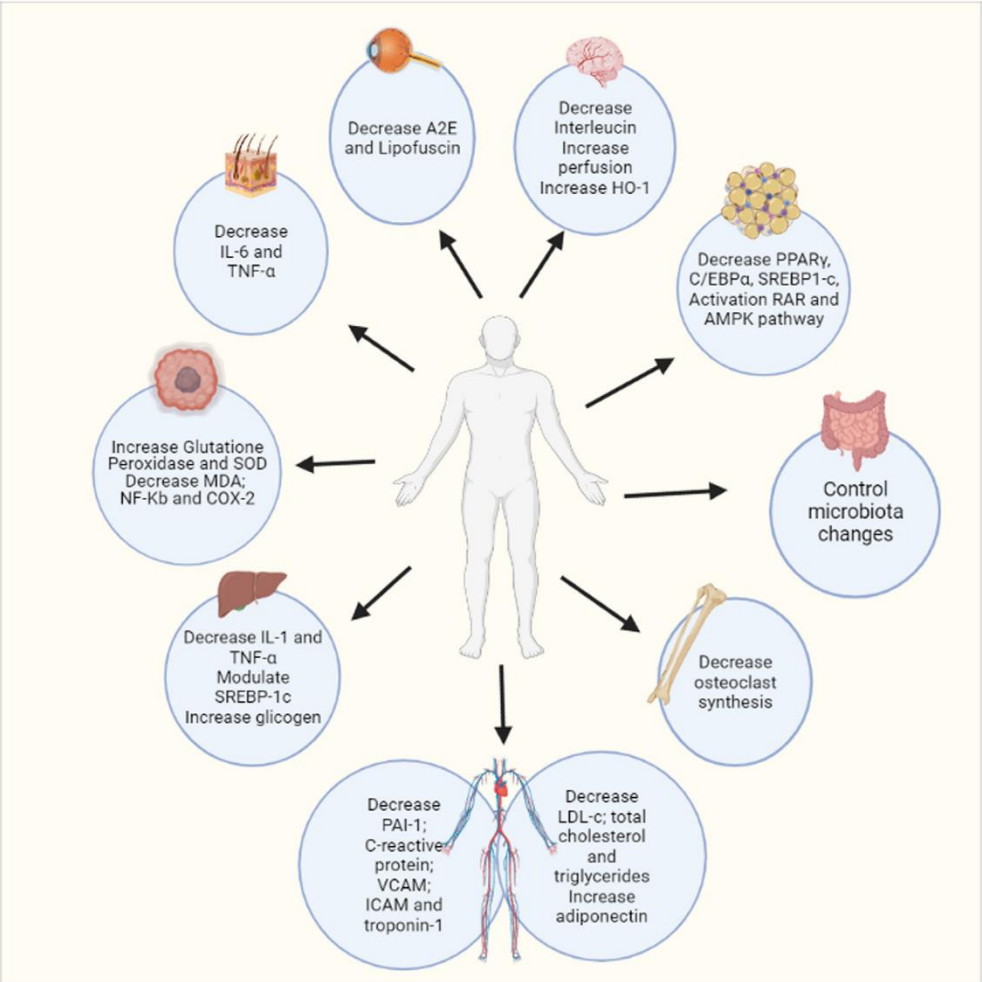

FIGURE 5.4 Potential health benefits of carotenoids via different mechanisms and action in multiple targets. Own authorship created by Biorender.com.

Macular xanthophylls have also been found to reduce lipofuscin formation in cultured retinal cells (Nilsson et al., 2003; Sundelin & Nilsson, 2001). Lipofuscin is a by-product of photoreceptor catabolism accumulated in RPE cells with age and associated with macular degeneration. One of its components, the fluorophore A2E, can promote singlet oxygen formation and light-mediated oxidative stress upon exposure to blue light, a process that has also been shown to be suppressed in vitro by lutein and mainly by zeaxanthin (Kim et al., 2006; Sundelin & Nilsson, 2001). Of note, inactivation of proteasome and changes in expression of inflammation-related genes MCP-1, IL-8, and CFH induced by light were attenuated by both xanthophylls in A2E-containing RPE cells, a model for mimicking the lipofuscin-mediated photooxidation in vitro (Bian et al., 2012). This result indicates that lutein and

zeaxanthin may modulate inflammatory responses in retinal cells in culture. Indeed, in addition to direct antioxidant properties, in vitro evidence is accumulating to support that carotenoids can interfere in cellular signaling pathways related to inflammation and oxidative stress, with implications on their putative protective role against chronic diseases as further exemplified in this chapter section (Kaulmann & Bohn, 2014).

Anticarcinogenic activities of carotenoids and their metabolites have been demonstrated in many cancer cell lines inculture. The mechanisms whereby these compounds have been implicated in cancer are mainly related to their ability to regulate the expression of proteins and transcription factors involved in signaling processes, including cell proliferation and apoptosis, either as direct modulators or as redox agents (Palozza et al., 2009). For instance, it is known that retinoids generated upon the metabolism of pro-vitamin A carotenoids are essential regulatory molecules in cell differentiation and growth. By activating nuclear RAR and RXR receptors that function as transcription factors, they regulate gene expression and may mediate carotenoids' effects on cancer development and progression. In the study of Prakash et al. (2004), the decreased expression of RARβ in normal bronchial cells exposed to smoke-borne carcinogens was reversed after treatment with either β-carotene or its metabolite 140-apo-β-caroten-140-oic acid, which was attributed to their conversion to retinoic acid (Prakash et al., 2004).

Intact carotenoids and other nonretinoid breakdown products may also modulate different cancer-related molecular pathways. Lycopene has been shown to effectively suppress the growth of breast (MCF-7 and T-47D) and endometrial (ECC-1) cancer cells through downregulation of cyclins D1 and D3, regulatory proteins of cell cycle progression (Nahum et al., 2001). In contrast to this direct modulation, Gansukh et al. (2019) observed that changes in the expression of apoptosis-related proteins in β-cryptoxanthin-treated cervical carcinoma (HeLa) cells were accompanied by intracellular ROS production and loss of the integrity of the mitochondrial membrane, suggesting a redox mechanism. The redox regulation of NF-κB induced by β-carotene, with increased expression of NF-κB target genes involved in apoptosis, has been reported to be implicated in the growth-inhibitory effects of the carotenoid in human leukemia (HL-60) and colon adenocarcinoma (LS-174 and WiDr) cells (Palozza et al., 2003).

There has been considerable interest in the potential role of lycopene in prostate carcinogenesis. To investigate mechanisms of occurrence and prevention of

prostate cancer, Liu et al. (2008) used a coculture model of primary human prostate cancer stromal (6S) cells expressing androgen receptors with primary normal prostatic epithelial cells. In this system, lycopene treatment (0.31.0 μmol/L) inhibited the pro-proliferative and anticell death effects of the androgen dihydrotestosterone (DHT), indicating the involvement of this carotenoid in the insulin-like growth factor IGF-1 and androgen signaling pathways, considered critical pathways to drive prostate cancer progression (Chang et al., 2014). Alternatively, no direct androgen outcomes were observed in the study of Ivanov et al. (2007), which reported inhibitory effects of physiological doses (0.20.8 μmol/L) of lycopene on cell proliferation in both androgenresponsive (LNCaP) and androgen-independent (PC-3) prostate cancer cells, as mediated by downregulation of cyclins D1 and E. In fact, a number of different mechanisms have been proposed to contribute to the chemopreventive action of lycopene on the prostate and other cancer cell lines, with detailed reviews summarizing in vitro findings found elsewhere (Applegate et al., 2019; Palozza, Parrone, et al., 2011; Palozza, Simone, et al., 2011).

The enhancement of gap junction intercellular communication, via upregulated expression of connexin protein Cx43, has been reported to be one of the mechanisms by which carotenoids can inhibit neoplastic transformation in vitro, as demonstrated in oral (Livny et al., 2002) and liver cancer (Liu et al., 2009) cells. Also of note is carotenoids' ability to induce phase II detoxifying enzymes by interacting with the transcription factor Nrf2. Lycopene and, to a lesser extent, phytoene, phytofluene, β-carotene, and astaxanthin, were shown to induce phase II enzymes such as NAD (P)H:quinone oxidoreductase and γ-glutamylcysteine synthetase in mammary (MCF-7) and hepatocellular carcinoma cells (HepG2), through activation of the antioxidant response element transcription system dependent on Nrf2 (Ben-Dor et al., 2005).

Overall, studies conducted in cultured cells have shown positive effects of carotenoids against carcinogenesis at physiologically attainable concentrations, whereas high doses have produced completely opposite outcomes. This observation is likely related to the pro-oxidant effects exhibited by high doses of carotenoids under certain conditions (Wang & Russel, 1999). In vitro studies have shown that β-carotene eccentric cleavage products generated under oxidative conditions can function as antagonists of retinoid receptors, and disrupt the retinoid signaling pathway (Eroglu et al., 2012). These in vitro findings have been proposed to have implications for the

detrimental consequences of pharmacological doses of β-carotene in cancer as observed in famous CARET and ATBC human clinical trials (see Section 5.3).

Some of the transcription factors and signaling cascades potentially affected by carotenoids play a key role in regulating cellular processes in adipose tissue. These include adipocyte differentiation, capacity for lipid storage and metabolism, adipokine secretion profile, and inflammatory status, which may impact obesity management and obesityassociated disorders (Bonet et al., 2015). Lobo et al. (2010) observed that β-carotene, upon conversion to retinoic acid, reduced the lipid content of mature adipocytes (3T3-L1) through RAR activation and subsequent downregulation of PPARγ and C/EBPα, key lipogenic transcription factors. Using the 3T3-L1 preadipocyte model, Liu, Liu, Xie, et al. (2017) showed that zeaxanthin inhibited adipogenesis and intracellular lipid accumulation in a dose-dependent manner (515 μM) by downregulating PPARγ, C/EBPα, SREBP1-c, and their downstream target genes. These effects were accompanied by activation of the AMPK pathway associated with lipogenesis suppression. In a further investigation, Liu et al. (2019) demonstrated that, through AMPKα1 activation, zeaxanthin treatment (2 μM) promoted adipocyte browning. Increased production of antioxidant enzymes and reduced the levels of ROS, lipid peroxidation, and mitochondrial oxidative damage were also noticed, contributing to the in vitro antiobesity effect of zeaxanthin.

Additionally, lycopene in vitro antiinflammatory effect has been reported and may be relevant in the obesity context. Lycopene pretreatment (2 μM) reduced the TNF-α-mediated expression of pro-inflammatory cytokines and chemokines in cultured adipocytes (3T3-L1) by a mechanism involving the blockage of NF-κB activation (Gouranton et al., 2011). A similar result was found by Marcotorchino et al. (2012) on macrophages (RAW 264.7), as evidenced by a decrease of lipopolysaccharide (LPS)-induced secretion of the cytokine TNF-α in lycopene-pretreated cells, accompanied by diminished macrophage migration in vitro. The authors also observed that the antiinflammatory action of lycopene on macrophages positively impacted the inflammation status of adipocytes that were subsequently incubated in the former macrophageconditioned media, in comparison with those of control treatment. The downregulated expression of genes encoding proinflammatory cytokines, acute phase proteins, and chemokines in such adipocytes reflected this effect. Cytokines and chemokines are assumed to be involved in developing insulin resistance (Gregor & Hotamisligil, 2011). Remarkably, Fenni et al. (2019) demonstrated in vitro that all-trans

and 5-cis lycopene isomers, by attenuating the cellular inflammatory process, counteracted the TNFα-mediated impairment in glucose uptake in adipocytes, that is, restored the insulin sensitivity. These findings indicate that carotenoids can potentially attenuate conditions associated with a low-grade inflammatory basis such as obesity, type 2 diabetes, and cardiometabolic disorders with implications on cardiovascular disease onset.

Nonetheless, the antioxidant properties have also been associated with carotenoids' protective effects against coronary heart disease. These compounds are predominantly transported in LDL in blood circulation and are hypothesized to inhibit the formation of oxidized LDL that may lead to atherosclerosis. Enrichment with β-carotene, but not with lycopene and lutein, has been shown to protect LDL from oxidation by human endothelial cells (EaHy-1) in culture (Dugas et al., 1998).

Carotenoids are also found in brain cells and may offer neuronal cells protection from oxidative damage and neuroinflammation implicated in neurodegenerative diseases, as assessed in cellular models of neuroprotection. In a cellular model of Parkinson's disease, Ye et al. (2012) observed that astaxanthin was able to reduce the neurotoxin (MMP1)-induced oxidative stress in PC-12 cells exhibiting features of midbrain dopaminergic neurons, by a mechanism involving the HO-1/NOX2 axis. The upregulated expression of HO-1 by the xanthophyll in a dose-dependent manner (5, 10, and 20 μmol/L) was suggested to limit the oxidative damage mediated by NOX2 activation, contributing to astaxanthin neuroprotective effects. The degeneration of midbrain dopamine neurons precedes motor dysfunctions characteristic of Parkinson's disease (Masoud et al., 2015). Pretreatment with lutein protected cerebrovascular endothelial cells against β-amyloid peptide-mediated oxidative stress by modulating the NF-κB and Nrf2 expression, as demonstrated by Liu, Liu, Zhao, et al. (2017). Enhanced mitochondrial membrane potential and decreased caspase activities in cells in response to lutein suppressed the cell apoptosis, a critical step for neurodegeneration in Alzheimer's disease. Lutein has also been shown to alleviate the LPS-induced inflammation in cultured BV-2 microglia cells, which actively participate in neuroinflammation (Wu et al., 2015).

5.5 Conclusion

Several studies have been conducted using in vitro and in vivo models aiming at proving and better understanding the role of carotenoids in the remediation or prevention of noncommunicable diseases, focusing especially on inflammatory, anticancer, and antioxidant properties. To assure the beneficial health effects of carotenoids, individuals must have balanced and health diet, rich in fruits and vegetables. Only when carotenoids are ingested in adequate amounts, they can be absorbed, metabolized and transported to the target organs to exert their bioactivity.

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CAPÍTULO III

How does the food matrix affect the provitamin A carotenoid *in vitro* bioaccessibility in fruit-based baby food?

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Abstract

Carotenoid bioaccessibility of is an important predictor of absorption and provitamin A intake. Humans do not synthesize carotenoids which need to be acquired from the diet, mainly from green leafy vegetables and fruits with colors from yellow to red. The carotenoid composition of five industrialized fruit-based baby food was determined, and the carotenoid bioaccessibility was calculated after *in vitro* digestion by the Infogest 2.0 protocol. The provitamin A carotenoids were converted to RAE (retinol activity equivalent) to estimate the vitamin A value. Lipids and minerals positively affected the bioaccessibility of carotenoids, except for (all-*E*)-α-carotene. Interestingly, fiber content positively affected the bioaccessibility of (all-*E*)-α-carotene, while no effect was observed on the other carotenoids' micellarization. (All-*E*)-β-carotene bioaccessibility ranged from 5% for assorted fruits (AF) to 24% for apple, mango, and carrot (AMC) baby foods and from 1% for grape and banana (GB) to 15.5% for tropical fruits (TF) baby foods for total carotenoid bioaccessibility. Regarding vitamin A intake, AMC baby food had the highest RAE value, 15 per portion of 120 g. However, this value corresponds to only 3% of the dietary reference intake (DRI).

Keywords: carotenoid, baby food, fruits, bioaccessibility, digestion, vitamin A

1. Introduction

Carotenoids are fat-soluble pigments widely distributed in nature and impart color ranging from yellow to red to plants. Photosynthetic (plants, algae, cyanobacteria) and non-photosynthetic (fungi, bacteria, and some invertebrate animals) organisms can synthesize carotenoids that participate in cellular photoprotection (Rodriguez-Concepcion et al., 2018).

Humans do not synthesize carotenoids and must acquire them from their diet. However, to be absorbed in the small intestine, carotenoids need to be released from the food matrix, solubilized, and micellarized (Schweiggert & Carle, 2017). This transfer process from the food matrix to the micelles is known as bioaccessibility and determines the fraction of compounds available for absorption (Kopec & Failla, 2018).

Interaction with other food components can affect the bioaccessibility of carotenoids. Lipids tend to increase carotenoid bioaccessibility by facilitating their solubilization and incorporation into micelles. Conversely, some minerals can interfere with solubilization and micellarization processes, reducing the bioaccessibility of carotenoids (Shilpa et al., 2020).

Another factor affecting bioaccessibility is how carotenoids are deposited in plants. Carotenoids are located in chromoplasts, which can have different shapes:

globular, tubular, membranous, crystalloid, or a combination. The physical form that the carotenoid is accumulated inside the different chromoplasts (dissolved in lipid, crystalline, or liquid-crystalline) can facilitate or hinder its bioaccessibility (Schweiggert & Carle, 2017).

In the human body, carotenoids have several functions related to the individual's health, mainly due to their antioxidant capacity and provitamin A activity. One of the main problems related to vitamin A deficiency is night blindness, which results in permanent loss of vision in severe cases. In children, the deficiency is related to an increase in infectious diseases and is of particular concern due to the increased requirement of this vitamin during growth. At this stage, the consequences of their deficiency can be severe, leading to stunted growth and vision loss (Sherwin et al., 2012; Bailey et al., 2015).

The World Health Organization (WHO) estimates that approximately 1/3 of children under five years had vitamin A deficiency (VAD) in 2009. In addition, 76% of children were at risk of vitamin A deficiency (considering countries with a gross domestic product (GDP) lower than US$15,000 in 2005) (World Health Organization, 2009). In Brazil, the prevalence of VAD is 6%, below the global levels (Universidade Federal do Rio de Janeiro, 2021).

Food introduction is a crucial period in a child's life and can modulate the immune and metabolic response throughout life, increasing the predisposition to diseases even in adulthood (Hanley et al., 2010; Uauy & Castillo, 2003). In addition, it is a period of high risk for developing nutritional deficiencies, depending on the quality of food offered to the child (World Health Organization, 2002).

In Europe and the United States, industrialized baby food represents most food offered to babies from 6 months (Briefel et al., 2004; Hurley & Black, 2010; Okesli et al., 2011; Mesch et al., 2014). They are found as ready-to-eat preparations that may or may not require heating before consumption. The high consumption is mainly due to the practicality of handling and transportation (Czajkowska-Mysłek & Leszczyńska, 2017).

Despite the high consumption, few studies have evaluated the composition of carotenoids in baby food and their bioaccessibility. Thus, this work aimed to determine the composition and *in vitro* bioaccessibility of the carotenoids in industrialized fruit-based baby food; correlating the bioaccessibility of carotenoids with the other components of the food matrix (proximate composition and mineral composition). In

addition, the amount of vitamin A in the fruit-based baby food was calculated considering the conversion of α-carotene, β-carotene, and β-cryptoxanthin, expressed as Retinol Activity Equivalent (RAE). The adequacy of vitamin A intake was compared concerning the DRI for children aged 6 to 12 months.

2 Material and Methods

2.1 Samples

Five flavors of industrialized fruit-based baby foods (puree) were purchased in São Paulo/Brazil, from November/2020 to January/2021. Three units of 120 g each from three distinct batches were acquired, totaling nine units of each flavor. Before analyses, all the units from the same flavor were homogenized, forming a composite sample for each flavor. Analyzes were performed in quadruplicate. Table 1 presents the ingredients of the fruit-based baby foods and the nutritional information declared on the labels.

2.2 Reagents

The reagents used in the proximate composition, *in vitro* digestion enzymes – (pepsin (P6887); lipase from *Rhizopus niveus* (62310); and pancreatin (P7545) and bovine bile (B3883)), enzymatic activity determination, and carotenoid extraction, and the assay kit (TDF-100A) for fiber analysis were acquired from Sigma Aldrich (St. Louis, MO, USA). The analytical grade solvents were purchased from Synth (SP, Brazil). HPLC grade hexane, methanol and methyl tert-butyl ether (MTBE) were purchased from J.T. Baker (Phillipsburg, NJ, USA). The water used in all analyzes was purified using the Milli-Q system (Billerica, MA, USA).

Standards of (all-*E*)-lutein (89% purity), (all-*E*)-β-carotene (87% purity), (all-*E*)-α-carotene (98% purity) and (all-*E*)-lycopene (92%), were obtained from petals of marigold flowers (*Tagetes erecta*), pumpkin (*Cucurbita ssp*.), carrot (*Daucus carota*), and papaya (*Carica papaya*), respectively, and purified in an open chromatographic column (MgO (Merck, Germany):Hyflo Super Cel® (1:1), 20 cm). Purity of the standards was determined by HPLC-DAD.

Table 1 Ingredients of the fruit-based baby food and nutritional information declared on the label

Flavor (120 g portion)	Grape and Banana (GB)	Assorted Fruits (AF)	Tropical Fruits (TF)	Apple, Mango, and Carrot (AMC)	Papaya and Orange (PO)
Energy (kcal)	83	51	64	56	53
Carbohydrate (g)	19	12	14	12	12
Protein (g)	1	0	1.2	0.7	0.6
Fat (g)	0	0	0	0	0
Fiber (g)	1.7	1.2	1.6	1.9	1.5
Sodium (mg)	0	0	0	0	0
Ingredients	Banana (43%); sweet potato (25%); grape (20%); water, lemon juice, and ascorbic acid.	Apple (71%); water; papaya (7%); orange juice (1.6%); lemon juice; and ascorbic acid.	Mango (29%); banana (27%); water; pineapple (16%); out flour; orange juice (0.9%); lemon juice; and ascorbic acid.	Mango (26%); apple (25%); carrot (20%); water; sweet potato (12%); orange juice; lemon juice; and ascorbic acid.	Papaya (45%); pear (19%); water; apple (15%); out flour; orange juice (1.5%); lemon juice; and ascorbic acid.

2.3 Proximate composition

Moisture and ash were determined by the AOAC methods (2003). Total nitrogen was determined by the micro-Kjeldahl method, and protein content was calculated using a factor of 6.25. Total lipids were cold extracted and gravimetrically determined by the Bligh & Dyer method (1959). The AOAC 985.29 method (2003) was applied for determining total dietary fiber.

2.4 Minerals

Acid mineralization was performed to assess the mineral content of baby food as described by Silva et al. (2020) with modifications. The samples were mineralized with diluted nitric acid (4 mL) in a digestion block for 2 h at 110 °C. Then, 2 mL of diluted nitric acid and 2 mL of hydrogen peroxide were added to the tubes and

mineralized for another 2 h at 130 °C. Subsequently, the digest was transferred to a 25 mL volumetric flask and filtered through quantitative filter paper.

Lanthanum oxide (0.5% v/v) was added to the samples for Ca and Mg, and cesium chloride (0.1% v/v) was added for K analysis to avoid interference. The minerals were evaluated using a flame atomic absorption spectrometer (FAAS), model AAnalyst 200, with a deuterium lamp for background radiation correction and hollow cathode lamps for the evaluated elements. Analytical curves were built using a 1000 mg/L stock solution of each element (Fe, Zn, Ca, Mg, Cu, Mn, and K) (Quemis, Brazil) ranging from 0.25 – 3.00 (Fe); 0.05 -0.5 (Zn); 0.5 - 5.0 (Ca); 0.025 - 0.25 (Mg); 0.05 - 1.45 (Cu); 0.05 – 0.6 (Mn), and 0.1 – 1.2 (K) mg/L. Dilutions were performed when necessary, and quantification was performed in triplicate.

2.5 Carotenoid extraction

The extraction of carotenoids from baby food was carried by the method described by Dhuique-Mayer et al. (2018) adapted for this matrix. To avoid waste of organic solvent, the mass of the sample was defined in preliminary tests according to the predicted amount of carotenoids: 1 g (TF, AMC, and PO); 2 g (AF), or 5 g (GB). Three extractions were performed using a hexane/ethanol solution (3:4, v/v) and showed to be sufficient for exhaustively extracting the carotenoids. After each extraction, the organic phase was transferred to a separatory funnel containing water and hexane. The aqueous phase was discarded, and the hexane layer evaporated on a rotary evaporator (T < 35 °C).

The extract containing the carotenoids was then subjected to saponification with 10% KOH methanol (w/v) solution overnight under continuous stirring, following by washing for alkali removal. The saponified extracts were concentrated on a rotary evaporator and stored in an amber flask under nitrogen atmosphere at -18 °C until injection into the HPLC (see 2.7). The entire procedure was carried out under dim light to avoid carotenoid degradation.

2.6 *In vitro* bioaccessibility

Before the *in vitro* digestion, the enzymes' activities were determined according to the methods described by Minekus et al. (2014) in the supplementary material.

The Infogest 2.0 protocol adapted for lipophilic compounds was used for *in vitro* digestion (Brodkorb et al., 2019; Marques et al., 2021). Briefly, the sample homogenized with simulated salivary fluid (SSF) was subjected to the gastric phase, in which samples were incubated at 37 °C with simulated gastric fluid (SGF), lipase 60 U/mL, pepsin 2,000 U/mL, and 0.3 M $CaCl_2$ (pH adjusted to 3.0 with 5 M HCl) for 2 h under continuous agitation. Finally, in the intestinal phase, after the addition of simulated intestinal fluid (SIF), 10 mM bile, 100 U/mL pancreatin (in trypsin activity), 0.3 M $CaCl_2$, the pH was adjusted to 7.0 with 5 M NaOH, and the mixture was incubated at 37 °C for 2 h under continuous agitation.

The separation of the mixed micelles and the extraction of carotenoids from the aqueous phase containing the micelles was carried out according to the method described by Rodrigues et al. (2016) The chyme was centrifuged (4 °C, 20,000 g for 5 min), and the supernatant containing the micelles with carotenoids was transferred to Teflon centrifuge tubes. Carotenoids were extracted exhaustively using diethyl ether by vortexing. Ten milliliters of 10% NaCl was added to the ethereal extract, and the mixture was centrifuged at 4°C, 20,000 g for 5 min. The process was repeated until the supernatant was colorless. The extracts were combined, and the diethyl ether was evaporated in a rotary evaporator until dryness. The dry extract was stored under nitrogen atmosphere until analysis by HPLC-DAD (see 2.7).

Carotenoid bioaccessibility (% of carotenoids of the initial sample that was present in the micellar fraction after digestion) was calculated by the quotient between the concentration of carotenoids in the micellar phase and the concentration of carotenoids in the sample, according to the formula:

$$\text{Bioaccessibility (\%)} = \left(\frac{[\text{carotenoids}]_{\text{micelar phase}}}{[\text{carotenoids}]_{\text{sample}}} \right) \text{x } 100$$

2.7 Determination of carotenoids by HPLC-DAD-MS

The carotenoid analysis was performed by high-performance liquid chromatography (HPLC) (model 1200 Series, Agilent) coupled to a diode array

detector (DAD) (model G1315D, Agilent). Separation was achieved on a C_{30} YMC column (3 µm, 250 x 4.6 mm id) (Waters, USA) at 29 °C using a linear methanol/MTBE gradient from 95:5 to 70:30 in 30 min to 50:50 in 20 min and maintaining this proportion for 35 min, with a flow rate of 0.9 mL/min. Data were acquired between 280 and 600 nm, and the chromatograms were processed at 450 nm, 285 nm (phytoene) and 347 nm (phytofluene).

The identification of carotenoids was performed using the combination of the following characteristics: elution order on C_{30} column and UV-vis (λ_{max}, spectral fine structure, and *cis* peak intensity, when applied) and mass spectrum features, compared with standards or literature data (De Rosso and Mercadante, 2007; Rodrigues et al., 2016, 2019; Chisté et al., 2021). The identity of carotenoids was confirmed by mass spectrometry in another apparatus by using the same chromatographic conditions. In this case, the HPLC system was connected in series to a mass spectrometer (LCMS-2020, Shimadzu - Kyoto, Japan) equipped with a single quadrupole mass analyzer and an atmospheric pressure chemical ionization (APCI) source operating at positive mode. The system operated with an interface voltage of 4.5 kV and an interface temperature of 400 °C. Drying and nebulizing gas flows were set at 5 L/min and 2 L/min, respectively. Full scan MS spectra were acquired in the range of *m/z* from 100 to 601, in a scan rate of 1667 u/s.

Carotenoids were quantified by HPLC-DAD using seven-point analytical curves (constructed in triplicate) for (all-*E*)-lutein (1.9−21.3 µg/mL), all-*E*-α-carotene (7.8−19.6 µg/mL), all-*E*-β-carotene (3.0−13.9 µg/mL), and all-*E*-lycopene (0.05 – 0.7 µg/mL). The limits of detection (LOD) and quantification (LOQ) were, respectively, 0.02 and 0.07 µg/mL for (all-*E*)-β-carotene, 0.013 and 0.044 µg/mL for (all-*E*)-α-carotene, 0.017 and 0.056 µg/mL for (all-*E*)-lutein and 0.07 and 0.24 µg/mL for (all-*E*)-lycopene. The other carotenoids (9-*Z*)-β-carotene, (13-*Z*)-β-carotene, and (all-*E*)-β-cryptoxanthin were calculated as (all-*E*)-β-carotene equivalents.

2.8 Conversion and suitability for vitamin A

The conversion of provitamin A carotenoids into retinol activity equivalents (RAE) to calculate the vitamin A value was performed using the conversion factors of the Institute of Medicine (IOM) of the United States (IOM, 2001), i.e., 1 RAE (1 µg retinol) = 12 µg β-carotene, 24 µg of α-carotene, and 24 µg of β-cryptoxanthin. In

addition, the adequacy of vitamin A intake from the baby food was calculated in relation to the DRI (Dietary Reference Intake) for children aged 6 to 12 months, which is 500 RAE/day (FAO/WHO, 2001).

2.9 Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA). Means were compared using Tukey's test at 95% significance level. Pearson's correlation test was used for correlation analysis at 95% significance level. Statistical analyzes were performed using the Past 4.07b software (Oyvind Hammer, University of Oslo, Norway).

3 Results and discussion

3.1 Proximate composition

The results of the proximate composition are shown in Table 2 and include moisture, ash, proteins, lipids, and fibers, besides the energy expressed as calories.

Table 2 Proximate composition of industrialized fruit-based baby foods

	Moisture (%)	Ash (g/100g)	Protein (g/100g)	Fat (g/100g)	Fiber (g/100g)	Carbohydrate (g/100g)	Calories (kcal/100g)
GB	81.44 ± 0.140^{d}	1.61 ± 0.042^{a}	9.66 ± 0.521^{b}	0.93 ± 0.041^{d}	8.67 ± 0.097^{d}	79.13	67.51
AF	87.42 ± 0.269^{b}	1.02 ± 0.057^{b}	5.04 ± 0.186^{c}	0.96 ± 0.035^{d}	9.76 ± 0.298^{c}	83.22	44.86
TF	84.87 ± 0.204^{c}	1.87 ± 0.053^{a}	2.81 ± 0.04^{e}	2.74 ± 0.112^{a}	7.71 ± 0.293^{e}	84.87	57.46
AMC	86.84 ± 0.306^{b}	1.01 ± 0.043^{b}	14.89 ± 0.571^{a}	1.65 ± 0.071^{c}	10.76 ± 0.201^{b}	71.70	47.48
PO	88.12 ± 0.244^{a}	0.67 ± 0.050^{c}	4.07 ± 0.080^{d}	2.08 ± 0.064^{b}	12.21 ± 0.161^{a}	80.98	42.62

Values are mean ± standard deviation (n=4). Results are expressed on a dry basis. Carbohydrates were calculated by difference. Different letters in the same column indicate a significant difference among samples ($p < 0.05$). GB: Grape and Banana; AF: Assorted Fruits;

There was a significant difference among all the samples ($p<0.05$) for the protein content, with means varying between 2.1 and 14.89 g/100g (dry basis, d.b.) for TF and AMC, respectively. Zand et al. (2015) analyzed meat and vegetable porridges and found mean values of 3.2 g/100 g w.b. in meat and 2.3 g/100 g w.b. in vegetable porridges. For comparison purposes, considering the results expressed in wet basis (w.b.), the protein content ranged from 0.42 g/100 g (TF) to 1.96 g/100 g (AMC). These values are close to those obtained by Zand et al. (2015) for the vegetable porridges, while Randhawa (2012) reported similar results for industrialized fruit-based baby foods, ranging from 0.09 to 2.03 g/100 g of protein.

The lipid contents of GB and AF were the same, 0.93 g and 0.96 g lipids/100 g d.b. ($p>0.05$), respectively; while in the other samples, lipid contents varied from 1.65 (AMC) to 2.74 g/100 g d.b. (TF). Considering the lipid content expressed in f.w, the values ranged from 0.12 g/100 g (AF) to 0.41 g/100 g (TF). Similar values can be found in the literature for vegetable-based baby foods ranging from 0 to 0.6 g/100 g (w.b.) (Randhawa, 2012; Zand et al., 2015). Zand et al. (2015) found a higher lipid content for plant-based baby foods, with an average of 2.5 g/100 g because they were formulated with vegetables containing more lipids, such as corn and tomato, and addition of vegetable oil or milk.

Carbohydrates (calculated by difference) ranged from 9.43 in AMC to 14.7 g/100 g w.b. in GB. Similar values were found by Zand et al. (2015) with 7.4 g/100 g w.b. for plant-based and 8.1 g/100 g w.b. for meat-based samples. Randhawa (2012) also reported values close to those found in this study, varying between 7.4 to 16.6 g/100 g w.b. in fruit-based samples.

Fibers play a fundamental role in intestinal health; despite this, there is no recommendation for fiber intake in the baby's first year of life. The DRI starts at one year of life with a recommendation for a total dietary fiber intake of 19 g/day.25 In this study, total dietary fibers were evaluated, and a significant difference was observed among all samples when considering the values on fresh weight. The results ranged from 1.39 in TF to 1.90 g/100 g w.b. in PO, and Zand et al. (2015) found similar values of 1 g/100 g w.b. in plant-based and 1.3 g/100 g w.b. in meat-based baby food. Brooks et al. (2006) also found similar results in fruit-based baby foods, with total dietary fiber ranging from 0.23 to 2.9 g/100 g w.b.

TF showed the highest ash content among the samples (1.87 g ash/100 g d.b.), whereas the PO had the lowest one (0.67 g ash/100 g d.b.). The ash content in AF and

AMC was the same (1.02 and 1.01 g ash/100 g d.b.). The ash content reflects the quantity of inorganic micronutrients in the sample. To better understand this result, individual minerals were also analyzed, and the results are presented and discussed in 3.2.

The nutrient content in one portion of baby food (120 g) was calculated to allow the comparison with the nutritional information on the label of the industrialized baby food (Table 3).

Table 3 Comparison between the experimental data and the composition declared on the baby food label per portion (120g)

	Protein (g/120 g)			**Fiber (g/120 g)**			**Carbohydrate (g/120g)**		
	Experimental	Label	Variation (%)	Experimental	Label	Variation (%)	Experimental	Label	Variation (%)
GB	2.15	1	115.25	1.93	1.7	13.65	17.6	19	-7.34
AF	0.72	0		1.7	1.2	42	12.41	12	3.46
TF	0.51	1.2	-57.44	1.74	1.6	8.75	15.6	14	11.4
AMC	2.35	0.7	235.92	1.39	1.9	-26.74	11.32	12	-5.67
PO	0.58	0.6	-3.33	1.48	1.5	-1.6	11.54	12	-3.84

GB: Grape and banana; AF: Assorted fruits; TF: Tropical fruits; AMC: Apple, mango, and carrot; PO: Papaya and orange.

Protein content showed an expressive variation, reaching up to 235% of the label value (AMC), followed by the fibers, with a maximum variation of 42% in AS. The highest variation in carbohydrate content was 11.4% in TF, which is within the ± 20% variation allowed by Brazilian legislation (BRASIL, 2003b). No comparison was established for lipids because all the sample labels declared that they did not contain lipid, this is probably because the Brazilian legislation allows the rounding of values up to 0.5 to “0” (BRASIL, 2003b). The same occurred for protein in AF.

Interestingly, the variation between calories calculated with experimental data and the label values did not show a considerable variation; the maximum value found for TF (7.73%). This fact can be explained because carbohydrates were calculated by the difference (100% - the sum of lipids, water, ash, and proteins). In fact, the most significant differences were observed in the protein content, which consequently changed the relative amount of carbohydrates estimated by difference. Since these

two macronutrients have the same multiplication factor for calculating the calories (4 kcal/g), the total calories should not be affected.

Brazilian legislation (BRASIL, 2003a), for example, does not oblige the industry to analyze proximate composition for the nutritional information on the label; they can use data from nutritional composition tables to calculate the nutrient content. Therefore, labels may not accurately reflect the nutritional content of the product since fruits and vegetables can have enormous composition variation due to edaphoclimatic conditions. Thus, a substantial error can be triggered when considering the macronutrient content based on the label, underestimating or overestimating the accepted values of ± 20%.

The difference between the label and the actual content of nutrients can be particularly important in situations of food restriction. For instance, there are situations where it is necessary to know the exact content of proteins in foods, such as phenylketonuria or kidney disease, because the amount of protein in the diet must be tightly regulated (King & Levey, 1993; Firman et al., 2022). In these two cases, if the baby food labels do not provide the correct information, it may impair the correct calculation of the diet and possibly, compromise the health of these individuals.

The nutrient variations reported in this study may occur due to the composition of each baby food, the amount of fruit and vegetables, the different cultivars used in the production of baby food, as well as factors involved in planting such as soil, climate, degree of maturation of the fruit, among others (Randhawa, 2012).

3.2 Minerals

The results of the seven analyzed minerals are shown in Table 4. Mineral composition varied significantly in the evaluated samples. Potassium was the most abundant mineral, ranging from 712.9 mg/100 g (AF) to 1142.4 mg/100 g (GB) d.b. Copper and zinc showed the lowest levels, ranging from 0.18 to 0.40 mg/100 g and not detected to 0.92 mg/100 g d.b., respectively.

The variations in mineral concentrations among baby foods were due to their composition. As well as proximate composition, the mineral composition varies according to the fruits, their variety, and planting conditions. AF, composed of 71% apple, had the lowest mineral content. The data are justified since the apple contains lower amounts of minerals when compared to other fruits (Priyadarshani, 2017).

Table 4 Concentration of minerals in fruit-based baby foods

	Minerals (mg/100g d.b.)						
	Fe	**Zn**	**Ca**	**Mg**	**Cu**	**Mn**	**K**
GB	1.4 ± 0.08^{c}	0.4 ± 0.01^{c}	21.7 ± 0.55^{d}	92.3 ± 0.97^{b}	0.40 ± 0.006^{a}	3.4 ± 0.04^{b}	1142.4 ± 7.22^{a}
AF	0.8 ± 0.03^{d}	Nd	32.0 ± 1.52^{c}	40.0 ± 1.08^{d}	0.18 ± 0.007^{c}	0.3 ± 0.03^{e}	712.9 ± 43.86^{d}
TF	1.9 ± 0.11^{a}	0.9 ± 0.06^{a}	32.5 ± 1.48^{c}	106.4 ± 7.83^{a}	0.36 ± 0.005^{b}	4.8 ± 0.03^{a}	986.6 ± 55.27^{bc}
AMC	1.8 ±0.17^{ab}	0.5 ± 0.06^{bc}	80.9 ± 1.09^{a}	74.6 ± 0.9^{c}	0.36 ± 0.002^{b}	1.7 ± 0.01^{c}	1046.1 ± 53.99^{ab}
PO	1.5 ± 0.03^{bc}	0.6 ± 0.00^{b}	68.3 ± 3.61^{b}	68.4 ± 1.06^{c}	0.34 ± 0.020^{b}	1.1 ± 0.08^{d}	926.7 ± 42.51^{c}

Values are mean ± standard deviation. Results are expressed in d.b. (dry basis). nd: not detected. Different letters in the same column indicate significant difference among samples (p<0.05). GB: Grape and banana; AF: Assorted fruits; TF: Tropical fruits; AMC: Apple, mango, and carrot; PO: Papaya and orange.

Concerning potassium, for example, this was the only baby food with a content lower than 900 mg/100g d.b.

On the other hand, baby food with a greater variety of fruits (TF and AMC) had the highest mineral content, and both contained mango as an ingredient. In addition, TF, composed of mango, banana, and pineapple, had the highest Fe, Zn, Mg, and Mn levels. The mineral content of these fruits, depending on the variety and planting conditions, can be higher when compared to apples, for example (Priyadarshani, 2017).

The findings reinforce the importance of a varied diet to obtain a greater quantity and quality of nutrients. As shown, the highest contents were verified in the baby foods with a combination of different fruits. The same variation was observed in the evaluation of the proximate composition of baby food.

3.3 Carotenoid composition and *in vitro* bioaccessibility

Table 5 presents the carotenoid profile, and Table 6 shows the carotenoid content and *in vitro* bioaccessibility of the provitamin A carotenoids in fruit-based baby food. The carotenoid chromatograms are presented in Supplementary Material S1.

(All-*E*)-β-carotene is the carotenoid with the highest provitamin A activity and was found in all the analyzed samples. In TF, it accounted for 41.4% of the total

Table 5 Chromatographic and spectroscopic characteristics of carotenoids from fruit-based baby foods determined by HPLC-DAD-MS.

Peak[a]	Carotenoid	Retention Time (min)[b]	λ_{max} (nm)[c]			%III/II	$\%A_b/A_{II}$	$[M+H]^+$ (*m/z*)[d]
1	(all-*E*)-phytoene	18.5	275	288	300	0		545
2	(all-*E*)-α-cryptoxanthin	20.1	420	445	475	66		553
3	(*Z*)-phytofluene	21.1 - 21.2	330	347	359	62	46	543
4	5,6-epoxy-β-carotene	23.3 - 23.4	420	447	472	50		553
5	(all-*E*)-β-cryptoxanthin	23.8	425	448	476	33		553
6	(all-*E*)-phytofluene	23.8 – 24.1	330	348	368	91		543
7	(13-*Z*) or (15-*Z*)-α-carotene	24.9 – 25.0	418	440	467	31	43	537
8	(13-*Z*)-β-carotene	27.0-27.3	415	442	471	20	56	537
9	(all-*E*)-α-carotene	29.7	420	445	473	60		537
10	(all-*E*)-β-carotene	33.6 – 33.9	421	452	478	30		537
11	(9-*Z*)-β-carotene	35.4– 35.7	412	445	472	20	15	537
12	not identified	40.3	418	450	480	20		nd[e]
13	di-(*Z*)-γ-carotene	43.4 – 43.5	422	451	479	33	18	537
14	γ-carotene 1′,2′-epoxide	49.1- 49.3	435	462	495	53		537
15	(9-*Z*)-γ-carotene	56.2– 56.4	440	465	497	75	12	537
16	(all-*E)*-lycopene	66.6 – 67.7		445	470	502	78	nd

[a] Peak numbered according to Supplementary Material S1. [b] Retention time on C_{30} YMC column. [c] Gradient of methanol/MTBE. Data acquired between 280 and 600 nm. [d] Protonated molecule. [e] not detected.

carotenoid content. Its isomer (9-*Z*)-β-carotene was found in AF, TF, and AMC, while (13-*Z*)-β-carotene was found in all the samples.

(All-*E*)-β-cryptoxanthin was present in three samples, GB and the other two containing papaya (PO and AF). (All-*E*)-α-carotene was detected in the baby foods containing banana (GB and TF) and sweet potato (GB and AMC).

In the literature, the carotenoid contents of baby foods present a wide variation, with the results of this study being intermediate values. Dhuique-Mayer et al. (2018) reported an average concentration of β-carotene of 54.8 µg/g in an industrialized baby food made from β-carotene enriched sweet potatoes. Doka et al. (2014) found a variation from 1.8 mg/100g to 3.6 mg/100g of total carotenoids in fruit-based baby foods. The concentration of β-carotene and β-cryptoxanthin in baby food ranged from

Table 6 Concentration and in vitro bioaccessibility of provitamin A carotenoids in fruit-based baby foods.

	GB		AF		TF		AMC		PO	
Carotenoids	**Concentration (µg/g)[a]**	**Bioaccessibility (%)**	**Concentration (µg/g)**	**Bioaccessibility (%)**	**Concentration (µg/g)**	**Bioaccessibility (%)**	**Concentration (µg/g)**	**Bioaccessibility (%)**	**Concentration (µg/g)**	**Bioaccessibility (%)**
(all-*E*)-β-cryptoxanthin	0.04 ± 0.00	0[c]	0.10 ± 0.00	4 ± 0.04	nd		nd		0.47 ± 0.01	7 ± 0.14
(13-*Z*)-β-carotene	0.04 ± 0.00	0	0.08 ± 0.00	5 ± 0.03	0.18 ± 0.01	10 ± 0.28	0.88 ± 0.01	10 ± 0.23	0.40 ± 0.01	8 ± 0.09
(all-*E*)-α-carotene	0.01 ± 0.00	5 ± 0.23	nd		0.07 ± 0.00	8 ± 0.45	0.46 ± 0.01	14 ± 0.76	nd	
(all-*E*)-β-carotene	0.04 ± 0.00	5 ± 0.18	0.10 ± 0.00	5 ± 0.24	0.41 ± 0.01	10 ± 0.6	1.23 ± 0.07	24 ± 0.96	0.44 ± 0.01	9 ± 0.09
(9-*Z*)-β-carotene	nd[c]		0.08 ± 0.00	5 ± 0.02	0.18 ± 0.00	11 ± 0.42	0.85 ± 0.03	10 ± 0.12	nd	
(9-*Z*)-γ-carotene	nd			nd		nd		nd	0.39 ± 0.012	9 ± 0.11
γ-carotene 1′,2′-epoxide	nd		0.09 ± 0.003	5 ± 0.06		nd		nd	0,43 ± 0,013	9 ± 0.41
Total carotenoid[b]	0.27 ± 0.00	1 ± 0.04	0.62 ± 0.01	6 ± 0.06	0.99 ± 0.01	15 ± 0.42	6.78 ± 0.09	11 ± 0.32	2.55 ± 0.07	14 ± 0.38

Values are mean ± standard deviation. [a] Carotenoid concentration before *in vitro* digestion. [b] Total carotenoid content represents the sum of individual contents of all the carotenoids in Table 5. [c] When the carotenoid concentration in the micellar phase was below the LOD, the bioaccessibility was considered zero. nd: not detected. GB: Grape and Banana; AF: Assorted Fruits; TF: Tropical Fruits;

6.61 µg/100 g to 10.9 µg/100 g for β-carotene and 0.75µg/100g to 1.79 µg/100g for β-cryptoxanthin in another study (Jiwan et al., 2010). These results demonstrate the importance of individually evaluating fruit-based baby food, as there is a wide variation in the concentration of carotenoids.

Some carotenoids were only detected in samples before *in vitro* digestion, while others only after this process. In addition to these facts, the fruit-based baby foods evaluated were poor in lutein and zeaxanthin, important carotenoids for the baby's eye health and cognitive development (Sherwin et al., 2012; Bailey et al., 2015) Thus, we decided to focus only on calculating and discussing the bioaccessibility of provitamin A carotenoids (Table 6).

Provitamin A carotenoids (Figure 1), the ones possessing a non-substituted β-ring terminal group, can be converted into retinol by the action of the enzyme β-carotene-15,15'-oxygenase 1 (BCO1) and 9',10' oxygenase 2 (BCO2) (Catharine Ross & Moran, 2020). (All-*E*)-β-carotene is capable of being cleaved into two molecules of

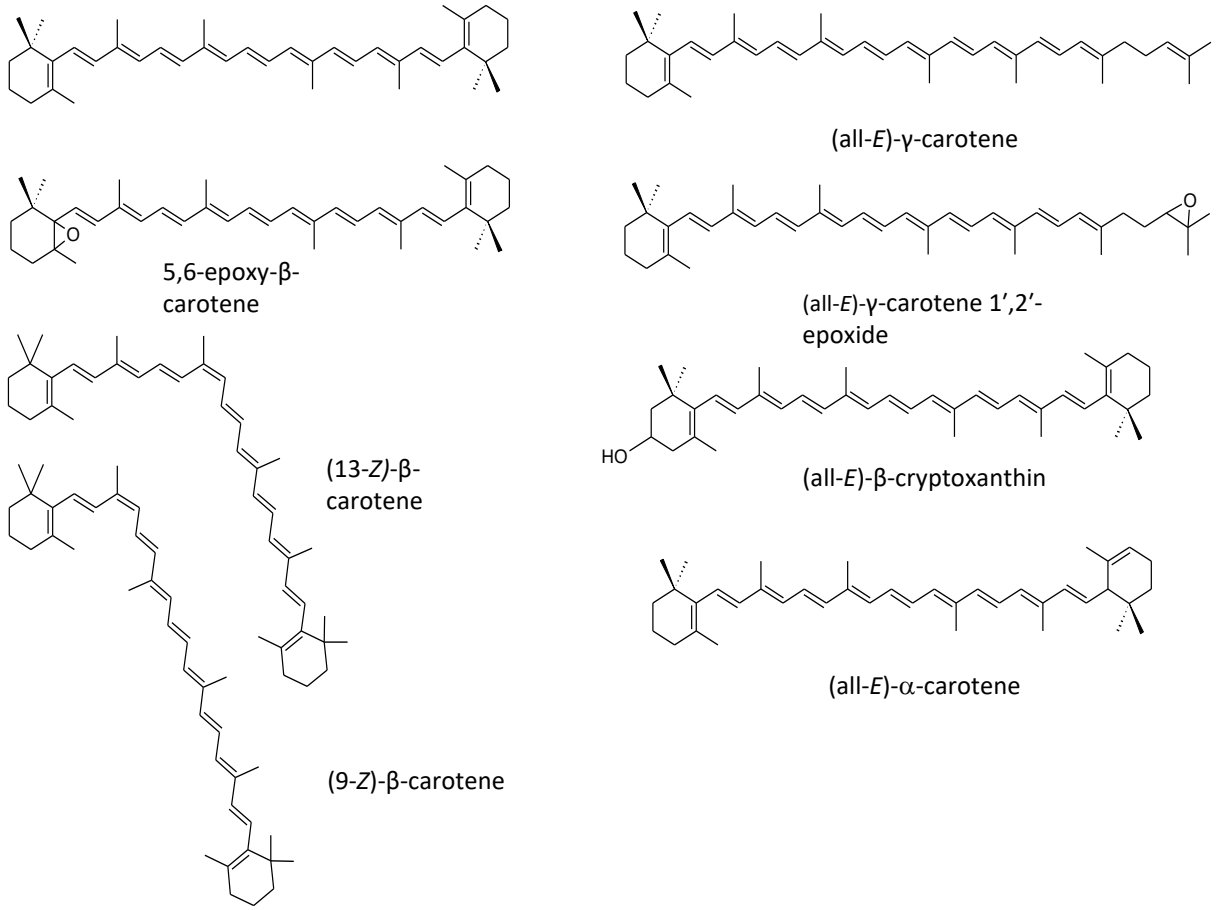


Fig. 1 Carotenoids with provitamin A activity. The central enzymatic cleavage of (all-*E*)-β-carotene can generate two retinol molecules, all other provitamin A carotenoids can only yield one molecule.

retinal, which are reduced to retinol at the point where both the provitamin A and the vitamin A pathways convene; the carotenoids (all-*E*)-α-carotene, (all-*E*)-α-cryptoxanthin, (all-*E*)-β-cryptoxanthin, and (all-*E*)-γ-carotene result in only one molecule of retinal. Among these provitamin A carotenoids, the most consumed in the diet are (all-*E*)-α and (all-*E*)-β-carotene, followed by (all-*E*)-β-cryptoxanthin (Bohn, 2012).

Table 6 shows the bioaccessibility of individual carotenoids. The extensive range of bioaccessibility values reflects the variability of the matrix. The chromatograms of the carotenoids after *in vitro* digestion can be found in Supplementary Material S2.

The bioaccessibility of (all-*E*)-β-cryptoxanthin was significantly lower ($p<0.05$) than that of (all-*E*)-β-carotene (AF and PO). This behavior is inconsistent with what was previously reported in the literature, in which β-cryptoxanthin, a xanthophyll, showed higher bioaccessibility than β-carotene, a carotene, in papaya and tomato

(Schweiggert et al., 2012). This difference is probably related to the composition of baby food and the interaction between the matrix components, as in this study, unlike the previously mentioned one, foods were not evaluated separately.

Bioaccessibility is affected by different factors, including heat treatment. Baby food undergoes thermal treatment during its production in the industry (sterilization and pasteurization), which leads to the loss of food matrix original structure and the rupture of the cell walls, increasing the release of carotenoids from the chromoplasts and, consequently, increasing their bioaccessibility (Priyadarshani, 2017).

(All-*E*)-β-carotene showed the highest bioaccessibility in AMC (24%) baby food, sample which also presented the highest amount of (all-*E*)-β-carotene before digestion (1.23 µg/g). A strong positive correlation (0.99) between the amount of (all-*E*)-β-carotene in the baby food and bioaccessibility was observed (Figure 2). This result agrees with the literature, showing that the greater the amount of β-carotene in the food matrix, the greater its bioaccessibility (Priyadarshani, 2017).

Although AMC had the highest β-carotene bioaccessibility, it was not the baby food with the highest total carotenoid bioaccessibility. AMC contains carrot in its composition, in which carotenoids are deposited in the solid-crystalline form, which may reduce the effectiveness of carotenoid release and micellarization (Schweiggert et al., 2012).

TF and PO baby foods showed the highest total carotenoid bioaccessibility, 15.53% and 14.37%, respectively. These samples had a higher lipid content, and a strong positive correlation was observed between the lipid content and the bioaccessibility of total carotenoids (Figure 2), even with lipids in small amounts, 2.08 g/100 g d.w. (PO) and 2 .74 g/100 g d.w. (TF). Lipids are known to affect the bioaccessibility of carotenoids. This can occur by stimulating the secretion of bile salts, increasing the hydrophobicity of the medium, and promoting the formation of micelles (Priyadarshani, 2017). In our study, the amount of bile was fixed, and the lipids probably influenced the carotenoids' bioaccessibility by increasing the medium hydrophobicity or helping the formation of the mixed micelles.

The isomers (9-*Z*)-β-carotene and (13-*Z*)-β-carotene also showed a strong positive correlation between their bioaccessibility and lipid content (Figure 2), which can be related to the easier solubilization of the *cis*-isomers in lipids when compared to (all-*E*)-β-carotene (Ferruzzi et al., 2006).

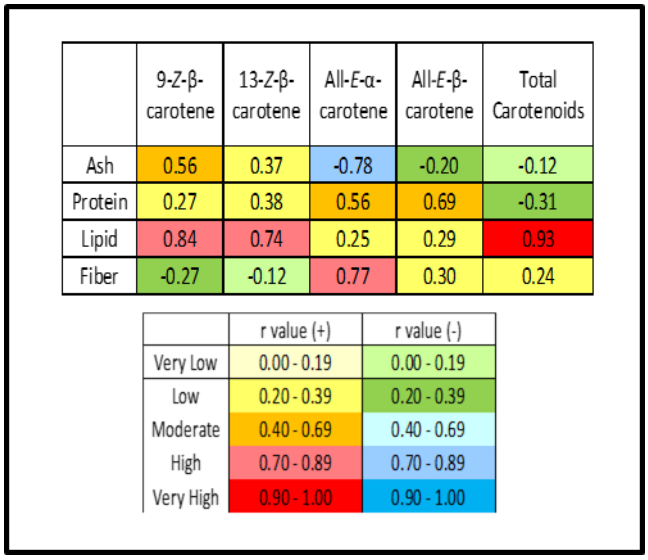


Fig. 2 Pearson correlation between bioaccessibility of individual and total carotenoids and proximate composition of baby food. Pearson's r values are indicated according to the color legend ($p<0.05\%$).

Another factor that influences micellarization is the amount and type of carotenoids in the sample. Overall, (*Z*)-isomers of carotenes are micellarized more efficiently than (all-*E*) isomers (Ferruzzi et al., 2006). Furthermore, a higher concentration of (9-*Z*)-β-carotene was correlated to a higher total bioaccessibility of carotenes ((all-*E*)- and (9-*Z*)- isomers) (Levin & Mokady, 1995). Considering only the carotenes (α- and β-carotene) (Table 7), the results agree with the literature. In samples with a higher concentration of (*Z*)-isomers, a greater total bioaccessibility of carotenes was observed in general. Furthermore, it is interesting to note that the ML sample, which contains only (13-*Z*)-β-carotene, showed greater bioaccessibility than the AF sample, which contains both isomers, but in smaller amounts. This data demonstrates that the total amount of (*Z*)-isomers may be more important for increasing the bioaccessibility of carotenes than the presence of one of the specific isomers.

Fibers affect the bioaccessibility of carotenoids mainly by increasing the viscosity of the medium and thus inhibiting the action of bile salts and lipases, preventing carotenoids from being released from the food matrix and micellarized (Shilpa et al., 2020). Our study did not find a significant correlation between fiber content and carotenoid bioaccessibility, except for (all-*E*)-α-carotene, which showed a strong positive correlation with fibers (Figure 2). This could probably be explained due to the industrial processing of baby food. The fruits are ground, homogenized, and heat treated. These operations modify the structure of the fibers and may have contributed

Table 7 Concentration of (9-Z) and (13-Z)-β-carotene and total in vitro bioaccessibility of carotenes ((all-E)-α-carotene, + (all-E)- and (Z)-isomers of β-carotene).

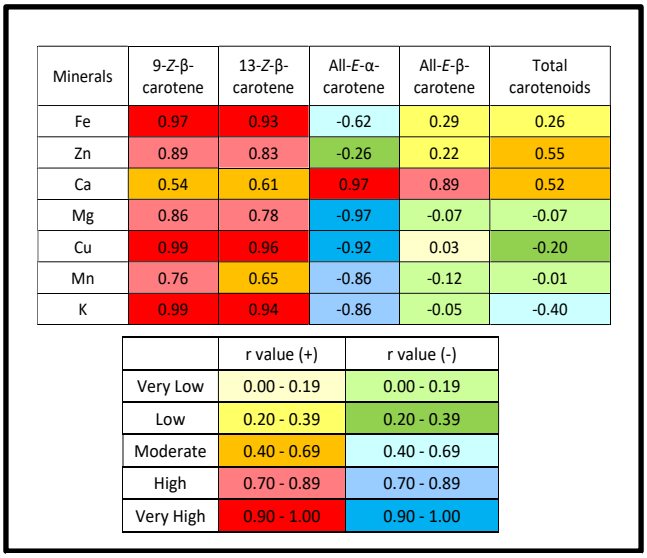
	(9-*Z*)-β-carotene (µg/g)	**(13-*Z*)-β-carotene (µg/g)**	**Bioaccessibility of carotenes (%)**
GB	0	0	1 ± 0.04
AF	0.08 ± 0.001	0.08 ± 0.002	5 ± 0.09
PO	0	0.40 ± 0.012	9 ± 0.09
TF	0.18 ± 0.008	0.18 ± 0.004	10 ± 0.44
AMC	0.85 ± 0.034	0.88 ± 0.015	18 ± 0.52

Values are mean ± standard deviation. GB: Grape and banana; AF: Assorted fruits; PO: Papaya and orange, TF: Tropical fruits; AMC: Apple, mango, and carrot

to reducing their action on the bioaccessibility of carotenoids (Elleuch et al., 2011). Surprisingly, a higher amount of fibers favored the bioaccessibility of α-carotene, possibly by altering solubility and interaction of α-carotene, which has a ring in the anterior plane (ε ring), with the fibers. However, the literature regarding the impact of fibers on α-carotene bioaccessibility is scarce, and future studies are needed to verify the behavior and possible interaction of these two compounds during processing and digestion.

(All-*E*)-α-carotene also showed a strong negative correlation with ash content. For the other carotenoids, no correlation with ash was observed. Ash corresponds to the inorganic compounds in the sample, so seven minerals were determined to understand better how ash affected the carotenoid bioaccessibility. The correlation of minerals with the carotenoid bioaccessibility can be seen in the Figure 3.

Divalent minerals negatively affect the micellarization of carotenoids, mainly by precipitating fatty acids and bile salts, preventing the solubilization of carotenoids, altering the viscosity of the medium, and increasing surface tension. Minerals can also reduce the size and quantity of micelles, thus affecting the more non-polar carotenoids, such as (all-*E*)-β-carotene (Biehler et al., 2011; Corte-Real et al., 2016, 2018) However, minerals must be consumed in high doses in a single meal, achieved only by mineral supplementation, to have this action on carotenoids, n. When in the food



Minerals	9-*Z*-β-carotene	13-*Z*-β-carotene	All-*E*-α-carotene	All-*E*-β-carotene	Total carotenoids
Fe	0.97	0.93	-0.62	0.29	0.26
Zn	0.89	0.83	-0.26	0.22	0.55
Ca	0.54	0.61	0.97	0.89	0.52
Mg	0.86	0.78	-0.97	-0.07	-0.07
Cu	0.99	0.96	-0.92	0.03	-0.20
Mn	0.76	0.65	-0.86	-0.12	-0.01
K	0.99	0.94	-0.86	-0.05	-0.40

	r value (+)	r value (-)
Very Low	0.00 - 0.19	0.00 - 0.19
Low	0.20 - 0.39	0.20 - 0.39
Moderate	0.40 - 0.69	0.40 - 0.69
High	0.70 - 0.89	0.70 - 0.89
Very High	0.90 - 1.00	0.90 - 1.00

Fig. 3 Pearson correlation between bioaccessibility of individual and total carotenoids and minerals composition of baby food. Pearson's r values are indicated according to the color legend ($p < 0.05\%$).

matrix, minerals can interact with other chelating compounds such as organic acids (phytic and oxalic acid, for example), phosphate, and fiber, which also reduces their effect on carotenoids and other lipophilic compounds (Corte-Real et al., 2018).

Minerals in low concentrations, such as those found in food, increase the solubilization of carotenoids, even divalent minerals, especially in processed foods such as juices. They act as a cofactor for lipases and help precipitate fatty acids from the surface of the lipid droplet, facilitating lipolysis (Hu et al., 2010; Corte-Real et al., 2017). A very strong positive correlation between calcium and the bioaccessibility of (all-*E*)-α-carotene and a strong positive correlation between calcium and the bioaccessibility of (all-*E*)-β-carotene were observed (Figure 3), demonstrating the effect of low mineral concentration in the micellarization of carotenoids.

The minerals showed a correlation ranging from moderate (Ca) to strong (Zn, Mg, and Mn) and very strong (Fe, Cu, and K) with the bioaccessibility of the *cis* isomers of β-carotene. This probably occurred due to the increase in lipolysis caused by these minerals present in small amounts in the food matrix and because the *cis* isomers are more soluble in lipids.

On the contrary, (all-*E*)-α-carotene showed a strong negative correlation with Mn and K and a very strong negative correlation with Mg and Cu. Although other nutrients could also have contributed to the low solubilization of (all-*E*)-α-carotene in

lipids, such as fibers, minerals might have influenced the binding forces and interactions.

3.4 Contribution of industrialized baby food to vitamin A intake

Table 8 shows the RAE (Retinol Activity Equivalent) values calculated for the fruit-based baby. The DRI for vitamin A is 500 RAE/day for children aged 6 to 12 months (FAO/WHO, 2001). The ingestion of the analyzed fruit-based baby foods contributes very little to the vitamin A intake, with the highest contribution found in AMC with an adequacy of 2.9% of the DRI and the lowest in the GB with 0.13% of the DRI. Dhuique-Mayer et al. (2018) found adequacy values above 100%, but they analyzed industrialized baby food based on sweet potatoes fortified with β-carotene.

Table 8 RAE in baby food (120 g portion) and adequacy to the DRI for children aged 6 to 12 months.

	RAE in the portion (120 g)	Adequate intake (%)
GB	1	0.13
AF	1	0.29
TF	4	0.88
PO	5	1.01
AMC	15	2.9

GB: Grape and banana; AF: Assorted fruits; PO: Papaya and orange, TF: Tropical fruits; AMC: Apple, mango, and carrot.

Breastfeeding is considered the gold standard food for infant feeding and should be maintained for up to two years of life or more (World Health Organization, 2003; Agostoni et al., 2009; Lessen & Kavanagh, 2015). According to the WHO (2003), food introduction should be complementary to breastfeeding and include two to three meals a day (one salty baby food and one to two fruit-based baby food) from six to eight months of age. Therefore, breastfeeding mostly contributes to nutrient intake and can contribute up to 70% of the daily requirement in the case of vitamin A (World Health Organization, 2003; Brasil, 2005). In this case, fruit-based baby food analyzed herein could offer up to 5.8% of the DRI (AMC) if consumed twice a day. It is recommended that the baby continues breastfeeding during the introduction of food; however, this can be replaced by infant formulas or segment formulas if it is not possible to maintain breastfeeding (World Health Organization, 2003; Agostoni et al., 2009).

For example, 22.4% of children between 6 and 12 months in the United States continue to be breastfed, against 54% who consume infant formula (Decker et al., 2022). Infant formulas contain preformed vitamin A, most commonly added in the forms of retinol acetate and palmitate. These forms have a higher absorption rate than provitamin A carotenoids (between 70% and 90%) (Rodas Mendoza et al., 2003; Loughrill et al., 2016;). Formulas can provide around 60 µg of retinol in 100 mL (60 RAE) (Rodas Mendoza et al., 2003); thus, the consumption of 200 mL of infant formula would correspond to 120 RAE or approximately 25% of the DRI and, like breastfeeding, contributes to the adequate intake of vitamin A.

The conversion values of provitamin A carotenoids into RAE determined by IOM consider the efficiency of β-carotene conversion, estimated at 50%, and the β-carotene absorption rate measured by serum concentration, considered 14 %.25 The conversion value for the other provitamin A carotenoids was obtained by extrapolation, considering that they have half the activity of β-carotene (IOM , 2001). The calculated RAE values may be underestimating or overestimating the vitamin A DRI. In this study, we did not evaluate the absorption of carotenoids. However, by looking at the bioaccessibility results for the provitamin A carotenoids, the absorption rate of 14% would not be achieved, resulting in overestimated RAE values for the analyzed baby food, except for AMC. In this sense, within the experimental conditions used in this *in vitro* study, the provitamin A conversion to retinol would be lower than those obtained by the RAE calculated according to the IOM.

This variability and uncertainty in the exact values of RAE can affect the health of individuals who experience or are at risk of vitamin A deficiency and need to be exposed to foods with higher amounts of RAE. In addition, it may affect public policies on vitamin A supplementation, which may consider the intake of fruits and vegetables containing provitamin A carotenoids and, thus, underestimate the need for supplementation using smaller doses.

Conclusions

In conclusion, there was a difference between the label nutritional information and the content of nutrients experimentally determined in baby food available on the

market, especially for protein content. This can difficult an accurate estimate of the nutrient intake of infants fed baby food.

Minerals, even in low contents, can affect the bioaccessibility of individual carotenoids from baby food. This influence can be positive, as observed for the cis isomers of β-carotene, or negative, for α-carotene. As expected, lipids had a positive correlation with the bioaccessibility of total carotenoids.

In addition, future studies are necessary to verify in more details the relationship between the bioaccessibility of α-carotene with fibers, ashes, and minerals, given that a positive correlation was observed with fibers and a strong negative correlation with ash and minerals (Mn, K, Mg, and Cu). However, due to the scarcity of data regarding interactions on α-carotene bioaccessibility in the literature, it was not possible to suggest possible mechanisms leading to these behaviors.

Moreover, attention still should be directed to the standardization of *in vitro* methods that should be able to provide good estimates of vitamin A for all age groups.

Fruit-based baby foods do not contribute significantly to children's vitamin A intake. Except for AMC, the other samples do not reach 2% of the DRI in a 120 g portion. For children who continue to be breastfed or on infant formula, fruit-based baby food can complement the intake of provitamin A carotenoids and contribute to an adequate vitamin A intake. Thus, breastfeeding or infant formula consumption are essential for an adequate intake of vitamin A by babies and the prevention of vitamin A deficiency.

Finally, the IOM report in 2001 with the establishment of RAE was a critical mark at public health and academic levels. Nonetheless, significant advances have been made since its release. These include progress not only in the analytical assessment of the carotenoid composition of foods of diverse origins and its systematic documentation, but also in the understanding of factors impacting the digestion, absorption, and bioconversion of carotenoids to vitamin A once they are consumed. In this sense, we have learned from *in vitro* and in vivo studies the factors related to the carotenoid structure, food matrix, and the individuals that may ultimately impact the bioconversion and subsequent processes. Therefore, this knowledge should be considered in developing more relevant DRI values for vitamin A shortly in the near future.

Conflicts of Interest
There are no conflicts to declare.

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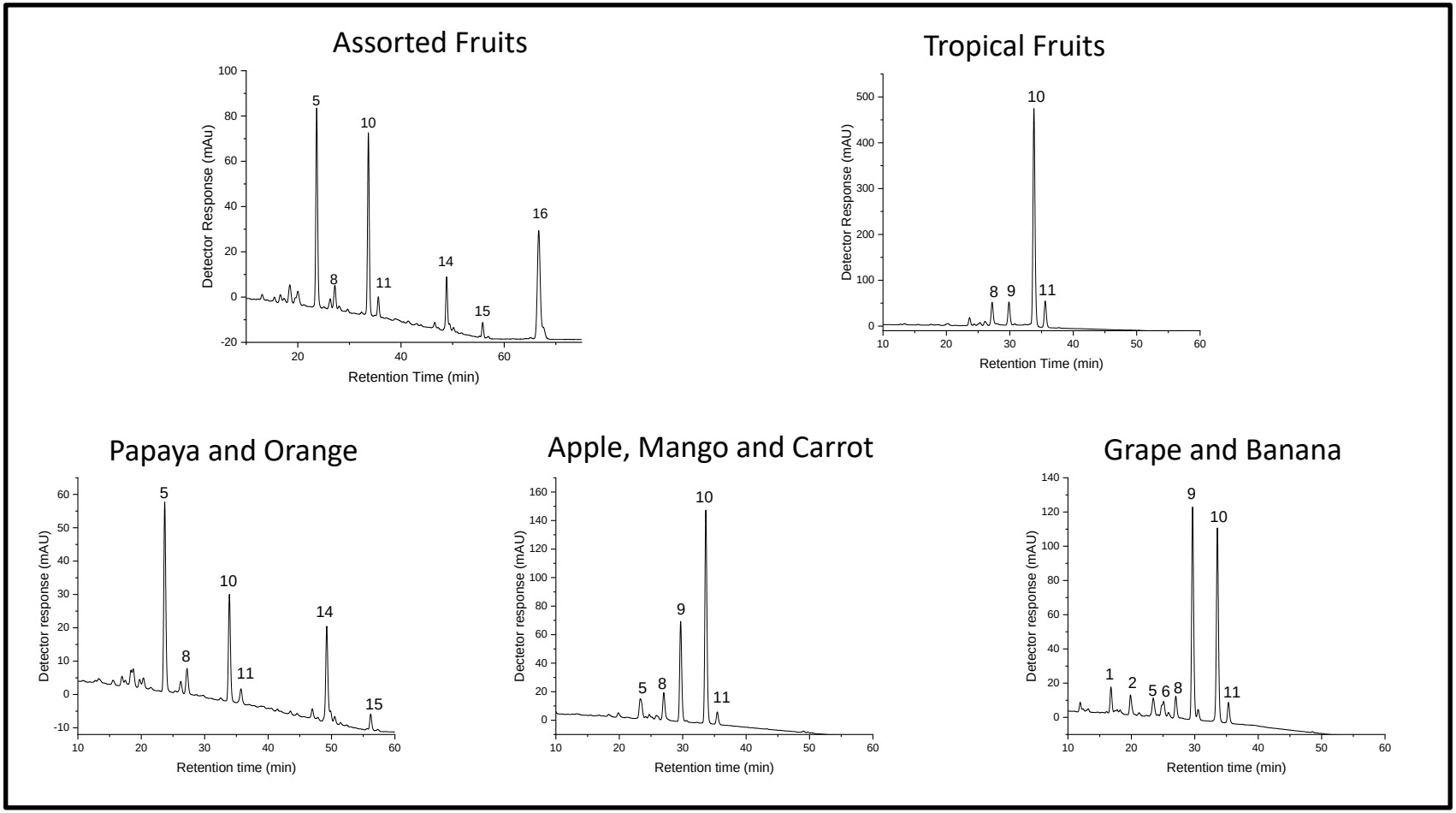
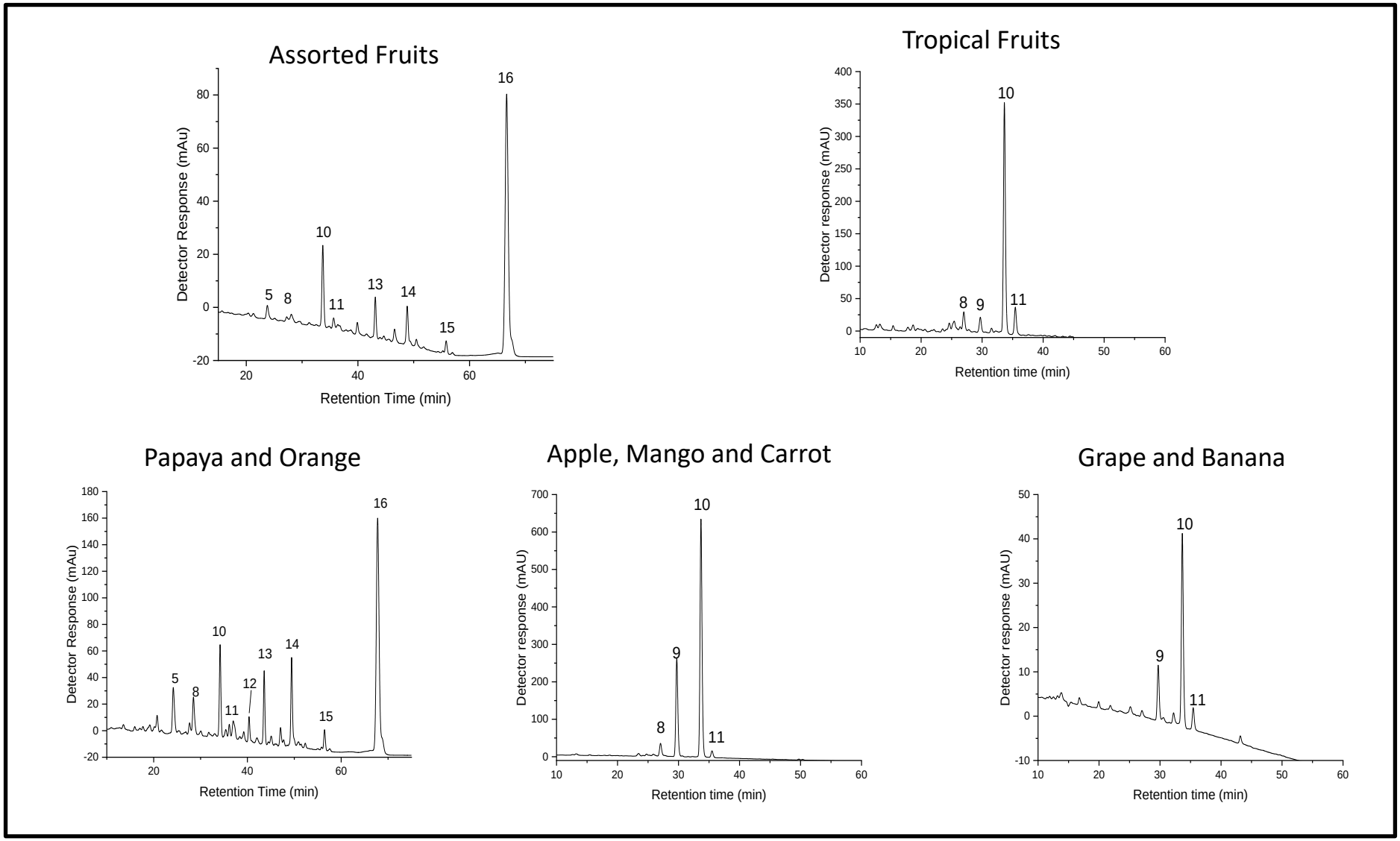
Supplementary Material

S1 Chromatograms of baby food before *in vitro* digestion, obtained from HPLC-DAD, processed at 450 nm. Peaks numbered according to Table 5.



S2. Chromatograms of baby food after *in vitro* digestion, obtained from HPLC-DAD, processed at 450 nm. Peaks numbered according to Table 5.



CAPÍTULO IV

Carotenoids in homemade baby food: composition and *in vitro* bioaccessibility

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Abstract

Vitamin A is an important nutrient involved in the growth and development of babies and children and its deficiency can have serious consequences for health. The aim of this study was to determine the composition and *in vitro* bioaccessibility of carotenoids from fruits and homemade fruit-based baby food. The retinol activity equivalent (RAE) from the fruits and baby foods was calculated based on the content of provitamin A carotenoids. In general, carotenoid content was higher in homemade baby food than in individual fruits and vegetables, mainly for all-*E*-β-carotene. However, the bioaccessibility of carotenoids was higher in fruits and vegetables than in the homemade baby food. This is justified by the strong negative correlation between the amount of carotenoids and bioaccessibility, observed mainly for all-*E*-β-carotene, which was the predominant provitamin A carotenoid among the samples. Furthermore, the diversity of fruits and vegetables used in the formulation of the baby foods may affect the bioaccessibility of carotenoids, since the food matrix of the baby foods may be more complex than that of fruits and vegetables alone, changing the release of carotenoids from this matrix.
Although the bioaccessibility of vegetables was percentually higher than that of baby food, the amount of carotenoids present in the bioaccessible fraction was higher. Thus, the homemade baby food was superior in relation to the concentration of carotenoids and RAE when compared to vegetables alone. And they can be considered better choices for provitamin A carotenoid intake.

Keywords: Provitamin A; bioaccessibility; baby food; carotenoids.

1. Introduction

Complementary feeding should start concomitantly with breastfeeding after the child is six months old. The start of complementary feeding is necessary to meet the needs of macro and micronutrients that are no longer met by exclusive breastfeeding due to the maturation of the body [1–3]. The most consumed foods in this phase are baby food, soups, and purées, which can be industrialized or homemade [4].

Vitamin A plays an essential role in the growth and development of babies and children. Vitamin A deficiency is one of the main causes of blindness in children, in addition to contributing to the increase in infections [5].

According to the World Health Organization (WHO)[5], 33.3% of the children under five years old has vitamin A deficiency worldwide. In Brazil, there was a reduction in the incidence of vitamin A deficiency in children under 5 years old, from 17.4% in 2009 to 6% in 2019.[6,7]

Vitamin A can be obtained from animal sources such as (eggs, milk) or vegetables and fruits in the form of provitamin A carotenoids, namely β-carotene, α-

carotene, and β-cryptoxanthin.[8] The activity of these carotenoids is measured in retinol activity equivalents (RAE) using the conversion rate of 1 RAE = 1 μg retinol; 12 μg of β-carotene, and 24 μg of α-carotene and β-cryptoxanthin.[9]

Few studies have demonstrated the concentration of provitamin A carotenoids in baby food, and most have not calculated their bioconversion into vitamin A or retinol activity equivalent. The major carotenoids in baby food are usually α-carotene and β-carotene, and their concentration depends on the composition of baby food.[10,11]

Bioaccessibility studies are carried out to assess the carotenoids that will be available to be absorbed by the enterocytes. Thus, bioaccessibility is defined as the fraction of a nutrient or bioactive compound that will be available for absorption by enterocytes after being released from the food matrix.[12] Considering the carotenoids that are liposoluble compounds, the bioaccessible fraction in that transferred from the food matrix to the mixed micelles during digestion[13].

Industrialized baby food is usually submitted to heating, homogenization, and sterilization, which affect the release of carotenoids from the food matrix and consequently their bioaccessibility.[13–15] Thus, homemade, which is often raw, and industrialized baby food may have different bioaccessibility for carotenoids, for example, the bioaccessibility of β-carotene ranged from 0.5% in homemade baby food to 6.34% in sterilized baby food.[16]

In view of the above, the aim of this study was to evaluate the composition and the *in vitro* bioaccessibility of carotenoids in homemade baby food produced with ingredients like commercially available industrialized baby food sold in São Paulo/Brazil. In addition, the composition and *in* vitro bioaccessibility of carotenoids from the fruits, vegetables, and cereals used in the formulation of the baby food were assessed separately.

2. Material and methods

2.1 Samples

Eleven fruits pearl pineapple (*Ananas comosus*); silver banana (*Musa paradisiaca*); organic silver banana; gala apple (*Malus domestica Borkh*); papaya (*Carica papaya*); Tommy Atkins mango (*Mangifera indica*); pear Williams (*Pyrus communis*); lime orange (*Citrus ourantifolia*); Tahiti lemon (*Citrus latifolia*), and organic

Victoria grape (*Vitis vinifera);* three vegetables, white sweet potato (*Ipomoea batatas*), organic white sweet potato (*Ipomoea batatas*), and carrot (*Daucus carota*); and one cereal, industrialized oat flour, were purchased in the market in Campinas, São Paulo/Brazil , in July 2021. The fruits and vegetables were sanitized with 2% sodium hypochlorite solution, then peeled (except de grapes, that were homogenized with the peel) and homogenized in a KitchenAid domestic mixer. Before homogenized, the carrot, sweet potato, and organic sweet potato were chopped into pieces (1 x 2 x 1 cm) and cooked in boiling water for 7 min. After sanitization, the orange and lemon were squeezed and strained using a fruit juicer to obtain fruit juice.

2.2 Formulation of homemade baby food

Five homemade baby foods were prepared using the 15 ingredients described in 2.1. The formulation of the baby foods and the percentages described on the labels of the similar flavor of industrialized baby food are presented in Table 1. Lemon juice and organic lemon juice were used to adjust the pH of the baby food according to that of industrialized baby food (GB = 4.25, AF = 3.89, TF = 4.08, PO = 4, and AMC = 4.21). No water was used for the preparation of the homemade baby food to simulate domestic consumption of homogenized and mixed fruits.

Table 9 Composition of homemade baby food according to the label of industrialized baby food.

Homemade baby food	Composition
Grape and banana (GB)	Organic banana (43%), organic sweet potato (25%), organic grape (20%)
Assorted fruits (AF)	Apple (71%), papaya (7%), orange juice (1.6%)
Tropical fruits (TF)	Mango (29%); Banana (27%); pineapple (16%); oat flour (5%); orange juice (0.9%)
Apple, mango, and carrot (AMC)	Mango (26%); apple (25%); carrot (20%); Sweet potato (12%); orange juice (1.5%)
Papaya and orange (PO)	Papaya (45%); pear (19%); apple (15%); oat flour (5%); orange juice (1.5%)

2.3 Reagents

The reagents used in the proximate composition, *in vitro* digestion, determination of enzymatic activity, and carotenoid extraction were purchased from Sigma Aldrich (St.Louis , MO, USA). Analytical grade solvents were acquired from Synth (SP, Brazil). For fiber analysis, the test kit (T DF-100A) from Sigma-Aldrich (St Louis, MO, USA) was purchased. The enzymes pepsin (P6887), lipase (62310), and pancreatin (P7545), and bovine bile (B3883) were acquired from Sigma Aldrich (St.Louis , MO, USA). Hexane (HPLC grade) was purchased from JTBaker (Phillipsburg, NJ, USA). The water used in all analyzes was purified by the Milli-Q system (Billerica, MA, USA).

Standards of all-*E*-lutein (89% purity), all-*E*-β-carotene (87% purity), and all-*E*-α-carotene (98% purity) were obtained from *Tagetes* flowers (*Tagetes erecta*), pumpkin (*Cucurbita ssp),* and carrot (*Daucus carota*), respectively, and purified on an open chromatographic column (MgO (Merck, Germany): Hipoflosupercel (1:1), 20 cm). Purity was determined by HPLC-DAD.

2.4 Proximate Composition.

Proximate composition of fruits (except lemon and organic lemon), vegetables, and cereal was determined. The proximate composition of baby food was calculated based on the results obtained for the individual plants considering the formulation presented in Table 1.

Moisture was determined by drying the sample in an oven using the AOAC 950.46 method.[17]Ashes were determined by muffle burning.[18] The determination of total nitrogen was performed by the micro-Kjeldhal method, using a factor of 6.25 to calculate the percentage of protein.[19] Total lipids were determined using the Bligh & Dyer cold extraction gravimetric method.[20] For total dietary fiber analysis, an assay kit was used according to the AOAC 985.29 method.[21] Carbohydrates were calculated by the difference.[22]

2.5 Minerals.

For the evaluation of mineral content, acid mineralization was performed as described by Silva et al,[23] with modifications. The samples and diluted nitric acid (4mL)

were mineralized in a digester block for 2 h at 110 º C. Then, 2 mL of diluted nitric acid and 2 mL of hydrogen peroxide were added and mineralized for another 2 h at 130 °C.

To avoid possible interferences, lanthanum oxide (0.5% v/v) was added for Ca and Mg analysis, and cesium chloride (0.1% v/v) for K analysis. The minerals were evaluated using a flame atomic absorption spectrometer (FAAS), model AAnalyst 200, with a deuterium lamp for background radiation correction and hollow cathode lamps for the evaluated elements. For the construction of the analytical curves, 1000 mg/L (Quemis, Brazil) stock solutions of each element (Fe, Zn, Ca, Mg, Cu, Mn, and K) were used. The analytical ranges varied from 0.25 – 3.00 (Fe), 0.05 -0.5 (Zn), 0.5 - 5.0 (Ca), 0.025 - 0.25 (Mg), 0.05 - 1.45 (Cu), 0.05 – 0.6 (Mn), and 0.1 – 1.2 (K) mg/L. Dilutions were performed when necessary and quantifications were carried out in triplicate.

2.6 Extraction of carotenoids.

The extraction of carotenoids from baby food was performed using the method described by Dhuique- Mayer et al,[16] adapted for this sample. To avoid waste of solvent, the mass of the sample used was defined in preliminary tests and according to the predictability of carotenoids in the plants: 5 g (pineapple, banana, sweet potato, apple, pear, orange juice, lemon juice, organic sweet potato, organic banana, organic grape, organic lemon juice, and GB) and 2 g (carrot, papaya, mango, AF, TF, PO, and AMC). Three extractions were performed using hexane/ethanol (3:4, v/v) and 1 g of celite. After each extraction, the organic phase was transferred to a separatory funnel containing water and hexane. The aqueous phase was discarded, and the hexane evaporated on a rotary evaporator. The extract containing the carotenoids was then subjected to saponification overnight with 10% KOH in methanol under continuous stirring. The extracts were washed until all alkali was removed, then were dried on a rotary evaporator and stored in an amber flask under nitrogen atmosphere until injection into the HPLC (item 2.8).

2.7 *In vitro* bioaccessibility.

Before starting the *in vitro* simulated digestion, the analysis of the activity of the enzymes was carried out according to the supplementary material of Minekus et al.[24] *In vitro* simulated digestion analysis was carried out according to the Infogest 2.0

[25]method, adapted for lipophilic compounds by Marques et al.[26] Micelle separation and carotenoid extraction were performed according to Rodrigues et al.[27] After extraction from the micelles, carotenoids were identified and quantified as described in 2.8.

The bioaccessibility of carotenoids (percentage of carotenoids in the micellar fraction after digestion) was calculated by the quotient between the concentration of carotenoids in the micellar phase and the concentration of carotenoids in the sample, according to the formula below:

$$Bioaccessibilty\ (\%) = \left(\frac{[carotenoids]_{micelar}}{[carotenoids]_{sample}}\right) x\ 100$$

2.8 Identification and quantification of carotenoids

The separation of carotenoids was performed by high performance liquid chromatography HPLC, model 1200 Series (Agilent) with diode array detector (DAD) model G1315D (Agilent). The identity of carotenoids was confirmed by HPLC-DAD-MS (LCMS-2020, Shimadzu). The mass spectrometer was equipped with an APCI interface operated in positive mode with nebulizer gas flow at 2 mL/min and block heating temperature of 300 ºC. The interface voltage was 4.5 kV and the temperature was 400°C. The scan rate was 1667 u/s and the *m/z* range was 300-601. The UV-visible spectrum was obtained between 280 and 600 nm and the chromatograms were processed at 450 nm, 285 nm (phytoene) and 347 nm (phytofluene).

The separation took place on a C $_{30}$ YMC column (3 µm, 250 x 4.6 mm id.) (Waters, USA) using a linear gradient of methanol/MTBE from 95:5 to 70:30 in 30 min to 50: 50 in 20 min and maintaining this proportion for 35 min, with a flow rate of 0.9 mL/min and column temperature of 29 °C.

The identification of carotenoids was carried out using a combination of characteristics: order of elution in a C30 column, UV-vis spectrum (λ_{max}, fine structure and *cis* peak intensity*,* when applied), and mass spectrum, compared with standards or data published in the literature.[28–31]

Carotenoids were quantified by HPLC-DAD using seven-point analytical curves (constructed in triplicate) for all-*E*-lutein (1.9−21.3 µg.mL^{-1}), all-*E*-α-carotene (7.8−19.6 µg.mL^{-1}), all-*E*-β-carotene (3.0−13.9 µg.mL^{-1}), and all-*E*-lycopene (0.05–0,7 µg.mL^{-1}). The limits of detection (LOD) and quantification (LOQ) were, respectively, 0.02 and 0.07 µg.mL^{-1} for (all-*E*)-β-carotene, 0.013 and 0.044 µg.mL^{-1} for (all-*E*)-α-

carotene, 0.017 and 0.056 μg.mL^{-1} for (all-*E*)-lutein and 0,0,7 and 0,24 μg.mL^{-1} for (all-*E)*-lycopene. The other carotenoids (9-*Z*)-β-carotene, (13-*Z*)-β-carotene, and (all-*E*)-β-cryptoxanthin were calculated as (all-*E*)-β-carotene equivalents.

2.9 Conversion into RAE (Retinol Activity Equivalent) and percentage of adequacy to DRI (dietary reference intake) of vitamin A for age

The conversion of provitamin A carotenoids to retinol activity equivalents (RAE) was made using the conversion factors suggested by the Institute of Medicine (IOM) in the United States.[9] The conversion factors were: 1 RAE (1 μg of retinol) = 12 μg of β-carotene; 24 μg of α-carotene, and 24 μg of β-cryptoxanthin. In addition, the adequacy of vitamin A intake was calculated in relation to the DRI for children aged 6 to 12 months, which is 500 RAE/day.[32]

2.10 Statistical analysis.

Statistical analysis was performed by analysis of variance (ANOVA). Means were compared using Tukey's test and means with $p < 0.5\%$ were significant different. For correlation analysis, Pearson's correlation test was used and considered significant at $p < 0.05\%$. Statistical analyzes were performed using Past 4.07b software (Oyvind Hammer, University of Oslo, Norway).

3 Results and discussion

3.1. Proximate composition

The results of the proximate composition of fruits, vegetables, and cereal are presented in Table 2 and for homemade baby food in Table 3. The proximate composition of the homemade baby foods was calculated from the average composition of the fruits, vegetables, and cereal; therefore, no statistical analysis was carried out for mean comparison.

As expected, oatmeal had the lowest moisture content (4.12%) and orange juice and carrots had the highest (90.28% and 90.87%, respectively). Ashes varied from 1.46 g/100 g in oats to 4.02 g/100 g in carrots. Regarding proteins, oat flour had the highest concentration (14.85 g/100 g) and pear the lowest (0.57 g/100 g). Oat flour

also had the highest concentration of total lipids with 7.91 g/100g, while orange juice had the lowest lipid content (0.23 g/100 g). Orange juice also had the lowest fiber content (1.16 g/100 g), whilst carrot had the highest content (19.13 g/100 g). Carbohydrates of oat flour showed the lowest content (66.1 g/100 g) and organic grape the highest (92.71 g/100 g).

Since the proximate composition of homemade baby food (Table 3) was calculated from the results of the individual ingredients, these values were dependent on the composition and proportions of the ingredients in each sample.

Calories ranged from 40 kcal/100 g to 71.7 kcal/100 g (wet basis) in AMC and GB, respectively. UB contains 20% of organic grapes which had the highest carbohydrate content, explaining the higher caloric content of this baby food. Comparing with the label a baby food of similar composition (table 4), the calories of GB and AMC did not vary significantly. However, TF and PO showed higher calories in homemade baby food possibly because of fruit and vegetables varieties differences between homemade and industrialized products.

The ash content varied from 0.27 g/100 g (AF) to 0.56 g/100 g (GB) (wet basis). GB is formulated with organic fruits and presented the higher ash content among the homemade baby food, while AF has ingredients of high moisture contents. Ash contents aren’t declared on the labels and therefore no comparison was made.

The protein variation was from 0.22 g/100 g to 1.15 g/100 g (wet basis) in AF and TF, respectively. TF contains oat flour, an ingredient with high protein content, while AF contains apple, the ingredient with the lowest protein content. Compared to the data on the industrialized baby food label, the proteins showed values close to the values described on the labels, except for homemade PO, which had 108% more protein than the industrialized baby food label.

Table 2 Proximate composition of fruits, vegetables, and cereals.

(mg/100g)	Moisture[1]	Ash[2]	Protein[2]	Fat[2]	Fiber[2]	Carbohydrates by difference[2]
Pineapple	86.77 ± 0.135^{c}	1.89 ± 0.047^{c}	4.41 ± 0.74^{e}	0.70 ± 0.035^{e}	8.65 ± 0.381^{d}	84.35
Oat flour	4.12 ± 0.152^{i}	1.46 ± 0.072^{d}	14.86 ± 0.203^{a}	7.91 ± 0.131^{a}	9.68 ± 0.385^{d}	66.1
Banana	73.23 ± 0.278^{g}	2.54 ± 1.022^{b}	2.69 ± 0.077^{b}	0.30 ± 0.012^{g}	6.83 ± 0.274^{e}	87.63
Organic banana	71.50 ± 0.349^{h}	2.40 ± 0.084^{b}	3.36 ± 0.146^{g}	0.60 ± 0.021^{e}	11.64 ± 0.408^{c}	81.99
Sweet potato	81.89 ± 0.185^{e}	2.01 ± 0.058^{c}	3.95 ± 0.112^{ef}	1.20 ± 0.056^{c}	10.78 ± 0.396^{c}	82.06
Organic sweet potato	80.81 ± 0.270^{f}	2.55 ± 0.094^{b}	9.20 ± 0.458^{b}	1.03 ± 0.023^{d}	14.81 ± 0.721^{b}	72.41
Carrot	90.87 ± 0.241^{a}	4.02 ± 0.125^{a}	5.16 ± 0.175^{d}	2.87 ± 0.112^{b}	19.13 ± 0.291^{a}	68.81
Apple	85.00 ± 0.074^{d}	2.05 ± 0.049^{c}	1.58 ± 0.055^{h}	1.30 ± 0.036^{c}	10.69 ± 0.506^{cd}	84.37
Mango	86.23 ± 0.076^{c}	2.45 ± 0.065^{b}	3.73± 0.123^{cefg}	0.97 ± 0.036^{d}	11.49 ± 0.425^{c}	81.36
Papaya	85.33 ± 0.082^{d}	3.97 ± 0.077^{a}	4.10 ± 0.172^{e}	1.00 ± 0.046^{d}	10.79 ± 0.399^{c}	80.14
Pear	85.13 ± 0.384^{d}	1.49 ± 0.020^{d}	0.57 ± 0.021^{i}	0.38 ± 0.003^{f}	14.35 ± 0.628^{b}	83.21
Orange juice	90.28 ± 0.185^{b}	3.94 ± 0.135^{a}	6.73 ± 0.291^{c}	0.23 ± 0.001^{h}	1.16 ± 0.041^{g}	87.94
Organic grape	82.19 ± 0.221^{e}	3.94 ± 0.129^{a}	1.42 ± 0.032^{h}	0.97 ± 0.032^{d}	5.36 ± 0.130^{f}	92.71

Values are mean ± standard deviation (n=3), except for carbohydrates which were calculated by difference. Different letters in each column indicate significant differences among the samples ($p < 0.05$).
[1] Wet basis. [2]Dry basis.

Table 3 Proximate composition of homemade baby food.

	Calories[a] (Kcal/100g)	**Ash[b] (g/100g)**	**Protein[b] (g/100g)**	**Fat[b] (g/100g)**	**Fiber[b] (g/100g)**	**Carbohydrate[b] (g/100g)**
GB	71.7	0.56	0.90	0.16	2.33	16.67
AF	41.8	0.27	0.22	0.15	1.31	9.98
TF	68.6	0.40	1.16	0.46	1.25	14.97
AMC	40.0	0.29	0.38	0.16	1.40	9.25
PO	58.5	0.43	1.04	0.49	2.18	12.48

[a] Calories calculated by the sum of the average content of protein, lipids, and carbohydrates, using conversion factors of 4 kcal/g for protein and carbohydrates and 9 kcal/g for lipids. [b]Calculated with data from Table 2 for the ingredients using formulation presented in Table 1. GB: grape and banana; AF: assorted fruits; TF: tropical fruits; AMC: apple, mango, and carrot; PO: papaya and orange. Wet basis.

AF had the lowest lipid content (0.15 g/100 g) and PO had the highest (0.49 g/100 g), which can be explained by the fact of apple as the main ingredient of AF and papaya and orange juice low amounts of lipids. On the other hand, despite PO has similar ingredients to AF (papaya, orange, and apple), it also contains oat flour which is the ingredient with the highest lipid content. The labels do not show the lipid values, probably because the Brazilian legislation allows the rounding of values up to 0.5 g/100 g to “0”.[22]

Fibers ranged from 1.25 g/100 g in TF to 2.32 g/100 g in GB (wet basis). Organic banana and organic sweet potato had high fiber contents and were the ingredients of the organic homemade baby food GB. On the other hand, FT contains ingredients with low fiber content (pineapple, banana, oat flour, and orange juice), leading to a lower fiber content. GB and PO showed the greatest variations in fiber content in relation to the label, containing 64% and 74% more fibers, respectively.

Carbohydrate varied from 9.24 g/100 g to 16.7 g/100 g in AMC and GB, respectively. The organic grape in GB was the ingredient with the highest carbohydrate content, which explains why the GB had the highest content. AMC contains carrots, which have a low carbohydrate content, which justifies the lower carbohydrate content in this baby food. Compared to the label, the greatest variation was found for TF, with 28.28% more carbohydrate in the homemade baby food than on the label (Table 4).

Table 4 Comparison between nutritional composition of homemade and industrialized baby food (label) of similar flavors.

	Protein (g/120 g)			Fiber (g/120 g)			Carbohydrate (g/120 g)			Calorie (kcal/ 120 g)		
	Homemade	Industrialized	Difference (%)	Homemade	Industrialized	Difference (%)	Homemade	Industrialized	Difference (%)	Homemade	Industrialized	Difference (%)
GB	1.08	1.00	8.43	2.79	1.70	64.37	20.00	19	5.28	86.03	83	3.65
AF	0.27	0.00	Nc	1.57	1.20	30.69	11.87	12	-1.09	50.15	51	-1.67
TF	1.39	1.20	15.49	1.50	1.60	-6.20	17.96	14	28.28	82.29	64	28.58
AMC	0.46	0.70	-34.34	1.68	1.90	-11.74	11.10	12	-7.54	47.97	56	-14.34
PO	1.25	0.60	108.84	2.62	1.50	74.65	14.98	12	24.79	70.16	53	32.38

Labels do not contain lipids, so no comparison was made for this macronutrient. Averages/portion of 120 g.GB: Grape and banana; AF: Assorted fruits; TF: Tropical fruits; AMC: Apple, mango, and carrot; PO: Papaya and orange. nc: not calculated.

The differences observed between homemade baby food and the labels of industrialized food can be explained by the difference in the cultivars of the plants used, as well as in the processing to which they were submitted.[33]

The homemade baby food processing consisted only in mixing the fruits in a food processor after sanitization until making a homogeneous puree, except carrot and sweet potato, which were cooked as described in section 2.2 before homogenization. On the other hand, industrialized baby food goes undergoes thermal processing, cooking and sterilization, which can more easily alter the food composition, for example, favoring lipid oxidation. In addition, cooking can result in physical changes in the food matrix, disrupting the cell structures and making nutrients easier to extract or to be released from the food matrix.

3.2. Minerals

Mineral contents of the ingredients are shown in Table 5. The iron content ranged from 0.31 mg/100 g in pear to 4.21 mg/100 g in oat flour. Oat flour also had the highest concentrations of manganese and zinc, 4.56 mg/100 g and 1.86 mg/100 g, respectively, while orange juice had the lowest concentration of these minerals, 0.11

mg/100 g of manganese and 0.11 mg/100g of zinc. In contrast, orange juice had the highest potassium content (2,488.4 mg/100 g) while oat flour had the lowest, with 10 times less potassium (219 mg/100 g).

Pear had the lowest concentration of calcium (28.3 mg/100 g) in opposition to carrot which showed the highest content (214.5 mg/100 g). Magnesium ranged from 0.28 mg/100 g to 153.8 mg/100 g for apple and organic banana, respectively. On the other hand, organic banana had the lowest copper content (0.17 mg/100 g), while apple had the highest (0.87 mg/100 g).

Table 6 presents the mineral contents for homemade baby food. GB had higher amounts of iron (1.15 mg/100 g), zinc (0.36 mg/100 g), and magnesium (86.8 mg/100 g). AF showed the lowest levels for six 6 out seven analyzed minerals, except for copper which presented the highest concentration (0.64 mg/100 g). TF had the highest value for manganese and potassium (2.28 mg/100 g and 1259.4 mg/100 g, respectively) and the lowest for copper (0.336 mg/100 g). The highest concentration of calcium (74 mg/100 g) was found in AMC.

The composition of each baby food influenced the amount of minerals. Considering calcium, for example, carrot was the vegetable with the highest mineral content, thus, the baby food containing carrots (AMC) was also the one with the highest calcium content. AF had the highest cooper content, as well as apple, the main ingredient used in baby food. Potassium was found in greater amounts in orange juice and banana, ingredients of TF, which in turn had the highest mineral content.

Table 5 Mineral composition of fruits, vegetables, and cereals.

	Mineral (mg/100 g, dry basis)						
Sample	**Fe**	**Zn**	**Ca**	**Mg**	**Cu**	**Mn**	**K**
Pineapple	0.67 ± 0.033^{e}	nd	98.91 ± 0.952^{c}	114.44 ± 9.821^{b}	0.40 ±0.028^{e}	9.23 ± 0.327^{a}	930.13 ± 84.563^{e}
Oat flour	4.21 ± 0.167^{a}	1.86 ± 0.024^{a}	31.84 ± 1.994^{fg}	121.29 ± 0.760^{b}	0.55 ± 0.008^{cd}	4.56 ± 0.105^{b}	219.05± 19.992^{g}
Banana	0.74 ± 0.040^{e}	0.29 ± 0.020^{f}	3.89 ± 0.296^{i}	114.23 ± 2.226^{b}	0.46 ± 0.001^{de}	1.64 ± 0.035^{d}	2246.89 ± 36.902^{b}
Organic banana	0.72 ± 0.004^{e}	0.35 ± 0.018^{e}	17.23 ± 0.954^{h}	153.8 ± 6,538^{a}	0.17 ± 0.001^{f}	1.98 ± 0.035^{c}	1483.00 ± 87.714^{d}
Sweet potato	1.57 ± 0.056^{c}	0.44 ± 0.017^{d}	119.29 ± 7.249^{b}	82.41 ± 5.043^{c}	0.55 ± 0.054^{cd}	0.92 ± 0.017^{e}	721.47 ± 36.876^{f}
Organic sweet potato	2.48 ± 0.251^{b}	0.85 ± 0.004^{c}	66.67 ± 1.198^{d}	82.22 ± 3,800^{c}	0.57 ± 0.047^{c}	0.53 ± 0.019^{f}	1438.84 ± 27.560^{d}
Carrot	1.50 ± 0.038^{c}	0.94 ± 0.019^{b}	214.15 ± 3.640^{a}	114.17 ± 8.342^{b}	0.62 ± 0.041^{c}	0.41 ± 0.001^{fg}	608.17± 22.736^{f}
Apple	0.69 ± 0.037^{e}	nd	10.55 ± 0.954^{hi}	0.28 ± 0.027^{d}	0.87 ± 0.037^{a}	0.20± 0.005^{gh}	640.82 ± 45.401^{f}
Mango	0.61 ± 0.036^{e}	0.42 ± 0.006^{d}	52.63 ± 0.182^{e}	72.58 ± 2.398^{c}	0.40 ± 0.022^{e}	0.48 ± 0.018^{f}	1622.66 ± 128.001^{cd}
Papaya	1.6 ± 0.113^{c}	0.19 ± 0.005^{g}	64.75 ± 0.900^{d}	147.6 ± 4,283^{a}	0.21 ± 0.002^{f}	nd	1679.82 ± 95.080^{c}
Pear	0.31 ± 0.000^{f}	0.31 ± 0.013^{f}	28.3 ± 0.176^{g}	0.79 ± 0.039^{d}	0.73 ± 0.046^{b}	0.18± 0.017^{gh}	928.14 ± 47.077^{e}
Orange juice	1.03 ± 0.033^{d}	0.11 ± 0.010^{h}	35.72 ± 0.383^{f}	106.9 ± 3,258^{b}	0.62 ± 0.039^{c}	0.11 ± 0.004^{h}	2488.4 ± 114.890^{a}
Organic grape	1.12 ± 0.018^{d}	nd	101.54 ± 2440^{c}	0.78 ± 0.032^{d}	0.80 ± 0.05^{ab}	0.18 ± 0.014^{g}	104697 ± 54.132^{e}

Values are mean and standard deviation. Different letters in the same column indicate significant difference ($p<0.05\%$). nd: not detected

Table 6 Mineral composition of homemade baby food calculated from the average mineral composition of fruits, vegetables, and cereals presented in Table 5.

	Mineral (mg/100 g)						
Sample	**Fe**	**Zn**	**Ca**	**Mg**	**Cu**	**Mn**	**K**
GB	1.15	0.36	44.38	86.84	0.38	1.02	1206.79
AF	0.62	0.01	12.59	12.24	0.64	0.14	612.38
TF	0.70	0.29	34.05	77.23	0.34	2.29	1259.40
AMC	0.84	0.35	74.00	53.27	0.52	0.37	827.63
PO	1.11	0.24	38.22	74.28	0.40	0.29	1076.67

GB: Grape and banana; AF: Assorted fruits; TF: Tropical fruits; AMC: Apple, mango, and carrot; PO: Papaya and orange.

3.3 Carotenoid composition

The carotenoid composition of the homemade baby foods and their ingredients is presented in Table 7 and Figure 1. The chromatograms of the carotenoids from the ingredients (fruits and vegetables) are shown in Supplementary Material S1.

β-carotene was found in all ingredients, except in oat flour that showed no carotenoid. β-carotene was the major carotenoid in 10 ingredients, except in sweet potato and organic sweet potato whose major carotenoid was luteoxanthin, and papaya and orange juice that had β-cryptoxanthin as the major carotenoid. In pineapple, β-carotene was the only identified carotenoid.

Sweet potatoes and carrots were cooked in water before analysis to simulate home consumption. This can affect the concentration of carotenoids since cooking can decrease the content of all-*E*-β-carotene and increase the formation of *cis isomers.*[34] 9-*Z*-β-carotene was identified in a few samples, namely: carrot, mango, regular lemon juice, and organic grape. Lemon juice was squeezed under regular light. Mango and grape were not exposed to any factor that could induce isomerization, 9-*Z*-isomers could have been formed during analysis or sample preparation, or even be intrinsic to the sample.[35]

Most studies with sweet potatoes were carried out with the orange variety, which has β-carotene as the major carotenoid.[36] However, in Brazil, the white variety is the most consumed, therefore, its major carotenoid is luteoxanthin. Other carotenoids have also been identified in sweet potato and are in accordance with the

findings of this study, such as β-epoxides, violaxanthin, and neoxanthin.[37,38]In addition, two carotenoids were reported for the first time, auroxanthin was identified in regular and organic sweet potato, and cryptochrome in organic sweet potato.

β-cryptoxanthin was previously identified as the major carotenoid in papaya, followed by β-carotene.[39,40] The results are similar to those observed in our study. Dugo et al[41] also identified β-cryptoxanthin as the major carotenoid in orange juice. In addition, these authors identified violaxanthin, lutein, luteoxanthin, and zeaxanthin as the main carotenoids. Gama and Sylos[42] also found β-cryptoxanthin as the major carotenoid in orange juice, followed by ζ-carotene. Apart from luteoxanthin and ζ-carotene, other carotenoids were also identified in this study, including a high concentration of β-carotene. Previous studies had also demonstrated the presence of violaxanthin, with β-carotene appearing as major carotenoid in some varieties and violaxanthin in others.[43–45]

Table 7 Chromatographic, spectrophotometric, and spectroscopic characteristics of baby food carotenoids obtained by HPLC-DAD-MS.

Peak	Carotenoid	Retention time (min)[a]	λ max (nm)[b]				%III/II	%A_B /A_{II}	[M + H]$^+$ (*m/z*)
1	all-*E*-neoxanthin	8.0 – 8.1		415	440	468	92		600
2	all-*E*-violaxanthin	10.8 - 11.2		415	438	468	88		601
3	all-*E*-lutein	13.6		420	445	472	59		568
4	9-*Z*-lutein	14.5 - 14.7	327	410	435	462	55	28	568
5	9-*Z*-violaxanthin	15.1 - 15.2	322	420	442	472	82	41	601
6	luteoxanthin	16.4 - 17.0		400	420	450	100		600
7	5,6-epoxy-β-cryptoxanthin	17.7		400	425	452	50		569
8	all-*E*-α-cryptoxanthin	17.7- 18.0		420	445	475	67		553
9	9-*Z*-β-cryptoxanthin	18.9 – 19.0	340	420	445	470	25	67	553

10	auroxanthin	18.7		380	400	420	100		600
11	cryptochrome	19.1 - 19.2		380	400	428	100		584
12	*Z*-luteoxanthin	20.6 - 20.7	342	405	428	455	32	21	600
13	*Z*-phytofluene	21.6 - 21.7	250	330	350	370	69	45	543
14	5,6-epoxy-β-carotene	23.3 - 23.4		420	447	472	50		553
15	all-*E*-β-cryptoxanthin	23.1 - 24.2		425	448	476	33		553
16	13-*Z*-α-carotene	23.9 – 24.0	333	415	440	467	29	60	537
17	5,8-epoxy-β-carotene	25.7 - 25.8		405	425	452	60		553
18	di-*Z*-α-carotene	26.1 - 26.3	338	420	448	472	29	34	537
19	13-*Z*-β-carotene	27.0-27.5		415	442	471	20	56	537
20	not identified 1	28.3 - 28.4	342	410	435	462	50	15	
21	all-*E*-α-carotene	30.1 - 30.2		420	445	473	60		537
22	all-*E* ζ-carotene	32.7 - 32.8		380	400	428	100		541
23	all-*E*-β- carotene	33.6 - 33.9		421	452	478	30		537
24	9-*Z*-β-carotene	35.4 - 35.9		412	445	472	20	15	537
25	not identified 2	36.7 - 36.9		430	455	485	73		
26	all-*E*-*δ*-carotene	43.2		424	450	480	62		537
27	di-*Z*-γ-Carotene	43.4		422	451	479	33	19	537
28	*Z*-γ-carotene isomer 1	46.7 - 46.9	360	445	460	500	45	42	537
29	γ-carotene 1′,2′-epoxide	48.8 - 49.2		435	462	495	54		537

30	all-*E*-γ-carotene	49.1 - 49.3		422	450	472	60		537
31	not identified 3	49.9 - 50.0		422	450	480	20		537
32	*Z*-γ-carotene isomer 2	49.9 - 50.1	285	438	460	492	53	21	537
33	not identified 4	49.9 - 50.0		422	450	480	20		
34	not identified 5	50.9		418	445	470	14		
35	not identified 6	54.8 - 54.9	335	418	440	470	64	15	
36	9-*Z*-γ-Carotene	56.2 - 56.4	350	440	465	497	75	12	537
37	not identified 7	58.6 - 58.9		425	450	480	42		
38	all-*E*-lycopene	65.7 - 66.2		445	470	502	78		

a: Retention time on C_{30} YMC column at 29 ^{o}C
b: Gradient of methanol/MTBE (95::5). Data were acquired between 280 and 600 nm.

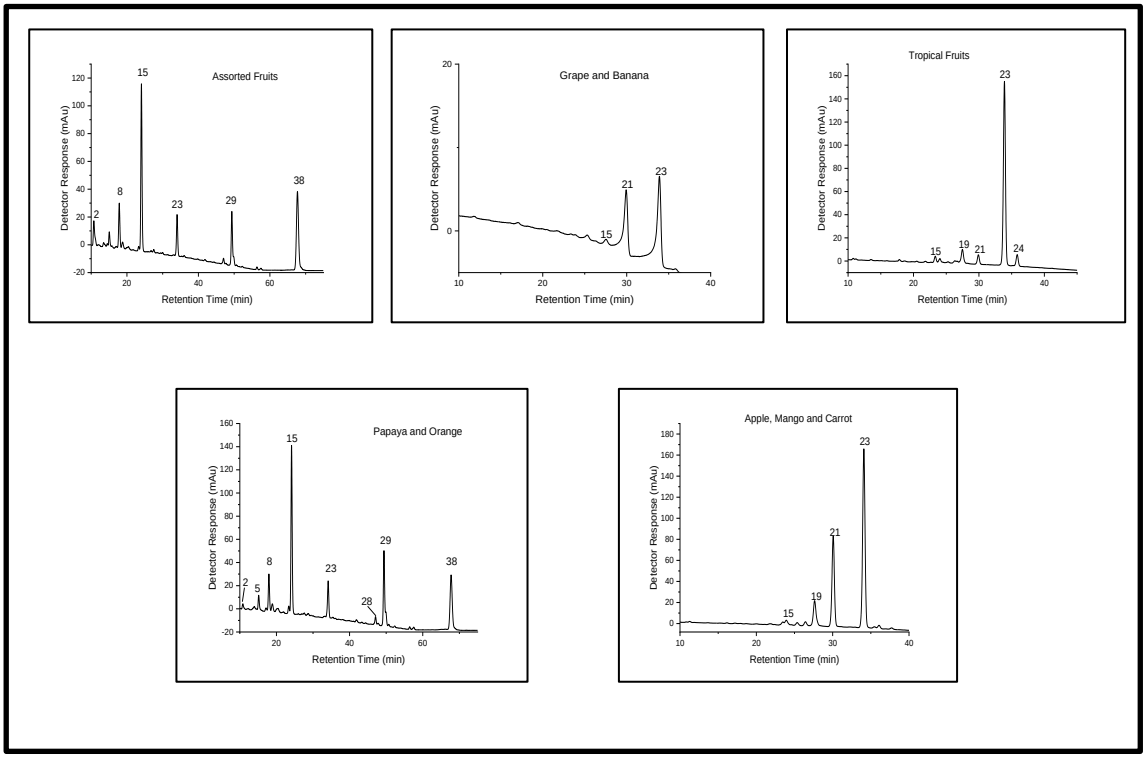

Figure 1 Chromatograms of carotenoids from homemade baby food, obtained by HPLC-DAD and processed at 450 nm. Peaks are numbered according to Table 7.

All-*E*-β-carotene was the predominant carotenoid also in the five homemade baby foods, followed by all-*E* β-cryptoxanthin present in four samples (except in GB). The samples containing papaya (AF and PO) showed all-*E*-β-cryptoxanthin as the major carotenoid. In the other samples, all-*E*-β-carotene was the major carotenoid.

As expected, the carotenoids in homemade baby food are directly related to the ingredients used in their preparations. Formation of new carotenoids was not observed after mixing the ingredients to prepare the baby food.

3.4 Carotenoid Bioaccessibility

The bioaccessibility of the ingredients can be found in Figure 2 and the chromatograms in supplementary material S2.

No carotenoid was found in the micelles after *in vitro* digestion of oat flour, banana, and organic bananas.

The bioaccessibility of all-*E*-β-carotene was calculated ranged from 5.1% for mango to 98.5% for pineapple, while organic sweet potato showed no bioaccessible all-*E*-β-carotene. The bioaccessibility of all-*E*-β-cryptoxanthin was calculated for carrot (37.7%), papaya (6.1%), orange juice (95%), and lemon juice (49%). All-*E*-β-cryptoxanthin and all-*E*- β-carotene bioaccessibility was the same ($p>0.05$) in carrot and orange juice. Laurora et al.[40] reported that all-*E*-β-cryptoxanthin is more bioaccessible in some papaya varieties, while in others it is all-*E*-β-carotene. In our study, all-*E*-β-carotene showed higher bioaccessibility than all-*E*-β-cryptoxanthin in the papaya. All-*E*-α-carotene bioaccessibility was 35.3% in carrot, 50.3% in pear, and 45.8% in lemon juice.

Pineapple

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
All-*E*-β-carotene	0.02 ± 0,000	98 ± 0.52
Total Carotenoids	0.02 ± 0,000	98 ± 0.52

Organic Sweet Potato

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
All-*E*-violaxanthin	0.08 ± 0.001	46 ± 0.91
Auroxanthin	0.08 ± 0.000	48 ± 1.04
γ-carotene 1',2'-epoxide	0.2 ± 0.000	44 ± 0.93
Total Carotenoids	0,17 ± 0.002	14 ± 0.29

Orange Juice

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
Lutein	0.02 ± 0.002	70 ± 2.09
Zeaxanthin	0.09 ± 0.001	96 ± 1.46
All-E-β-cryptoxantin	0.11 ± 0.007	95 ± 0.81
All-E-β-carotene	0.09 ± 0.001	94 ± 0.79
Total Carotenoids	0.68 ± 0.017	94 ± 0.85

Mango

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
All-*E*-β-carotene	0.69 ± 0.022	5 ± 0.05
Total Carotenoids	1.95 ± 0.027	5 ± 0.06

Pear

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
Lutein	0.003 ± 0.0003	36 ± 1.98
5,6-epoxy-β-carotene	0.017 ± 0.0001	48 ± 0.38
All-*E*-α-carotene	0.004 ± 0.0000	50 ± 0.27
All-*E*-β-carotene	0.017 ± 0.0002	49 ± 0.12
Total carotenoids	0.041 ± 0.0004	48 ± 0.33

Papaya

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
Z-phytofluene	0.43 ± 0.012	8 ± 0.08
All-*E*-β-cryptoxanthin	0.56 ± 0.017	6 ± 0.21
All-*E*-β-carotene	0.43 ± 0.013	8 ± 0.05
γ-carotene 1',2'-epoxide	0.46 ± 0.007	7 ± 0.22
Lycopene	0.54 ± 0.044	12 ± 0.73
Total Carotenoids	4.85 ± 0.109	5 ± 0.13

Sweet Potato

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
All-*E*-violaxanthin	0.08 ± 0.000	10 ± 0.06
All-*E*-β-carotene	0.09 ± 0.000	9 ± 0.05
Total Carotenoids	0.70 ± 0.029	2 ± 0.01

Carrot

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
Z-phytofluene	0.54 ± 0.031	34 ± 1.05
All-*E*-β-cryptoxanthin	0.45 ± 0.014	38 ± 0.74
13-*Z*-β-carotene	0.58 ± 0.037	34 ± 1.32
All-*E*-α-carotene	1.23 ± 0.068	35 ± 1.99
All-*E*-ζ-carotene	0.41 ± 0.007	39 ± 0.65
All-*E*-β-carotene	2.54 ± 0.111	34 ± 2.05
9-*Z*-β-carotene	0.42 ± 0.008	39 ± 0.67
Z-γ-carotene	0.42 ± 0.007	40 ± 0.72
Total Carotenoids	7.52 ± 0.277	31 ± 2.5

Apple

Carotenoid	Concentration(µg/g)	Bioaccessibility (%)
All-*E*-α-carotene	0.01 ± 0.000	42 ± 1.65
All-*E*-β-carotene	0.02 ± 0.000	35 ± 1.05
Total Carotenoids	0.18 ± 0.000	6 ± 0.18

Lemon Juice

Carotenoid	Concentration(µg/g)	Bioaccessibility (%)
All-*E*-β-cryptoxantin	0.02 ± 0,000	49 ± 0.12
All-*E*-α-carotene	0.004 ± 0.000	46 ± 0.28
All-*E*-β-carotene	0.02 ± 0.000	45 ± 0.17
Total Carotenoids	0.08 ± 0.001	38 ± 0.63

Organic Lemon Juice

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
All-*E*-β-carotene	0.02 ± 0.000	52 ± 0.86
Total Carotenoids	0.03 ± 0.000	26 ± 0.44

Organic Grape

Carotenoid	Concentration (µg/g)	Bioaccessibility (%)
All-*E*-β-carotene	0.03 ± 0.000	30 ± 0.16
Total Carotenoids	0.04 ± 0.000	37 ± 0.24

Figure 2. Carotenoid concentration and bioaccessibility in fruits and vegetables. Values are means ± standard deviation.

The *cis* isomers of β-carotene were bioaccessible only in carrots, 39.3% for 9-*Z*-β-carotene and 33.8% for 13-*Z*-β-carotene, showing values similar to the bioaccessibility of all-*E*-β-carotene (34.3%). The high micellarization of *cis* isomers is related to their solubility, which facilitates the incorporation onto the micelles.[46] Even showing a high bioaccessibility of the *cis* isomers, the absolute concentration of all-*E*-β-carotene is still higher, being 0.87 µg/g for all -*E* -β-carotene, and 0.2 µg/g and 0.16 µg/g for 13-*Z*-β-carotene and 9-*cis*-β-carotene, respectively.

The bioaccessibility of total carotenoids ranged from 2.35% in sweet potato to 98.5% in pineapple. Despite previous studies identifying large amounts of xanthophylls in pineapple,[43–45]we found only all-*E*-β-carotene. The pineapple was processed to simulate the baby's ingestion, in this way, there was rupture of the plant cells and alteration of the fibers structure, facilitating the release of β-carotene from the matrix, which may explain its high bioaccessibility.

Sweet potato contains a large amount of starch granules that can be gelatinized forming large aggregates during heating.[47] These aggregates can “trap” the carotenoids inside, making their solubilization and micellarization difficult. Probably, this heating effect, in addition to the presence of xanthophylls, may have affected the matrix release and micellarization of β-carotene and total carotenoids, thus leading to low bioaccessibility.

In organic sweet potato, the bioaccessibility of the xanthophylls all-*E*-violaxanthin and auroxanthin was 46.5% and 48.4%, respectively. The micellarization of these xanthophylls may have impaired the incorporation of all-*E*-β-carotene, since xanthophylls are more polar than carotenes and are incorporated into the outermost part of the micelle. Usually, the higher the concentration of xanthophylls in the micelle, the lower the bioaccessibility of β-carotene.[14,48]

The cooking and homogenization of carrots may have facilitated the release of all-*E*-β-carotene from the matrix, increasing the bioaccessibility of this carotene. Hedrén et al, [49]demonstrated an increase in the bioaccessibility of β-carotene after cooking and homogenizing carrots, going from 3% in raw carrots to 27% after processing. We did not analyze the raw carrot, but our cooked carrot showed a bioaccessibility of 34%, close to the result found by Hedrén et al.[49]

The bioaccessibility of all-*E*-β-carotene and total carotenoids in papaya was among the lowest values in comparison with the other evaluated fruits and vegetables. However, this result agrees with literature data, which is approximately 5%.[50]

Nevertheless, when considering the concentration of all-*E*-β-carotene in the micellar phase, it was higher in papaya (0.034 µg/g) than in pineapple (0.017 µg/g), which showed the highest bioaccessibility.

After the *in vitro* digestion of orange juice, several carotenoids that were not present in the fresh juice were identified. These carotenoids, peaks 31, 33, 34, 35, and 37 (Supplementary material S3, Table 7), are probably derived from violaxanthin, zeinoxanthin, and β-cryptoxanthin, as they were previously identified by Petry and Mercadante.[51] However, in our study it was not possible to confirm the identity of these carotenoids.

Figures 3 and 4 show the bioaccessibility of homemade baby food. No carotenoids were detected after *in vitro digestion* in the GB. The bioaccessibility of individual carotenoids in the samples varied in order of magnitude (Figure 3), which reflects the variability of the matrices.

All-*E*-β-carotene was found in all samples and its bioaccessibility ranged from 3% (AMC) to 29% (PO). A strong negative correlation (-0.72) was observed between the initial carotenoid concentration and bioaccessibility, that is, the higher the concentration of all-*E*-β-carotene in the food before *in vitro* digestion, the lower its bioaccessibility. The same behavior was not observed for total carotenoids, which showed a weak correlation (-0.33) with bioaccessibility.

In samples containing 13-*Z*-β-carotene, its bioaccessibility was equal (AMC) or higher (TF) than the bioaccessibility of the *trans* isomer. These results agree with the literature, in which the *cis* forms of β-carotene present greater micellarization than the *trans* isomer.[46]

Total carotenoid bioaccessibility ranged from 2% (AMC) to 23% (PO), following the same trend as all-*E*-β-carotene. The differences found in the bioaccessibility of the samples are related to the food matrix, considering their proximate and the mineral compositions.

The minerals affected the bioaccessibility of some carotenoids (Figure 5). The bioaccessibility of all-*E*-β-cryptoxanthin showed a very strong negative correlation with iron (-0.98) and manganese (-1), and strong negative correlation with magnesium (-0.87), indicating that the higher the concentration of these minerals, the lower the bioaccessibility of all-*E*-β-cryptoxanthin. On the other hand, there was a strong positive correlation with copper (0.76), indicating that copper favors the bioaccessibility of all-*E*-β-cryptoxanthin. All-*E*-α-carotene bioaccessibility showed a very strong negative

correlation with iron (-0.85) and a very strong positive correlation with potassium (0.90). For the bioaccessibility of all -*E* -β-carotene and total carotenoids, a moderate positive correlation with manganese was verified, but only weak (negative for iron and zinc and positive for potassium) or very weak (negative for calcium and positive for magnesium and copper) correlation with the other analyzed minerals, demonstrating that the minerals had no significant effect on the bioaccessibility of all-*E*-β-carotene and total carotenoids.

Assorted Fruits

Carotenoids	Concentration (µg/g)	Bioaccessibility (%)
All-*E*-β-cryptoxanthin	0.23 ± 0.005	14 ± 0.07
All-*E*-β-carotene	0.20 ± 0.002	17 ± 0.06
γ-carotene 1′.2′-epoxide	0.21 ± 0.002	16 ± 0.08
Lycopene	0.21	24
Total carotenoids	1.04 ± 0.016	16 ± 0.07

Apple. Mango and Carrot

Carotenoids	Concentration (µg/g)	Bioaccessibility (%)
13-Z-β-carotene	1.09 ± 0.02	3 ± 0.03
All-E-α-carotene	0.65 ± 0.02	2 ± 0.13
all-E-β-carotene	1.74 ± 0.05	3 ± 0.15
Total carotenoids	5.55 ± 0.05	2 ± 0.07

Papaya and Orange

Carotenoids	Concentration (µg/g)	Bioaccessibility (%)
Z-phytofluene	0.29 ± 0.00	28 ± 0.14
All-*E*-β-cryptoxanthin	0.42 ± 0.01	20 ± 0.14
Not identificated 1	0.28 ± 0.00	30 ± 0.21
All-*E*-β-carotene	0.29 ± 0.00	29 ± 0.21
γ-carotene 1′.2′-epoxide	0.32 ± 0.01	32 ± 0.28
Lycopene	0.2	49
Total carotenoids	3.28 ± 0.01	23 ± 0.18

Tropical Fruits

Carotenoids	Concentration (µg/g)	Bioaccessibility (%)
13-Z-β-carotene	0.21 ± 0.00	16 ± 0.24
All-E-β-carotene	0.33 ± 0.00	11 ± 0.35
Total carotenoids	1 ± 0.00	7 ± 0.16

Figure 3 Concentration and bioaccessibility of carotenoids in homemade baby food. Values are mean ± standard deviation.

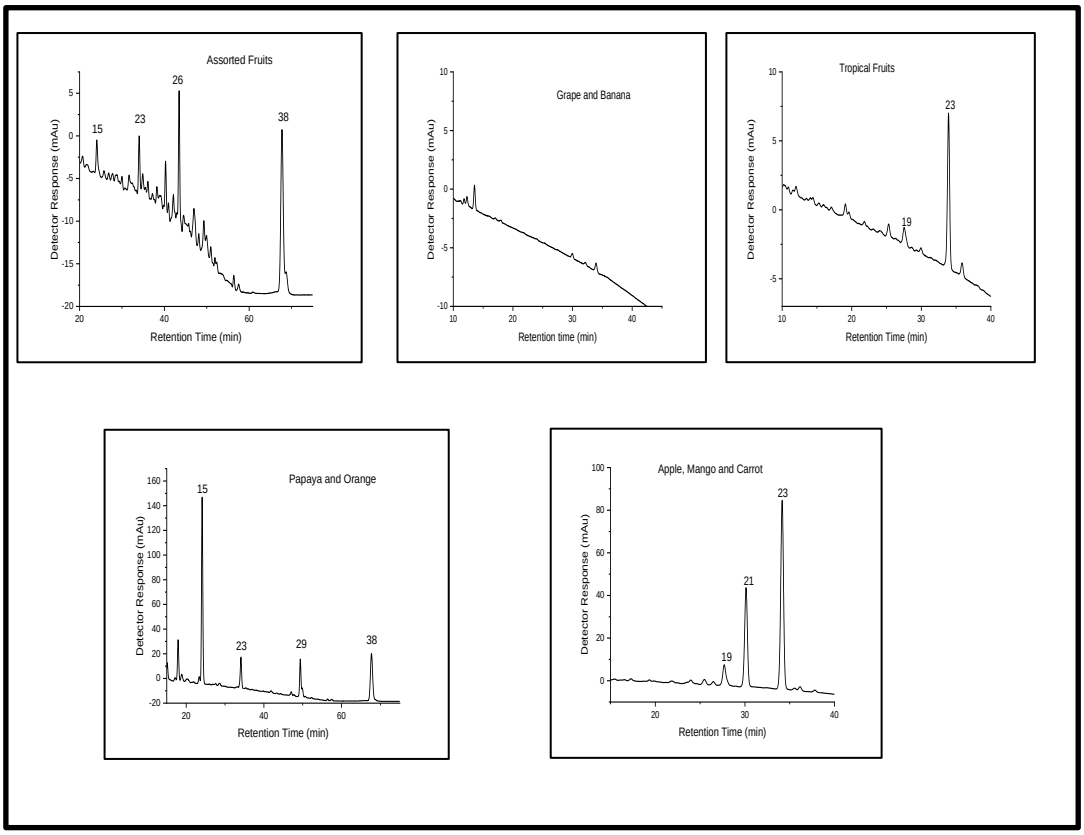

Figure 4 Chromatograms of carotenoids from digested homemade baby food, obtained by HPLC-DAD and processed at 450 nm. Peaks are numbered according to Table 7.

Minerals	All-*E*-β-cryptoxanthin	All-*E*-α-carotene	All-*E*-β-carotene	Total carotenoids
Fe	-0.98	-0.85	-0.38	-0.36
Zn	-0.24		-0.30	-0.35
Ca	-0.31	0.27	-0.14	-0.01
Mg	-0.87	0.09	0.11	0.16
Cu	0.76	-0.37	0.14	0.03
Mn	-1	0.08	0.58	0.57
K	0.57	0.90	0.26	0.30

	r value (+)	r value (-)
Very Low	0.00 - 0.19	0.00 - 0.19
Low	0.20 - 0.39	0.20 - 0.39
Moderate	0.40 - 0.69	0.40 - 0.69
High	0.70 - 0.89	0.70 - 0.89
Very High	0.90 - 1.00	0.90 - 1.00

Figure 5 Pearson's correlation between individual and total carotenoids and minerals in homemade baby food. The r values are indicated according to the color legend ($p < 0.05\%$).

Divalent minerals are known to reduce the micellarization of carotenoids, mainly iron, zinc, magnesium, and calcium. However, they need to be in high amounts in foods to have this effect (50 to 100 mg/mL). On the other hand, monovalent minerals such as potassium can increase the bioaccessibility of carotenoids.[52]In our study, this effect was observed for all-*E*-α-carotene and all-*E*-β-cryptoxanthin in which iron negatively affected the bioaccessibility while potassium favored it, mainly of all-*E*-α-carotene.

The proximate composition of the ingredients and homemade baby food also influenced the carotenoid bioaccessibility (Figure 6).

Fibers negatively affected the bioaccessibility of all-*E*-β-cryptoxanthin, all-*E*-β-carotene and total carotenoids. Several studies have already demonstrated a negative correlation between the amount of fiber and the bioaccessibility of carotenoids, especially β-carotene.[53–55] The fibers act by increasing the viscosity of the medium and preventing micellarization of carotenoids, and, consequently, reducing their bioaccessibility.

The bioaccessibility of *all-trans*-α-carotene was negatively affected by the presence of lipids, which differs from the literature that associates lipids with higher bioaccessibility of carotenoids[56]. However, the analyzed fruits and vegetables have low lipid concentrations, which may have had little effect on bioaccessibility when considering the whole food composition.

	All-*E*-β-cryptoxanthin	13-*Z*-β-carotene	All-*E*-α-carotene	All-*E*-β-carotene	Total carotenoids
Carbohydrate	-0.47	-0.43	0.59	-0.17	-0.21
Ash	-0.24	0.64	-0.94	-0.22	-0.16
Protein	0.10	0.00	-0.28	-0.07	-0.12
Fat	-0.48	0.24	-0.94	-0.43	-0.44
Fiber	-0.89	0.76	0.51	-0.53	-0.54

	r value (+)	r value (-)
Very Low	0.00 - 0.19	0.00 - 0.19
Low	0.20 - 0.39	0.20 - 0.39
Moderate	0.40 - 0.69	0.40 - 0.69
High	0.70 - 0.89	0.70 - 0.89
Very High	0.90 - 1.00	0.90 - 1.00

Figure 6 Pearson's correlation between individual and total carotenoids and proximate composition in homemade baby food. The r values are indicated according to the color legend ($p < 0.05\%$).

As can be seen in Figures 2 and 3, the concentration of carotenoids in the samples, especially all-*E*-β-carotene, was higher in the homemade baby foods than in the individual fruits and vegetables. The bioaccessibility, on the other hand, was percentually higher in the fruits and vegetables than in the homemade meals. This may have occurred due to the strong negative correlation observed between the amount of carotenoid present in the sample and its bioaccessibility, especially for all-*E*-β-carotene, which was the predominant carotenoid among the samples. Another factor that may have contributed to this difference, is the matrix of the baby foods, since the diversity of fruits and vegetables used, as well as their proportions in the baby food, can increase or reduce other components of the matrix that influence the bioaccessibility of carotenoids, such as fiber, for example.

3.5 Vitamin A

Table 8 and 9 show, respectively, the RAE (Retinol Activity Equivalent) values calculated for the ingredients (fruit and vegetable) and homemade baby food in the portion equivalent to an industrialized baby food (120 g). The DRI (Dietary Reference Intake) for vitamin A is 500 RAE/day for children aged 6 to 12 months.[32]

The fruits and vegetables that had the highest RAE were carrot (34 RAE), followed by mangoes (8 RAE); however, these values represent only 6.8% and 1.7% of the DRI of vitamin A. In homemade baby food, AMC containing mango and carrot as ingredients had the highest RAE value (26), corresponding to 5.2% of the DRI.

Considering the intake of two fruit-based baby food a day, as recommended by the WHO[57]for 6 to 8 months of age, GB provides 0.44% of the DRI and AMC, 10.4%. Regarding the consumption of fruits and vegetables, only carrots significantly contribute to the intake of vitamin A.

Table 8 Retinol Activity Equivalents (RAE) per 120 g serving and Suitability to Dietary Reference Intake (DRI) of fruits and vegetables.

	RAE in the portion (RAE/120 g)	**Adequacy to DRI (%)**
Pineapple	0.2	0.03

Banana	0.5	0.09
Organic banana	2	0.32
Sweet potato	1	0.17
Organic sweet potato	0.2	0.03
Carrot	34	6.8
Apple	0.4	0.1
Mango	7	1.43
Papaya	8	1.7
Pear	0.2	0.04
Orange juice	1.5	0.31
Lemon juice	0.3	0.06
Organic lemon juice	0.2	0.03
Organic grape	0.3	0.05

Table 9 Retinol Activity Equivalents (RAE) per 120 g serving and Dietary Suitability to Dietary Reference Intake (DRI) of homemade fruit-based baby food.

	RAE in the portion (RAE/120 g)	**Adequacy to DRI (%)**
GB	1	0.22
AF	3	0.64
TF	5	0.91
PO	5	1.01
AMC	26	5.2

GB: Grape and banana; AF: assorted fruits; TF: Tropical fruits; PO: papaya and orange; AMC: apple, mango, and carrot.

This adequacy, however, is based on the formula to calculate RAE values, which considers a rate of 16% for β-carotene absorption, in addition to limiting provitamin A carotenoids to α-carotene, β-carotene, and β-cryptoxanthin, not considering *cis* isomers or α-cryptoxanthin, for example. Thus, the values calculated for RAE and the adequacy in relation to the vitamin A, DRI for age may be underestimated or overestimated. The conversion factors, proposed by the IOM, may

make it difficult to obtain the recommended amount of vitamin A, according to the DRI.[58]

For example, AMC all-*E*-β-carotene bioaccessibility was only 3%; therefore, an absorption of 16% of the intake will not occur, resulting in overestimated RAE value for this sample. On the other hand, PO all-*E*-β-carotene bioaccessibility was 29%; therefore, the absorption of β-carotene by the enterocytes can be equal, greater, or lesser to 16%, consequently the RAE value may be achieved or not.

Regarding the fruits, this difference was even greater. Pineapple, for example, showed 98% bioaccessibility of all-*E*-β-carotene, and the calculated RAE value (0.2 RAE per serving) may possibly be below the actual absorption value of β-carotene. Thus, when consuming pineapple, the child may absorb more pro-vitamin A carotenoids than the calculated by the current IOM reference.

Although the IOM considers absorption (with measurements of β-carotene in the bloodstream after ingestion), the bioaccessibility data from our study may help to identify foods that have greater or lesser potential for provitamin A carotenoid intake and, consequently, should have revised RAE values.

In conclusion, homemade baby food is an alternative to feed babies concomitantly with breastfeeding, to complement the intake of vitamin A. Although the bioaccessibility of fruits and vegetables alone was higher than that of baby food, they showed a higher amount of bioaccessible provitamin A carotenoids than vegetables, that is, mixing different vegetables in a single food seems to favor the intake of provitamin A carotenoids.

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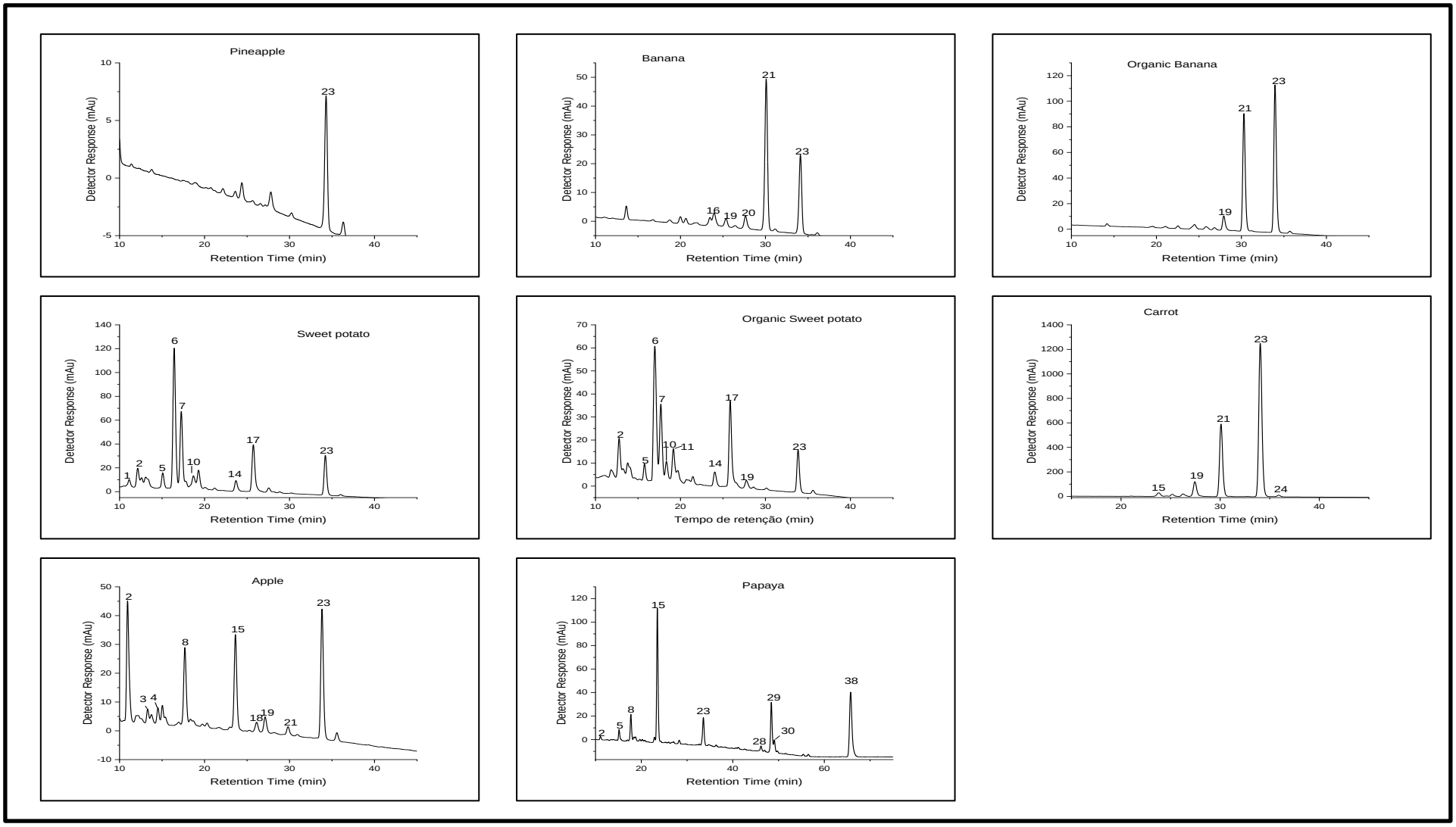
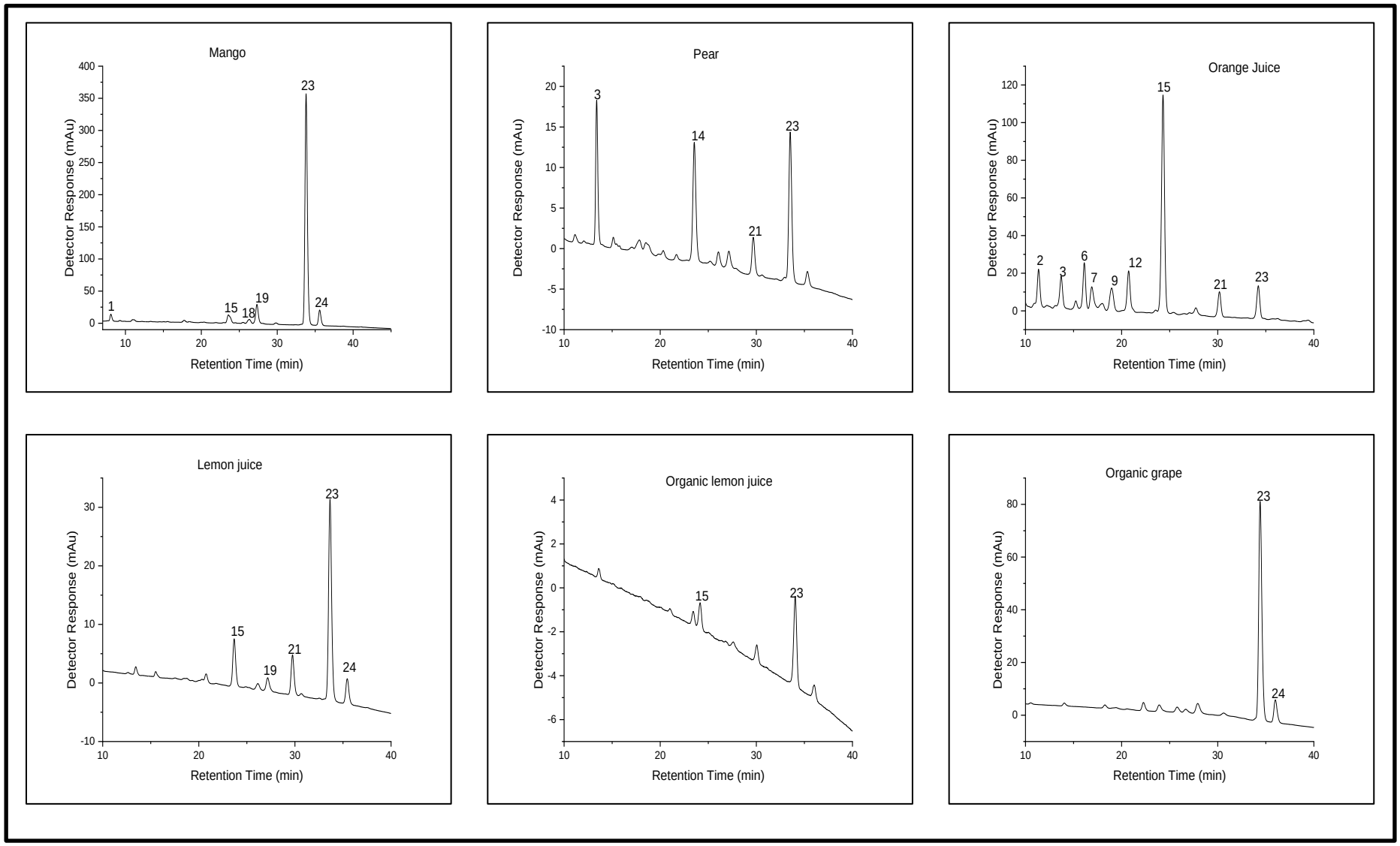
Supplementary material

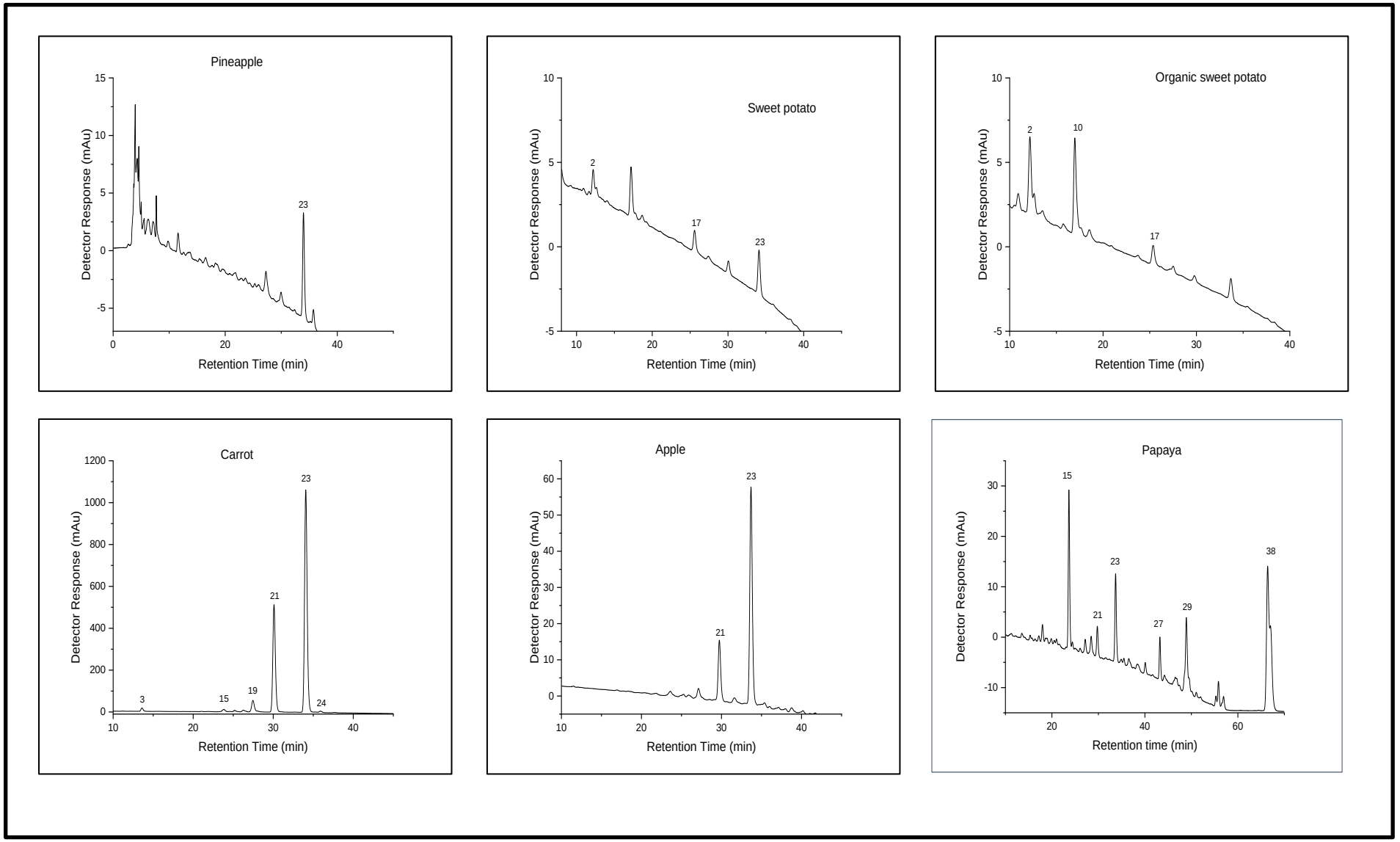
S 1. Chromatograms of fruits and vegetables before *in vitro* digestion, obtained from HPLC-DAD, processed at 450 nm. Peaks numbered according to Table 7



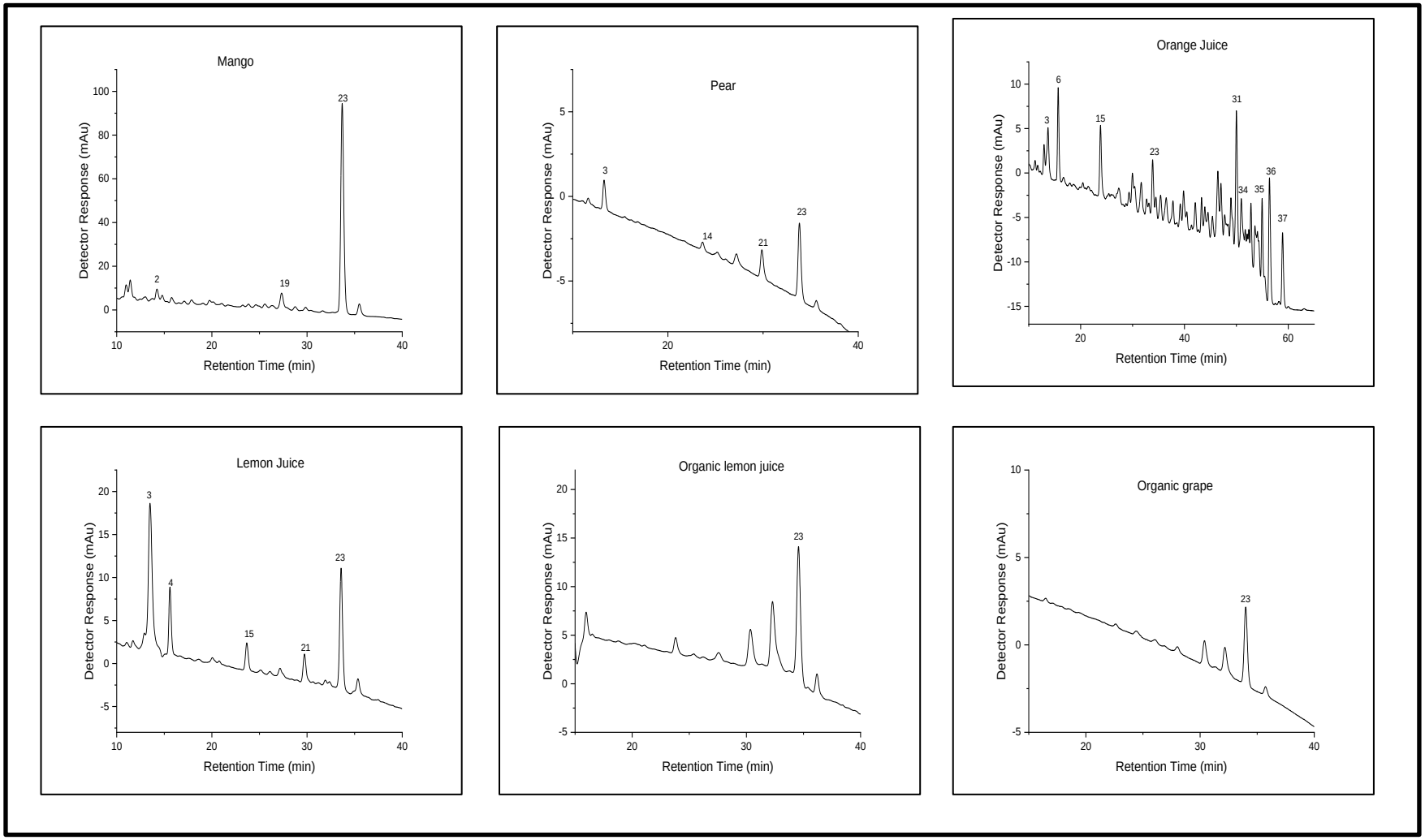
S 1 Cont. Chromatograms of fruits and vegetables before *in vitro* digestion, obtained from HPLC-DAD, processed at 450 nm. Peaks numbered according to Table 7



S 2 Chromatograms of fruits and vegetables after *in vitro* digestion, obtained from HPLC-DAD, processed at 450 nm. Peaks numbered according to Table 7



S 2 Cont Chromatograms of fruits and vegetables before *in vitro* digestion, obtained from HPLC-DAD, processed at 450 nm. Peaks numbered according to Table 7



DISCUSSÃO GERAL

As papinhas caseiras (capítulo IV) e as papinhas industrializadas (capítulo III) e os rótulos das papinhas apresentaram diferenças entre si (Tabela 1).

Tabela 1 Comparação da composição centesimal entre papinhas caseira, o rótulo das papinhas industrializadas e os resultados experimentais das papinhas industrializadas.

		Uva e Banana (UB)	**Frutas Sortidas (FS)**	**Frutas Tropicais (FT)**	**Maçã, manga e cenoura (MMC)**	**Mamão e Laranja (ML)**
Proteína (g/120 g)	Industrializada	2,15	0,72	0,51	2,35	0,58
	Caseira	1,1	0,26	1,39	0,46	1,25
	Rótulo	1	0	1,2	0,7	0,6
Lipídio (g/120 g)	Industrializada	0,22	0,14	0,5	0,25	0,3
	Caseira	0,19	0,18	0,55	0,19	0,58
	Rótulo	0	0	0	0	0
Fibras (g/120 g)	Industrializada	1,93	1,5	1,4	1,7	1,74
	Caseira	2,8	1,57	1,5	1,7	2,62
	Rótulo	1,7	1,2	1,6	1,9	1,5
Carboidrato (g/120 g)	Industrializada	17,6	12,4	15,6	11,32	11,54
	Caseira	20	11,87	18	11,1	15
	Rótulo	19	12	14	12	12
Calorias (kcal/120 g)	Industrializada	81	53,8	69	57	51
	Caseira	86	50,1	82,3	48	70,1
	Rótulo	83	51	64	56	53

Valores médios por porção do alimento que corresponde a 120 g.

Considerando as calorias, não houve grande variação entre as papinhas caseiras e industrializadas , por exemplo entre as papinhas uva e banana (UB) e frutas sortidas (FS). Para a UB a diferença foi de 6% e para FS de 7%. Já para as amostras frutas tropicais (FT), maçã, manga e cenoura (MMC) e mamão e laranja (ML), a diferença entre o valor calórico foi maior,19%, 16% e 37%, respectivamente.

Em relação as proteínas, os teores das papinhas industrializadas e caseiras foram semelhantes, com exceção da ML que apresentou 108% a mais proteína na papinha caseira.

Os lipídios variaram de 10% na FT à 93% na ML. Para as fibras, a variação foi de 0% na MMC à 51% na ML, enquanto para os carboidratos, a diferença foi de 1,94% a 29,9% nas MMC e ML, respectivamente.

As diferenças encontradas entre as papinhas caseiras e as papinhas industrializadas podem ser explicadas pela diferença nos cultivares das plantas utilizadas assim como nos diferentes tipos de processamento ao qual foram submetidas (Randhawa,2012).

As papinhas caseiras passaram apenas pelo processo de trituração, com exceção da cenoura e da batata-doce que foram cozidas. Já as papinhas industrializadas, passam pelos processos de cocção e esterilização, que podem alterar a composição dos alimentos, por exemplo oxidando lipídios. Além disso, a cocção pode resultar em alteração na matriz do alimento, rompendo a estrutura celular e facilitando extração dos nutrientes e compostos bioativos durante as análises ou tornando-os mais bioacessíveis.

A principal limitação destas comparações é o fato de que não utilizamos exatamente os mesmos ingredientes da indústria, apenas nos baseamos nas informações do rótulo quanto aos ingredientes e proporções utilizados, ou seja, não podemos fazer uma comparação direta entre estes valores de composição das papinhas caseiras e industrializadas, pois não se trata das mesmas amostras. Sendo assim, o principal fator associado a diferença na composição centesimal das papinhas caseiras e industrializadas neste estudo é provavelmente o cultivar dos vegetais utilizados para seu preparo, visto que plantas de variedades diferentes ou cultivadas em diferentes condições edafoclimáticas podem apresentar composições diferentes.

Com relação a composição de carotenoides, para as papinhas industrializadas (Capítulo III), o padrão de carotenoide majoritário foi igual ao encontrado nas papinhas caseiras (Capítulo IV), exceto para UB em que o α-caroteno foi o carotenoide majoritário nas papinhas industrializadas, enquanto na papinha caseira foi o β-caroteno.

Ao compararmos as papinhas caseiras com as industrializadas, observamos diferenças em relação a composição de carotenoides. Na papinha de FS industrializada foram detectados isômeros *cis* e epóxidos de carotenos, e menor teor

de carotenoides totais (40,8% a menos) e individuais, como por exemplo de all-*E*-β-caroteno (52,4% a menos) e all-*E*-β-criptoxantina (58,3% a menos) em relação a papinha caseira.

Na papinha FT, a quantidade de carotenoides totais foi semelhante nas duas papinhas (diferença de 1,1%). Porém, a papinha industrializada apresentou maior quantidade de all-*E*-β-caroteno (19,8% a mais) enquanto na papinha caseira foi identificada β-criptoxantina e na industrializada não.

Para a papinha de UB também foi observado maior teor de carotenoides totais na papinha industrializada em relação a caseira (12% a mais), porém, neste sabor, a papinha caseira apresentou maior teor de β-caroteno (153% a mais). Por outro lado, a papinha industrializada apresentou maior diversidade de carotenoides inclusive presença de α- criptoxantina e β-criptoxantina.

Já a papinha industrializada MMC apresentou maior teor de carotenoides totais (diferença de 18,2%) do que a caseira, além da presença de epóxidos de caroteno e do isômero 9-*Z*-β-caroteno. Porém, esta apresentou menor teor de all-*E*-β-caroteno (41,9% a menos), enquanto na papinha caseira foi encontrada β-criptoxantina, que não foi identificada na industrializada.

Por fim, na papinha ML caseira houve maior teor de carotenoides totais (28,7% a mais), além de maior diversidade de carotenoides, com presença da violaxantina nas formas all-t*rans* e *cis* e α-criptoxantina. Porém, a versão industrializada apresentou maior teor de all-*E*-β-caroteno (33,1% a mais) e all-*E*-β-criptoxantina (10,6% a mais).

O processamento realizado pela indústria nas papinhas (moagem, cocção, autoclavagem) afeta a composição dos carotenoides (CILLA et al., 2018). A estocagem também pode afetar a concentração dos carotenoides pela exposição a luz, calor e oxigênio. O processo da estocagem em vidro selado, que ocorre nas papinhas, está relacionado com a prevenção da oxidação dos carotenoides, devido à ausência de oxigênio, porém, ainda podem ocorrer alterações devido a luz e calor (MELÉNDEZ-MARTÍNEZ et al., 2022). Nas papinhas caseiras foi utilizado apenas o processo de moagem, exceto para a amostras que continham cenoura e batata-doce (convencional e orgânica) em que foi feita a cocção desses ingredientes.

O β-caroteno sofre diminuição em sua concentração, principalmente após a cocção que leva a formação de isômeros (O'SULLIVAN et al., 2010; RYAN et al., 2008) Em nosso estudo houve menor teor de all-*E*-β-caroteno em três das papinhas

industrializadas (FS, MMC e UB) e em duas das papinhas caseira (FT e ML). De fato, nessas papinhas foi observado maior teor de isômeros *cis*, como o 13-*Z*-β-caroteno e o 9-*Z*-β-caroteno. Na FS caseira (que apresentou maior teor de β-caroteno em relação a industrializada), por exemplo, não foi verificada a presença de *Z*-β-caroteno, já na FT caseira houve maior concentração dos isômeros *cis.*

O processamento pode também ser benéfico ao romper a parede celular e facilitar a liberação dos carotenoides (MELÉNDEZ-MARTÍNEZ et al., 2022). O simples processo de moagem realizado tanto nas papinhas quanto nas frutas, já leva ao rompimento da célula vegetal e dessa forma pode facilitar a extração dos carotenoides. Porém, os maiores efeitos do processamento podem ser observados na bioacessibilidade dos carotenoides.

A figura 1 mostra a comparação entre a bioacessibilidade das papinhas caseiras e industrializadas. Não foi feita a comparação da papinha UB, pois na amostra caseira não foi possível identificar carotenoides após a digestão. Para o carotenoide all-*E*-α-caroteno, só foi possível fazer a comparação na MMC.

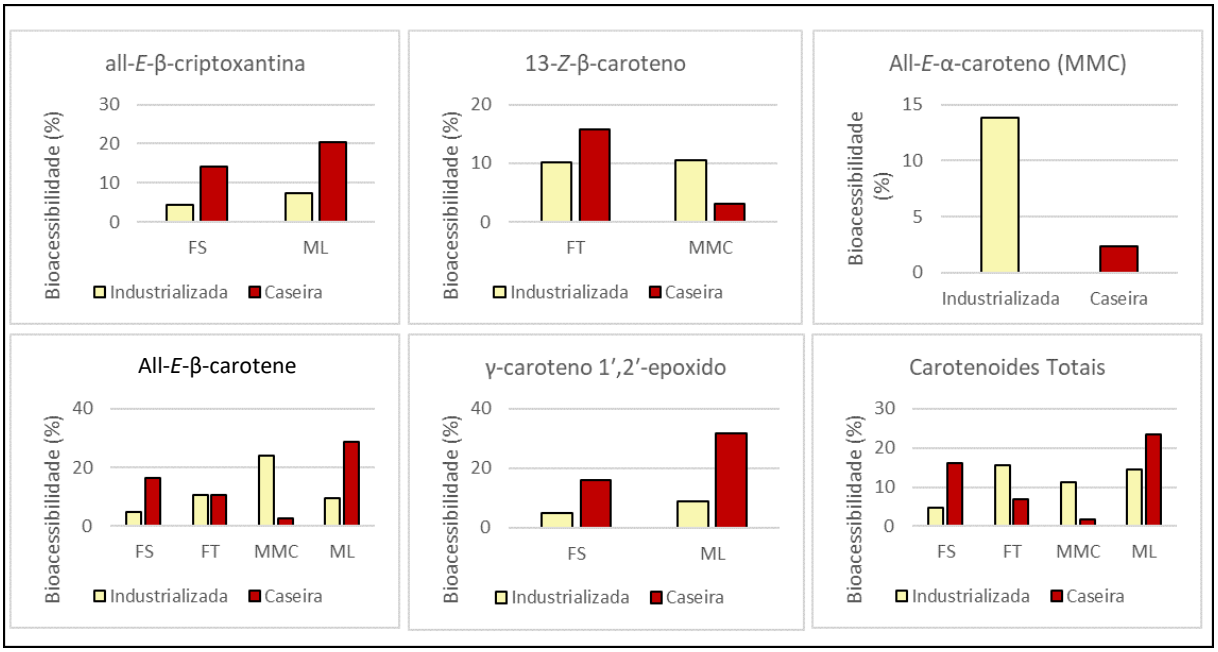


Figura 1. Comparação entre a bioacessibilidade (%) dos carotenoides individuais e totais nas papinhas industrializadas (amarelo) e caseiras (vermelho).
FS: Frutas Sortidas; FT: Frutas Tropicais; MMC: Maçã, manga e cenoura; ML: Mamão e laranja.

Pode-se notar que em FT e MMC houve maior bioacessibilidade dos carotenoides totais nas papinhas industrializadas, enquanto em FS e ML a bioacessibilidade foi maior nas papinhas caseiras. MMC também apresentou maior

bioacessibilidade na sua versão industrializada para o 13-*Z*-β-caroteno, o all-*E*-α-caroteno e o all-*E*-β-caroteno, demonstrando que para esta amostra os processos industriais favoreceram a bioacessibilidade dos carotenoides. Já FS e ML apresentaram maior bioacessibilidade para todos os carotenoides na papinha caseira.

Na papinha UB industrializada a bioacessibilidade dos carotenoides totais foi baixa (1,1%) e na caseira, nenhum carotenoide foi detectado na micela. Como a composição dessa papinha utiliza ingredientes com baixo teor de carotenoides o processo industrial pode ter favorecido a liberação destes da matriz, porém, mesmo assim em pequena quantidade. Enquanto nas papinhas caseiras, sem tratamento térmico, a pequena quantidade de carotenoides na amostra não conseguiu ser liberada da matriz ou micelarizada.

Com relação a all-*E*-β-criptoxantina, as papinhas caseiras tiveram bioacessibilidade maior do que as industrializadas, assim como para o γ-caroteno 1',2'-epóxido.

Considerando o all-*E*-β-caroteno, houve tanto maior bioacessibilidade nas papinhas caseiras (FS e ML), quanto nas industrializadas (MMC), e ainda a papinha FT que apresentou a mesma porcentagem de carotenoides bioacessíveis para os dois tipos de amostras. Portanto, não foi possível determinar uma correlação entre o processamento das papinhas industrializadas e caseiras em relação a bioacessibilidade do all-*E*-β-caroteno.

Quando comparadas com as papinhas industrializadas (Figura 2), as papinhas caseiras UB, FS e MMC apresentaram maior teor de RAE, e valores semelhantes em FT e ML. A maior diferença foi observada na papinha MMC com diferença de 10 RAE. Esta já havia sido a papinha com maior teor de RAE entre as industrializadas, o que se confirmou também entre as papinhas caseiras com um teor ainda maior. Dessa forma, as papinhas caseiras se mostraram melhores escolhas quando considerada a ingestão de carotenoides provitamina A.

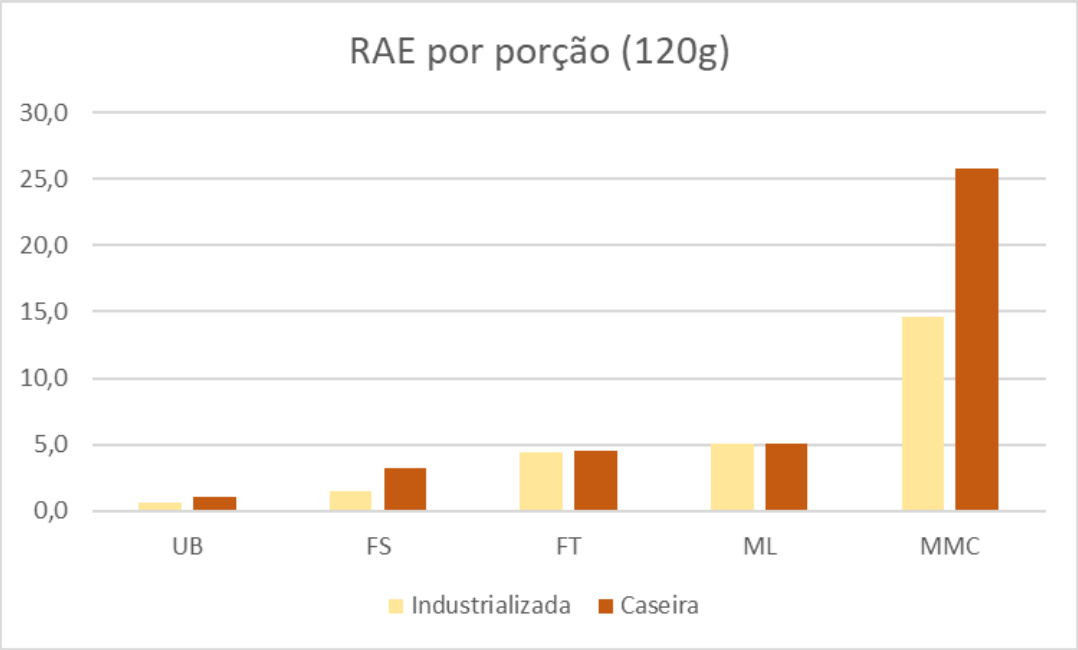


Figura 2. Comparação entre os valores de RAE nas papinhas caseiras e industrializadas.

UB: Uva e banana; FS: Frutas Sortidas; FT: Frutas Tropicais; MMC: Maçã, manga e cenoura; ML: Mamão e laranja.

CONCLUSÃO GERAL

As diferenças na composição observadas entre o rótulo, as papinhas caseiras e as papinhas industrializadas reforçam a importância das análises constantes e atualizações nos rótulos dos produtos, a fim de facilitar a escolha por parte dos consumidores.

Com relação a composição de carotenoides, o all-*E*-β-caroteno foi o carotenoide predominante, sendo identificado em todas as frutas, vegetais e papinhas analisados, tanto antes quanto após a digestão *in vitro* (com exceção da batata-doce orgânica que não apresentou all-*E*-β-caroteno após a digestão). O all-*E*-β-caroteno foi o principal carotenoide provitamina A identificado nesse estudo.

A bioacessibilidade apresentou diferenças entre as papinhas caseiras e industrializadas dependendo do carotenoide. Porém, considerando os carotenoides provitamina A, em geral, as papinhas caseiras apresentaram maior bioacessibilidade, com exceção da MMC que apresentou maior bioacessibilidade na versão industrializada, ou seja, o processamento industrial não favoreceu a bioacessibilidade dos carotenoides apesar do tratamento térmico.

Apesar de as amostras de frutas e vegetais terem apresentado maior bioacessibilidade dos carotenoides do que as papinhas caseiras, estas apresentaram maior valor de RAE e de carotenoides provitamina A do que os vegetais isoladamente, porém, o teor ainda é pequeno em relação a IDR.

Devido ao consumo das frutas e vegetais isoladamente ter apresentado menor teor de carotenoides provitamina A, o consumo das papinhas caseiras seria mais indicado para a ingestão de vitamina A, ou seja, a mistura diferentes vegetais em uma única refeição parece favorecer a ingestão de carotenoides provitamina A.

Porém, apesar dos resultados encontrados, a referência utilizada para cálculo do RAE não considera a bioacessibilidade e, posterior, absorção dos carotenoides provitamina A em diferentes matrizes alimentares, além de possuir mais de 20 anos de sua publicação. Como podemos observar em nosso estudo, as diferenças encontradas na bioacessibilidade podem estar levando a subestimar ou superestimar os valores de RAE e, consequentemente, os valores de ingestão de vitamina A conforme a IDR. Dessa forma, essa diferença, pode afetar tanto as políticas públicas de suplementação quanto a suplementação individual de vitamina A. Por se tratar de uma referência com mais de 20 anos, e por terem surgido diversos estudos sobre bioacessibilidade, bioatividade e biodisponibilidade dos carotenoides neste período, seria interessante uma revisão dos cálculos utilizados para redefinir as referências de RAE e IDR.

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