Universidade Estadual Paulista
"Júlio de Mesquita Filho"

Faculdade de Ciência Farmacêuticas

UMA INVESTIGAÇÃO EXPLORATÓRIA DO CONSUMO CRÔNICO DE SUCO DE LARANJA NA MICROBIOTA INTESTINAL DE MULHERES ADULTAS

Ana Carolina Delgado Lima

Dissertação apresentada ao Programa de Pós-graduação em Alimentos e Nutrição para obtenção do título de Mestre em Alimentos e Nutrição.

Área de concentração: Ciência dos Alimentos

Orientadora: Prof(a).Dr(a).Katia Sivieri

Araraquara
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TÍTULO DA DISSERTAÇÃO: Uma investigação exploratória do consumo crônico de suco de laranja na microbiota intestinal dos indivíduos saudáveis

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DEDICATÓRIA

Aos meus pais, José Humberto e Margarete, aos meus irmãos, minha madrinha Valquíria, meu namorado Gabriel e sua família por sempre acreditarem no meu potencial.

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Sumário

LISTA DE ABREVIATURAS

AGCC	Ácido Graxo de Cadeia Curta
DGEE	Denaturing Gradient Gel Electrophoresis
DP	Desvio Padrão
UFC	Unidade Formadora de Colônia
PCR	Polymerase Chain Reaction
ISL	Ingestão do Suco de Laranja
OMS	Organização Mundial da Saúde
SHIME	Simulator of Human Intestinal Microbial Ecosytem
SFCA	Short-Chain Fatty Acid
POJ	Pasteurized orange juice
Rr	Richeness
UFC	Unidade Formadora de Colônia
CFU	Colony Forming Unity
WHO	World Health Organization

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Capítulo 1: Revisão de Literatura

RESUMO

Introdução: A microbiota intestinal se mantem em equilíbrio (homeostase) durante a fase adulta do ser humano, porém pode se desestabilizar através do estresse, tratamentos quimioterápicos, medicamentos e dietas. A homeostase pode ser alcançada através da ingestão de probióticos, prebióticos e componentes bioativos, tais como os flavonóides cítricos do tipo hesperitina e naringina. Objetivo: Avaliar o efeito da ingestão crônica de suco de laranja pasteurizado (POJ) sobre a microbiota intestinal das mulheres adultas. Material e métodos: foram avaliadas dez mulheres adultas, que ingeriram suco de laranja pasteurizado durante 60 dias, com um período de esmaecimento de 30 dias. As fezes foram coletadas antes da ingestão de suco de laranja (basal) e depois a cada 30 dias até o final do experimento, totalizando 120 dias do período experimental. Durante todo o período experimental, foi realizada a avaliação antropométrica de todos os indivíduos que participaram no estudo. Para a avaliação da microbiota intestinal, foram realizados análises microbiológicas dependente de cultivo usando meios seletivos para bactérias anaeróbias totais, *Lactobacillus* spp., *Bifidobacterium* spp. e *Clostridium* spp. Uma avaliação independente de cultura foi realizada usando a desnaturação Gradient Gel Electrophoresis (DGGE). Para a avaliação dos metabolismos microbiano foram realizadas as análises de pH, amónio (NH4 +) e ácidos graxos de cadeia curta (AGCC).Resultados: A ingestão crônica de suco de laranja não influenciaram nas medidas de composição do corpo, melhorou os parâmetros bioquímicos. O POJ mostrou modulações da composição da microbiota e nas atividades metabólicas. Em particular, os números de *Bifidobacterium* spp e *Lactobacillus* spp nas fezes foram maiores após 60 dias de ingestão POJ. Análise de PCR-DGGE mostrou que a microbiota de todos os indivíduos estudados tinha uma composição semelhante em relação às bactérias totais. Em relação ao metabolismo microbiano, observou-se redução dos valores de NH4 + e aumento ($p \leq 0,05$) na produção de AGCC após 60 dias de ingestão POJ Conclusão: Este estudo demonstrou que crônica ingestão de POJ pode ser uma alternativa eficaz para uma alimentação saudável, com efeito, na modulação da microbiota intestinal de mulheres adultas.

Palavras-Chaves: Prebióticos, microbiota intestinal, AGCC.

ABSTRACT

Introduction: The gut microbiota remains balanced (homeostasis) during the adult stage of the human being, but can be destabilized through stress, chemotherapeutic treatments, medicines and diets. Homeostasis can be achieved through ingestion of probiotic, prebiotic, and bioactive components such as hesperitin and naringin-type citrus flavonoids. Objective: To evaluate the effect of chronic ingestion of pasteurized orange juice (POJ) on the intestinal microbiota of adult women. Material and Methods: Ten adult women were evaluated, which ingested pasteurized orange juice during 60 days, with a washout period of 30 days. The stool was collected before orange juice intake (basal) and then every 30 days until the end of the experiment, totaling 120 days of experimental period. Throughout the experimental period, the anthropometric evaluation of all individuals who participated in the study was performed. For the evaluation of the intestinal microbiota, plate count analyzes were performed using selective media for total anaerobic bacteria, *Lactobacillus* spp., *Bifidobacterium s*pp., and Clostridium spp. An independent culture evaluation was performed using Denaturing Gradient Gel Electrophoresis (DGGE). The pH, ammonium (NH4 +) and short chain fatty acids (SCFA) were evaluated for microbial metabolism. Results: Chronic intake of orange juice did not influence the body composition measurements but improved blood biochemical parameters. POJ showed positive modulations of the microbiota composition and metabolic activity. In particular, numbers of faecal *Bifidobacterium* spp and *Lactobacillus* spp were higher after 60 days of POJ intake. PCR-DGGE analysis showed that the microbiota of all the individuals studied had a similar composition in relation to total bacteria. In relation to the microbial metabolism, it was observed reduction of NH4+ values and increase ($p \leq 0.05$) in the production of SCFA after 60 days of POJ intake Conclusion: This study has shown that chronic intake of POJ can be an effective alternative for healthy eating, with effect on the modulation of the intestinal microbiota of adult women.

Keywords: Prebiotic, Intestinal Microbiota, SFCA.

INTRODUÇÃO

INTRODUÇÃO

Estima-se que o trato gastrointestinal seja habitado por 10^{11} células por mL de conteúdo luminal, compreendendo cerca de 400 a 500 espécies bacterianas (1). A microbiota intestinal é composta por bactérias anaeróbias estritas, que superam as anaeróbias facultativas e bactérias aeróbias (2). Os principais filos mais comuns encontrados são quatros, entre eles os Bacteroidetes, Actinobactérias, Proteobactérias e os Firmicutes. Os gêneros mais comuns encontrados são os *Bifidobacterium spp., Clostridium spp., Eubacterium spp. e Ruminococcus spp.* (3).

A composição da microbiota intestinal é influenciada por fatores imunológicos e dietéticos (2). A dieta possui uma influência muito grande sobre o ambiente do intestino, incluindo o pH, trânsito intestinal e mudanças na microbiota intestinal (1). A microbiota exerce as funções de degradação de componentes não digeríveis da dieta, como as fibras, modulação do sistema imunológico, síntese de vitaminas K e B_{12} e produção ácidos graxos de cadeia curta (AGCC) (4).

Os benefícios à saúde do hospedeiro dependem do equilíbrio entre bactérias comensais e patogênicas (5). Segundo Eckburgg et al. (6), a microbiota intestinal se mantém estável e equilibrada (homeostase) durante a fase adulta, porém pode se desestabilizar através do estresse, tratamentos quimioterápicos, remédios e dietas. No entanto o equilíbrio da microbiota pode se alcançada através da ingestão de alimentos probióticos, ingredientes prebióticos e compostos bioativos, tais como os compostos fenólicos (1). Os compostos fenólicos são transformados pela microbiota intestinal em metabólitos bioativos, os quais podem promover a manutenção da homeostase

intestinal e estimular o crescimento de bactérias benéficas (*Lactobacillus* spp. *e Bifidobacterium* spp.*) e* inibindo o de bactérias patogênicas (*Clostridium* spp.) (7).

Os compostos fenólicos são caracterizados pela presença de um ou mais anéis aromáticos ligados a pelo menos um radical hidroxila Os principais grupos de polifenóis são flavonóides, álcoois fenólicos, estilbenos e lignanas (8). As principais fontes de flavonóides incluem frutos (uvas, cerejas, maçã, frutas cítricas entre outros) e hortaliças (pimenta, tomate, espinafre, cebola e brócolis) (9). Os estudos *in vitro* têm demonstrado que os flavonóides do tipo hesperitina e naringina presente no suco de laranja tem apresentado uma variedade de atividades biológicas, entre elas antioxidantes, antiflamatória, antitumorais, redução de colesterol elevado e aumento da sensibilidade à insulina (10).

O suco de laranja juntamente com as frutas cítricas tem sido muito valorizado como parte essencial de uma dieta nutritiva e saborosa. Os sabores fornecidos pelo suco de laranja e pelas frutas cítricas estão entre os mais preferidos no mundo. Além disso, o suco de laranja e as frutas cítricas são fontes ricas de vitaminas, minerais e fibras alimentares que são essenciais para o crescimento e desenvolvimento nutricional global (11,12).

O consumo diário do suco de laranja está sendo associada com a redução do aparecimento de doenças crônicas, melhora no perfil lipídico, diminuição o colesterol total e marcadores de inflamatórios. Esses efeitos ocorrem devido à presença de compostos bioativos, tais como ácido ascórbico, carotenoides e flavonóides (hesperitina e naringina) presente no suco de

laranja (13,14). Estudos envolvendo a ingestão de suco de laranja e o efeito sobre a microbiota ainda são escassos.

O objetivo desse estudo foi avaliar o efeito da ingestão crônica do suco de laranja pasteurizado sobre a microbiota de mulheres adultas.

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Capítulo 1

REVISÃO DA LITERATURA

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1.1 Microbiota Intestinal

O trato gastrointestinal apresenta o maior número e diversidade de espécies de bactérias que colonizam o corpo humano. Nos indivíduos saudáveis as bactérias são encontradas de forma heterogênea em todo o trato gastrointestinal. O estômago e o intestino delgado apresentam um ambiente desfavorável para a colonização de bactérias devida ação bactericida do suco gástrico, enquanto isso na região do cólon as bactérias encontram condições favoráveis para a sua proliferação devido ao abundante suprimento nutricional e ausência de secreções (1,2).

A colonização bacteriana intestinal inicia-se durante o nascimento, quando o recém-nascido é exposto aos diferentes tipos de bactérias presente na mãe (canal vaginal) e no ambiente (3). O estabelecimento da microbiota intestinal dos recém-nascidos é um processo lento, pois depende de fatores intrínsecos (saúde do recém-nascido) e fatores extrínsecos (dieta da mãe, tipo de parto e tipo de aleitamento) no qual influenciam o processo de colonização e tipo de bactéria (4,5). Por volta dos 2 e 3 anos de idade a composição da microbiota passa a ser estável e igual a de um adulto (6).

Avalia-se que o trato gastrointestinal seja habitado por 10^{11} células por mL de conteúdo luminal, compreendendo cerca de 400 a 500 espécies bacterianas (7). A microbiota intestinal é composta por bactérias anaeróbias estritas, que superam as anaeróbias facultativas e bactérias aeróbias (8). Os principais filos mais comuns encontrados são quatros, entre eles os Bacteroidetes e os Firmicutes. Os gêneros mais comuns encontrados são os

*Bifidobacterium spp., Clostridium spp., Eubacterium spp. e Ruminococcus spp. (*9). A composição da microbiota intestinal é influenciada por fatores imunológicos e dietéticos (8). A dieta possui uma influência muito grande sobre o ambiente do intestino, incluindo o pH, trânsito intestinal e mudanças na microbiota intestinal (7).

A microbiota exerce as funções de degradação de componentes não digeríveis da dieta, como as fibras, modulação do sistema imunológico, síntese de vitaminas K e B12 e produção ácidos graxos de cadeia curta (AGCC) (10). Os AGCC podem ser obtidos de forma endógena (metabolismo de gorduras e carboidratos) ou exógena (absorção dos produtos formados pela fermentação bacteriana), sendo essa última a principal fonte de ACGC no organismo (11).

As fibras provenientes da dieta são compostas, principalmente, por polissacarídeos e em menor parte por oligossacarídeos, no qual não são digeridas pelas enzimas intrínsecas do estômago e do intestino humano. Todavia, grande parte das fibras que passam pelo trato gastrointestinal é degradada pela microbiota intestinal, ocorrendo à formação e liberação de ACGG (ácido acético, ácido propiônico e ácido butírico) como produto do metabolismo microbiano (12). Os AGCC são liberados na luz intestinal e rapidamente são absorvidos no ceco e no cólon, ou seja, a absorção é um processo muito eficiente, sendo que apenas 5% a 10% dos AGCC são excretados nas fezes (13,14). A mucosa intestinal (colonócitos), tecido hepático e o tecido muscular metabolizam e utilizam os AGCC (15).

O ácido acético é o mais abundante, no entanto ele é pouco metabolizado no cólon, pois ele é rapidamente absorvido e transportado para o fígado no qual vai ser utilizado por diversas vias: síntese dos ácidos graxos de

cadeia longa (lipogênese), cetogênese (síntese de corpos cetônicos), glutamina e glutamato (15,16). O ácido propiônico é metabolizado pelo fígado, sendo utilizado na inibição da síntese de colesterol e substrato para a via de formação do piruvato da gliconeogênese (17). Por fim, o ácido butírico é umas das principais fontes de energia para os colonócitos (mucosa intestinal), no qual estimula a proliferação celular do epitélio e intensifica a absorção de sódio e água (18).

Clemente (19) sugere que a microbiota intestinal desempenha um papel fundamental na saúde do hospedeiro, exercendo funções importantes sobre o processo metabólico e o sistema imune. Os benefícios à saúde do hospedeiro dependem do equilíbrio entre bactérias comensais e patogênicas (20). Segundo Eckburgg et al.(21), a microbiota intestinal se mantém estável e equilibrada (homeostase) durante a fase adulta, porém pode se desestabilizar através do estresse, tratamentos quimioterápicos, remédios e dietas.

A manutenção do equilíbrio da microbiota pode se alcançada através da ingestão de alimentos probióticos, ingredientes prebióticos e compostos bioativos, tais como os compostos fenólicos (7).

Os probióticos são microrganismos vivos que, quando administrados em quantidades adequadas, conferem benefícios à saúde do hospedeiro e estimulam o sistema imune a promover resistência gastrointestinal à colonização de patógenos (22,23). Os prebióticos são ingredientes alimentares não digeríveis, que são fermentados de maneira seletiva no cólon, no qual estimula o crescimento de bactérias benéficas deixando a composição da microbiota intestinal mais saudável (24).

De acordo com Dueñas et al. (25) a microbiota intestinal pode transformar os compostos fenólicos em metabólitos bioativos, os quais podem promover uma manutenção da homeostase intestinal e estimular o crescimento de bactérias benéficas (*Lactobacillus* spp. *e Bifidobacterium* spp.*) e* inibindo o de bactérias patogênicas(*Clostridium* spp.).

1.2 Compostos Fenólicos

Os compostos fenólicos são caracterizados pela presença de um ou mais anéis aromáticos ligados a pelo menos um radical hidroxila ou outros substitutos e podem ser divididos de acordo com o número de anéis fenólicos e com as estruturas às quais estão ligadas. Os principais grupos de polifenóis são flavonóides, álcoois fenólicos, estilbenos e lignanas (Figura1) (26).

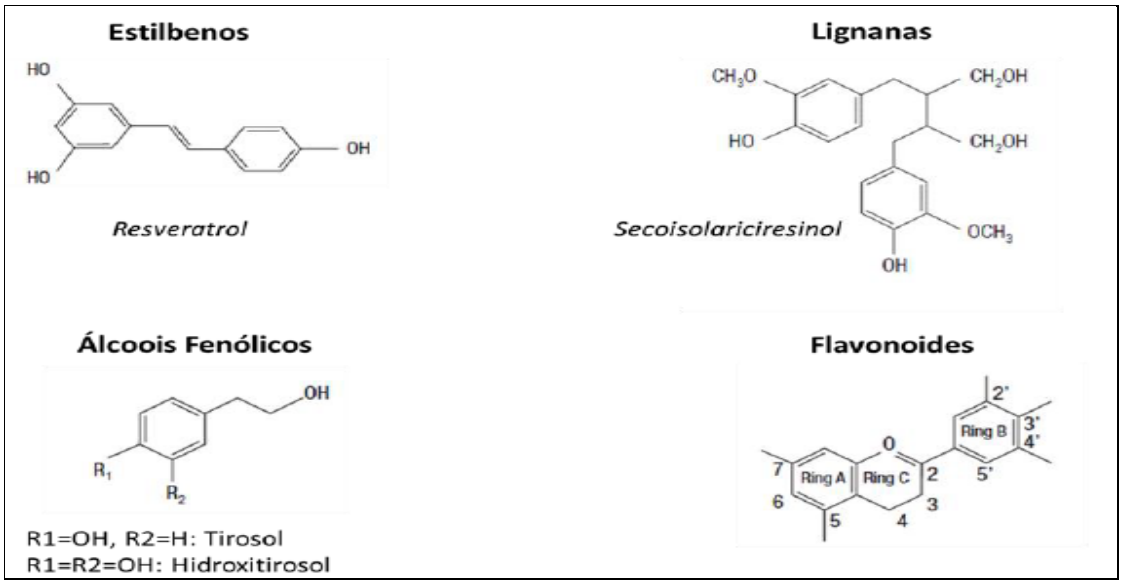

Figura 1: Estruturas químicas de polifenóis (26).

Os flavonóides são metabólitos secundários de plantas comumente encontradas nos frutos e nos vegetais consumidos regularmente por seres humanos (27). São pigmentos naturais importantes e tem como função principal proteger o organismo contra agentes oxidantes (28). Os flavonóides são constituídos por três anéis, onde os seus carbonos podem sofrer variações

químicas, como hidroxilação, hidrogenação metilação e sulfonação, proporcionando a formação de mais de quatro mil compostos flavonóides, que são agrupados em classes (29).

As principais fontes de flavonóides incluem frutos (uvas, cerejas, maçã, frutas cítricas entre outros) e hortaliças (pimenta, tomate, espinafre, cebola e brócolis) (30). E suas principais classes são as flavonas, isoflavonas, flavonóis e flavononas (31).

Em relação à estrutura química, os flavonóides são formados por um esqueleto de dois anéis aromáticos comumente designados como A e B, sendo as flavonas conectadas por um anel pirora (anel C) e as flavanonas por um anel dihidropirona (anel C) (Figura 2) (32).

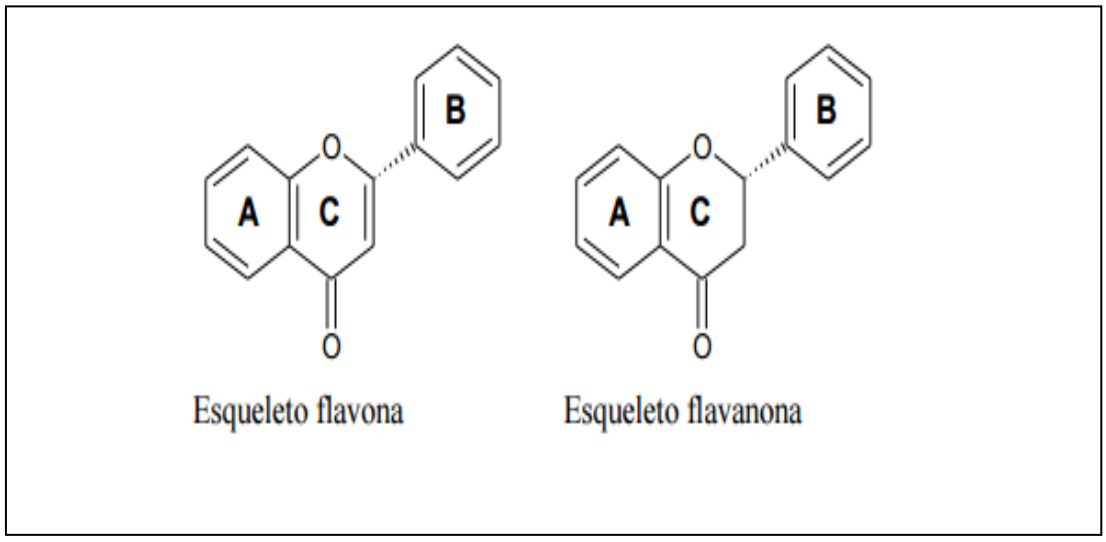

Figura 2: Estruturas químicas de duas classes de flavonóides (32).

A estrutura das flavonas e das flavanonas presentes nos flavonóides cítricos podem ser classificadas como agliconas ou glisídicos (32). As principais flavanonas são a hesperitina e naringina, presentes em frutas cítricas (33).

A absorção dos flavonóides depende da presença ou ausência do carboidrato na estrutura química, além disso, os flavonóides cítricos com a

hesperidina e a naringenina presente no suco de laranja, são compostos glicosilados que sofrem reação de hidrólise para a formação de suas angliconas (hesperitina e naringina) posterior à absorção (34).

Os flavonóides são degradados por enzimas presente na microbiota intestinal resultando em uma diversidade de metabólitos fenólicos bioativos, que são em seguida são absorvidos (35,25). Na parede intestinal, os flavonóides sofrem reações de conjugação, tais como glicuronidação e metilação (36,37) e são conduzidos dos enterócitos ao fígado pela circulação entero-hepática, onde sofrerão outras reações, como metilação, sulfatação e glicuronidação, formando metabólitos fenólicos (38, 39, 40,). Contudo os metabólitos podem ser excretados pela bile ou pela urina dependendo do metabólito formado (Figura 3) (42).

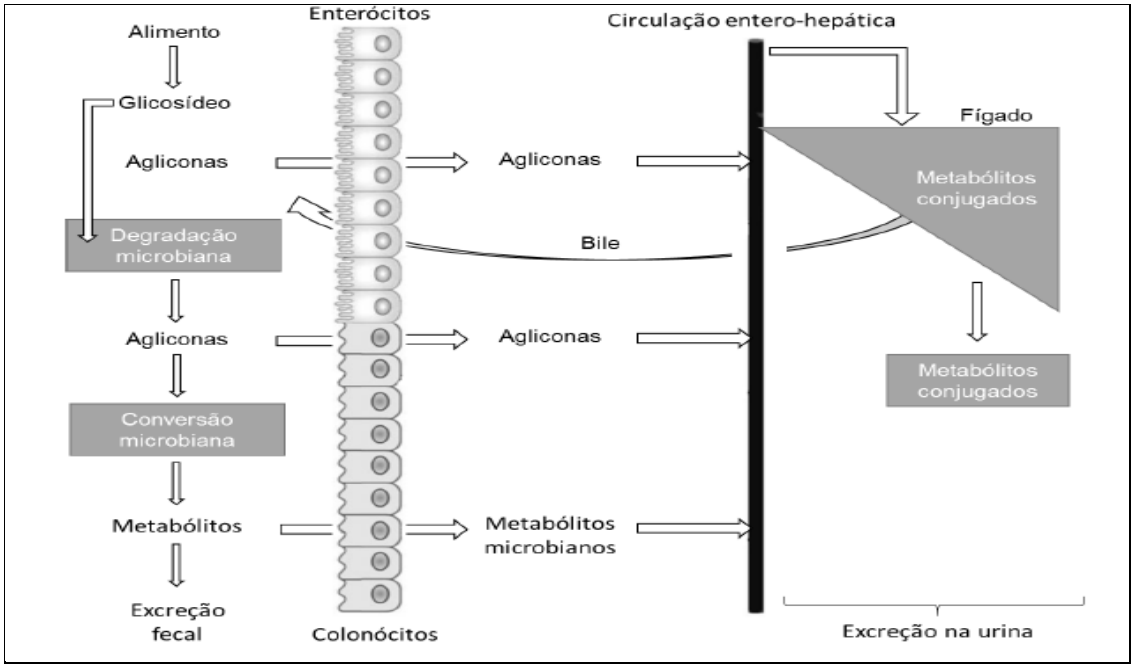

Figura 3: Metabolismo dos polifenóis do tipo flavonóides (42).

Os flavonóides das frutas cítricas são resistentes ao suco gástrico do estômago, sendo esses absorvidos no trato gastrointestinal após deglicosilação por enzimas de bactérias intestinais (43).Os estudos *in vitro* têm demonstrado

que os flavonóides do tipo hesperitina e naringina presente no suco de laranja tem apresentado uma variedade de atividades biológicas, entre elas antioxidantes, antiflamatória, antitumorais, redução de colesterol elevado e aumento da sensibilidade à insulina (44).

1.3 Suco de Laranja

O suco de laranja juntamente com as frutas cítricas tem sido muito valorizado como parte essencial de uma dieta nutritiva e saborosa. Os sabores fornecidos pelo suco de laranja e pelas frutas cítricas estão entre os mais preferidos no mundo. Além disso, o suco de laranja e as frutas cítricas são fontes ricas de vitaminas, minerais e fibras alimentares que são essenciais para o crescimento e desenvolvimento nutricional global (45,46).

Segundo a FAO (45), o suco de laranja é uma ótima fonte de vitamina C e outros nutrientes essenciais como carboidratos glicêmicos, fibras, potássio, folato, cálcio, tiamina, niacina, vitamina B6, fósforo, magnésio, cobre, riboflavina, e compostos fenólicos.

O consumo diário do suco de laranja está sendo associada com a redução do aparecimento de doenças crônicas, melhora no perfil lipídico, diminuição o colesterol total e marcadores de inflamatórios. Esses efeitos ocorrem devido à presença de compostos bioativos, tais como ácido ascórbico, carotenoides e flavonóides (hesperitina e naringina) presente no suco de laranja (47,48).

No quadro 1 pode-se observar resultados de recentes estudos clínicos realizados com suco de laranja. Observa-se que os principais efeitos do consumo crônico ou agudo de suco de laranja são: redução do colesterol total e do lipoproteínas de baixa densidade (LDL), aumento da atividade

psicomotora e da atividade antioxidante, diminuição da pressão arterial sistólica e diastólica, perda de peso e aumento do aporte da vitamina C.

Estudos envolvendo a ingestão de suco de laranja e o efeito sobre a microbiota ainda são escassos. Estudos *in vivo* ou *in vitro* mostrando a ingestão de suco de laranja estão sendo apresentados no quadro 2, no qual mostra que a ingestão de suco de laranja aumentou a população bacteriana de *Lactobacillus* spp., *Bifidobacterium* spp., e diminuiu a de Enterobactérias. Além disso, os estudos mostraram que o suco aumentou a produção de ácidos graxos de cadeia curta e diminui o conteúdo de íons amônio. O consumo regular de suco de laranja pasteurizado (POJ) pode modular a microbiota intestinal de mulheres adultas.

Quadro 1: Estudos clínicos, experimental e in vitro sobre o efeito da ingestão crônica ou aguda do suco de laranja.

Estudo	Resultado	Tipo de Estudo	Referência
Investigar a resposta pós prandial dos marcadores lipídicos em uma refeição rica em gorduras associado com dois tipos de bebida (água e suco de laranja) em mulheres com peso normal e sobrepeso/obesidade.	A ingestão de suco prolongou a lipemia pós prandial em mulheres com sobrepeso/obesidade.	Estudo agudo. 2 dias.	(49)
O efeito do consumo de suco de laranja no perfil lipídico do sangue e dos índices da síndrome metabólica.	O suco de laranja não afetou a sensibilidade à insulina, aos lipídeos circulantes e ao peso corporal.	Estudo crônico. 12 semanas	(50)
O suco de laranja rico em flavonóides associados com a melhoria aguda na função cognitiva em homens da meia idade.	O desempenho da função executiva e psicomotora foi significativamente melhor após o consumo de suco de laranja.	Estudo agudo. 2 dias	(51)
O suco de laranja vermelha na melhoria dos fatores de risco para síndrome metabólica.	Não teve alteração na obesidade abdominal, diminuiu a pressão arterial sistólica nos indivíduos normais e a diastólica em indivíduos com excesso de peso e teve um aumento da atividade antioxidante.	Estudo crônico. 8 semanas	(52)
Consumo crônico do suco de laranja rico em flavonóides associados á benefícios em idosos saudáveis.	O consumo de suco de laranja rico em flavonóides foi benéfico para a função cognitiva em idosos saudáveis.	Estudo crônico. 8 semanas	(53)
100% do consumo de suco de laranja estão associado a uma melhor qualidade da dieta, maior adequação de nutrientes, menor risco de obesidade e melhor saúde nos adultos.	Os resultados sugerem que o consumo moderado de 100% de suco de laranja ajuda os indivíduos a satisfazer as recomendações diárias do USDA.	Estudo agudo 1 dia	(54)
Influência da ingestão crônica do suco de laranja na pressão arterial e na composição corporal.	O consumo regular de suco de laranja aumentou significativamente o aporte de vitamina C na dieta. O suco e seus componentes auxiliam na prevenção da hipertensão e em menor prevalência de sobrepeso e obesidade.	Estudo crônico 13 meses	(55)
Um extrato rico em flavonóides de suco de laranja reduziu o estresse oxidativo em um modelo experimental de doença inflamatória intestinal	Os resultados mostraram que o suco de laranja pode ser utilizado como um alimento funcional em caso de inflamações causadas	Estudo agudo 4 dias	(56)

	pelo estresse oxidativo colônico. Uma nova abordagem saudável para evitar colite ulcerativa .		
Investigação de citocinas, estresse oxidativo, metabólico e biomarcadores inflamatórios após consumo de suco de laranja por indivíduos normais e com excesso de peso.	Redução do colesterol total e LDL, aumento do efeito anti-inflamatório e da atividade antioxidante. Redução da peroxidação lipídica em indivíduos normais e com excesso de peso.	Estudo crônico 8 semanas	(57)
Suco de laranja aliado a uma dieta de calorias reduzidas resulta em perda de peso e melhora os biomarcadores relacionados à obesidade.	O suco de laranja associado com dieta de redução de calorias não inibiu a perda de peso, no entanto melhorou a sensibilidade á insulina, o perfil lipídico e o estado inflamatório.	Estudo crônico 12 semanas	(58)
O consumo de suco de laranja pode levar a redução do estresse oxidativo e pode melhorar o sistema de defesa antioxidante.	Os resultaram mostraram que o consumo de suco de laranja com altas ou normais concentrações de polifenóis protegeu o DNA contra danos e a peroxidação lipídica, modificou várias enzimas antioxidantes. Além disso, houve perda de peso nos adultos obesos e não obesos.	Estudo crônico 12 semanas	(59)
Efeito da suplementação com oligofrutose (prebiótica) ou ácido ascórbico sobre a viabilidade de Lactobacillus paracasei spp.	Os sucos com suplementação de ácido ascórbico ou oligofrutose apresentaram características físico-químicas, aceitação e estabilidade de armazenamento igual ao suco de laranja puro. Contudo o ácido ascórbico e a oligofrutose não apresentaram efeito protetor nas culturas probióticas durante o armazenamento porem mostraram uma viabilidade de cultura probiótica superior a 10^6UFC/mL.	Estudo agudo 28 dias	(60)

Quadro 2: Estudos in vitro ou vivo do efeito da ingestão do suco de laranja sobre a microbiota intestinal

Estudo	**Resultado**	**Tipo de Estudo**	**Referência**
Efeito da formulação do suco de laranja sobre a funcionalidade prebiótica utilizando um sistema colônico in vitro.	O suco controle não apresentou alteração significativa nas populações bacteriana. O suco com adição de galactooligossacarídeo apresentou um aumento para *Bifidobacterium* spp., e diminui *Clostridum* spp. Por fim o suco com adição de açúcar apresentou um aumento de *Bifidobacterium* spp., e diminuiu *Clostridium* spp.	Estudo Agudo 2 dias	(61)
Investigar a capacidade de duas bactérias probióticas em catalisar as flavononas cítricas presente no suco de laranja.	Os resultados demonstraram que o potencial enzimático de *Bifidobacterium* spp., e *Lactobacillus* spp., podem estar envolvido no catabolismo do cólon de flavononas presente no suco de laranja	Estudo Agudo 48 horas	(62)
O suco de laranja (polifenóis) é altamente biodisponível em seres humanos.	Após a ingestão de suco de laranja houve um aumento significativo na quantidade de catabólitos urinários	Estudo Agudo 1 dia	(63)
Absorção, metabolismo e excreção do suco de laranja fermentado em ratos.	A urina apresentou 48,2% do ácido 4-O-glucósido de ácido ferúlico. Isto indica que o hidroxicinamato é mais biodisponível do que as flavononas em modelo de rato.	Estudo Agudo 36 horas	(64)
Influência do suco de laranja na microbiota intestinal humana.	Suco de laranja fresco: Aumentou *L*actobacillus spp., *Enterococcus* spp., e *Clostridium* spp., e diminui enterobactérias. Suco de laranja pasteurizado: Aumentou L*actobacillus* spp., e diminui enterobactérias..Aumento de ácidos graxos de cadeia curta e diminuição do conteúdo de íons amônio no tratamento com os sucos de laranja fresco e pasteurizado.	Estudo Crônico 28 dias	(65)

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Capítulo 2

AN EXPLORATORY INVESTIGATION OF CHRONIC CONSUMPTION OF ORANGE JUICE ON GUT MICROBIOTA OF ADULT WOMEN

An exploratory investigation of chronic consumption of orange juice on gut microbiota of adult women

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ABSTRACT

Introduction: The gut microbiota remains balanced (homeostasis) during the adult stage of the human being, but can be destabilized through stress, chemotherapeutic treatments, medicines and diets. Homeostasis can be achieved through ingestion of probiotic, prebiotic, and bioactive components such as hesperitin and naringin-type citrus flavonoids. Objective: To evaluate the effect of chronic ingestion of pasteurized orange juice (POJ) on the intestinal microbiota of adult women. Material and Methods: Ten adult women were evaluated, which ingested pasteurized orange juice during 60 days, with a washout period of 30 days. The stool was collected before orange juice intake (basal) and then every 30 days until the end of the experiment, totaling 120 days of experimental period. Throughout the experimental period, the anthropometric evaluation of all individuals who participated in the study was performed. For the evaluation of the intestinal microbiota, plate count analyzes were performed using selective media for total anaerobic bacteria, *Lactobacillus* spp., *Bifidobacterium* spp., and Clostridium spp. An independent culture evaluation was performed using Denaturing Gradient Gel Electrophoresis (DGGE). The pH, ammonium (NH4 +) and short chain fatty acids (SCFA) were evaluated for microbial metabolism. Results: Chronic intake of orange juice did not influence the body composition measurements but improved blood biochemical parameters. POJ showed positive modulations of the microbiota composition and metabolic activity. In particular, numbers of faecal *Bifidobacterium* spp and *Lactobacillus* spp were higher after 60 days of POJ intake. PCR-DGGE analysis showed that the microbiota of all the individuals studied had a similar composition in relation to total bacteria. In relation to the microbial metabolism, it was observed reduction of NH4+ values and increase ($p \leq 0.05$) in the production of SCFA after 60 days of POJ intake Conclusion: This study has shown that chronic intake of POJ can be an effective alternative for healthy eating, with effect on the modulation of the intestinal microbiota of adult women.

Keywords: Prebiotic, Intestinal Microbiota, SFCA.

1. Introduction

It is estimated that the gastrointestinal tract is inhabited by 10^{11} cells per mL of luminal contents, comprising of 400 to 500 bacterial species (1). The gut microbiota is composed of strict anaerobic bacteria, which outweigh the facultative anaerobic and aerobic bacteria (2). The main phyla found microbiota are Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria. The most common genera found are the *Bifidobacterium* spp., *Clostridium* spp., *Eubacterium* spp., and *Ruminococcus* spp. (3).

The composition of the intestinal microbiota is influenced by immunological and dietetic factors (2). The diet has a very big influence on the environment of the intestines, including pH, intestinal transit and changes in intestinal microbiota (1). The microbiota exerts the functions of degradation of non-digestible dietary components, such as fiber, modulation of the immune system, synthesis of vitamins K and B12 and producing short-chain fatty acids (SCFA) (4).

The benefits to the health of the host depends on the balance between commensal and pathogenic bacteria (5). According to Eckburgg et al. (6), the intestinal microbiota is steady and balanced (homeostasis) during adulthood, but can destabilize through stress, chemotherapy treatments, remedies, and diets. However the balance of microbiota can be achieved through the ingestion of probiotic foods, prebiotic ingredients and bioactive compounds, such as phenolic compounds (1). The phenolic compounds are transformed by the intestinal microbiota in bioactive metabolites, which may promote the maintenance of intestinal homeostasis and stimulate the growth of beneficial

bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.) and by inhibiting pathogenic bacteria (*Clostridium* spp.) (7).

The phenolic compounds are characterized by the presence of one or more aromatic rings linked to at least one hydroxyl radical. The main group of polyphenols are flavonoids, phenolic alcohols, stilbenes and lignans (8). The main sources of flavonoids include fruits (grapes, cherries, apples, citrus fruits, among others) and vegetables (pepper, tomato, spinach, onion and broccoli) (9). In vitro studies have shown that flavonoids of type hesperitin and naringin present in orange juice has presented a variety of biological activities, among them antioxidants, anti-inflammatory, antitumor, reduction of high cholesterol and increased insulin sensitivity (10).

The orange juice along with citrus fruits has been highly valued as an essential part of a tasty and nutritious diet. The flavors provided by orange juice and citrus fruits are among the most popular in the world. In addition, the orange juice and citrus fruits are rich sources of vitamins, minerals and dietary fiber that are essential for the growth and development of global nutrition (11, 12).

The daily consumption of orange juice is being associated with a reduction in the onset of chronic diseases, improved lipid profile, and decreased of the total cholesterol and inflammatory markers. These effects occur due to the presence of bioactive compounds, such as ascorbic acid, carotenoids and flavonoids (hesperitin and naringin) present in orange juice (13, 14). This is a first clinical trial reported on the intake of orange juice and the effect on the gut microbiota. Using a clinical trial we evaluated the effect of chronic intake of

pasteurized orange juice on the microbiota composition and metabolism of eutrophic healthy individuals.

2. Material and methods

2.1 Individuals

To evaluate the influence of the effect of chronic intake of pasteurized orange juice on the intestinal microbiota, 10 women were recruited among students and employees of the Faculty of pharmaceutical sciences of the Universidade Estadual Paulista (UNESP), selected among women with normal nutritional state, according to the classification of the World Health Organizatio (15). Individuals have framed the following inclusion criteria: women of 18 to 55 years, no smoking, apparently healthy, without the presence of diseases, no pregnant women, without regular use of medications, balanced diet (no food restriction, vegetarian diet, macrobiotics, etc.), without use of vitamins or dietary supplements, not be using probiotics or prebiotics in the last 3 months and antibiotics in the last 6 months. Before starting the study all participants signed an informed consent.

2.2 Orange juice

The pasteurized orange juice was provided by Citrosuco (Matão, Brazil), observing that the juice was 100% not from concentrate, pasteurized and no added sugar. The orange juice that was provided to the participants was bottling in transparent PET bottles, without any identification of the product. The juice was stored in bottles of 1L at -20° C and distributed weekly to the volunteers, who were responsible for thawing and consuming the juice at home, on volume of 300 ml.

2.3 Experimental protocol

Non-randomized study with temporal series intergroup design, with a basal time of 30 days, 60 days of orange juice consumption (300 ml/day) and washout of 30 days is totalizing 120 days trial period (Figure 1). All participants did not eat fruit and citrus juices or alcohol three days before the start of treatment with the juice. The project was approved by the Ethics Committee of the Faculty of pharmaceutical sciences of the Universidade Estadual Paulista (UNESP) (opinion of the Ethics Committee 1,644,906) and registered in Clinical Trials (ID: NCT 03032861).

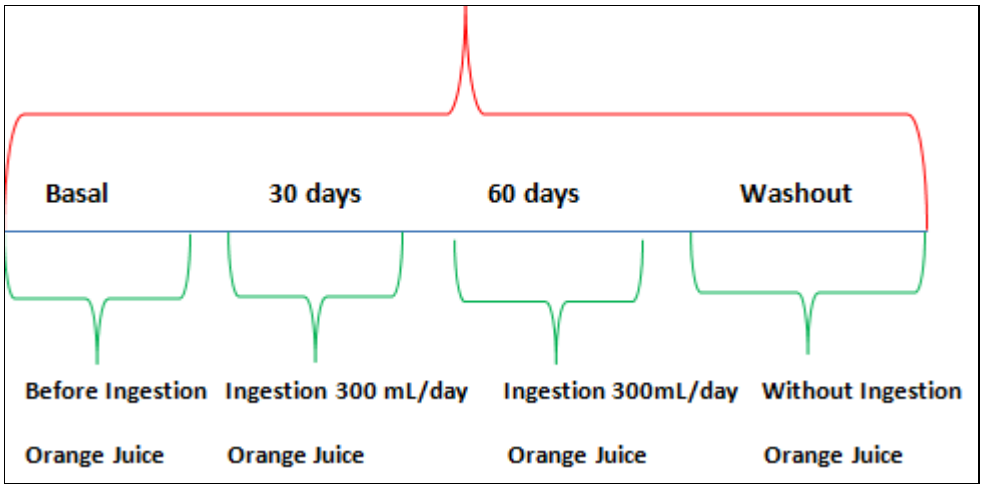


Figure 1: Model of the experimental design used with the women

2.4 Body composition

Measurements of body weight (kg), height (m), muscle mass (kg), fat mass (kg) and body fat percentage (%) were measured during the experimental protocol. For obtaining the weight, muscle mass, fat mass and percentage of body fat, we used the equipment of bioimpedance (Inbody 720 ®). The body mass index (BMI) was calculated by using the weight/height ratio 2, being considered a BMI ≤ thinness 18.5 kg/m2, eutrophy BMI between 18.5 -24.9

kg/m2, overweight BMI between 25.0 -29.9 Kg/m2 and obesity a BMI ≥ 30 Kg/m2, according to the World Health Organization's proposal.

2.5 Stool collection

Samples of fecal from the last 24 hours were collected the day before the beginning of the experiment (basal) and then every 30 days until the end of the experiment, 30 and 60 days of juice ingestion and without juice ingestion (washout),(Figure 1). The samples were stored at -20 ° C until analysis.

2.6 Microbiological analysis

The intestinal microbiota composition analysis was based on the enumeration of *Lactobacillus* spp., *Bifidobacterium* spp., total anaerobes and *Clostridium* spp. Serial dilutions in sterile peptone water were prepared and the inoculation held in selective growth media. Total anaerobic bacteria were determined by Standard Methods ‘agar’ and incubation at 37° C/48 h for anaerobic form, respectively, MRS agar with incubation at 37° C/48 h, anaerobically, was used to determine the number of *Lactobacillus* spp., agar BIM-25 with an incubation at 37° C/72 h, anaerobically, was used to determine the number of *Bifidobacterium* spp., and the Reinforced Clostridial agar 37° C/48 h for anaerobic form was used to determine the number of *Clostridium* spp.

Molecular methods (PCR–DGGE) were used to analyse the the effects of the orange juice ingestion on the community of total bacteria. The DNA of the samples used was extracted through the Fast kitQIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The initiators used to replicate the DNA were 968FGC (5 '-CCG GCC GCC GCC GGG CGC GGC GCA GCG GGG GGG GGG CGG CGC GAA GAA GAA CCT TAC-3 ') and 1401R (5 '-CGG TGT GTA

CAA GAC CC-3 ') (16). For the polymerization of DNA GoTaq ® Green Master Mix (Promega, USA) was used, the samples were then amplified in a thermal cycler (Applied Biosystems, USA) using the following conditions: initial denaturation at 95° C for 7 min; 35 cycles of denaturation at 94° C for 45 s, annealing at 56° C for 45 s, extension to 72° C for 1 min and final extension 72° C for 10 min, followed by cooling to 4° C. For electrophoresis an 8% polyacrylamide gel with a denaturing gradient of 45-65% was used during 16 hours at 75 V in a buffer ATE 1x at constant temperature of 60° C, as described by Dos Reis et al.,(17). The gels were stained with ethidium bromide as (18) and scanned (400 dpi) and analyzed using the BioNumerics software, version 6.0 (Applied Maths, Belgium). Distance matrices for each DGGE were based on the similarity coefficient of Pearson. These matrices were used for cluster analysis. The analysis was performed using the BioNumerics software, version 6.0.

2.7 Determination ammonium (NH_4^+), short-chain fatty acids (SCFAs) and pH in fecal samples

NH_4^+ was determined using ion-selective meter (model 710A, Orion) coupled to the ion-selective electrode of ammonium chloride (model 95-12, Orion). The samples were added to 0.5 mL of solution pH and ionic strength adjustment (Ammonia pH Adjusting Ionic Strength Adjuster Orion) and had the content of ammonium ions measured three times (19). Concentrations of SCFAs were determined in a 1:19 dilution of 100 µL supernatant. The SCFAs were analyzed using a gas 2010 Chromatograph Model (Shimadzu, Japan) equipped with a split/splitless injector, a flame ionization detector and an automatic sampler CombiPAL for head space analysis. The SCFAs were separated using a HPINNOWAX column (30 m x 0.25 mm x 0.25 µm) (Agilent Technologies, USA). The carrier gas was hydrogen and the flow rate was 1.45 mL/min. The temperature of the injector and detector was 240° C (20).

The analysis of pH was made from 1 gram (the same used to make ammonium chloride), in which the same was diluted in 9 mL of distilled water, measured by a pH electrode.

2.8 Gastrointestinal symptoms and bowel function scores

Weekly diary was applied to record the individual gastrointestinal symptoms (diarrhea, abdominal pain, bloating, nausea, vomiting, flatulence and blood in stool). During the entire study period, the participants were required to record the bowel function (consistency of stool, frequency of bowel movements, effort to evacuate and painful bowel movements). The consistency of the stool was classified by the Bristol stool form modified, 0 (watery) .1 (smooth) .2 (segmented) and 3 (dry). The average frequency of evacuations was calculated by the number of stools per week, 1 (daily) .2 (every other day) and 3 (less than 3 times a week). The effort to evacuate was classified as 1 (very easy), 2 (easy), 3 (hard) or 4 (very hard). And painful evacuations were classified as 2 (rarely) .3 (a few times) and 4 (always).

3. Statistical analysis

The results are presented in the form of medium ± DP and both were divided by time, in which they were subjected to repeated measures analysis of variance and Tukey average post-test with significance level $p < 0.05$. We used the program Sigma Stat statistical 5.0.

4. Results

4.1 Body composition

During all experimental period there was no change in weight and BMI (Table 1).

Table 1: Mean values and standard deviation of anthropometric data of women during 120 days of trial period.

Anthropometric Variables	Basal	30 days	60 days	Washout
Weight (kg)	63.4 ± 9.63	63.5 ± 9.23	63.6 ± 9.50	63.7 ± 9.01
BMI (kg/m^2)	24.1 ± 3.28	24.1 ± 3.12	24.1 ± 3.26	24.2 ± 3.04
Lean muscle mass(kg)	38.7 ± 4.31	39.3 ± 4.45	38.8 ± 4.52	39.6 ± 4.65
Lean body mass (kg)	22.3 ± 2.74	22.5 ± 2.77	22.4 ± 2.85	22.8 ± 2.88
Body fat mass(Kg)	23.0 ± 5.09	22.7 ± 5.03	23.3 ± 5.23	22.4 ± 4.85
%Body Fat	34.5 ± 5.13	33.9 ± 5.25	34.6 ± 5.13	33.5± 5.25

Basal: before juice ingestion, 30 and 60 days: juice ingestion, Washout: without juice ingestion. n=10.

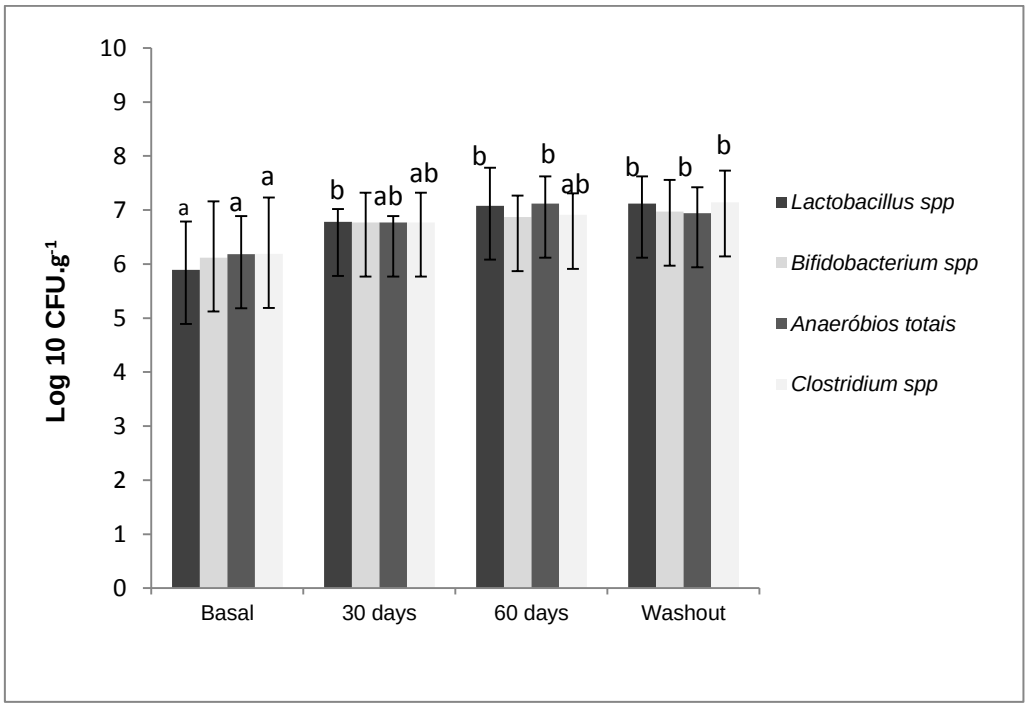
4.2 Effect of orange juice on the growth of intestinal bacteria

Levels of *Lactobacillus* spp., *Bifibobacterium* spp., total anaerobic and *Clostridum* spp., in women can be observed in Figure 2. Changes in intestinal microbiota of women were evaluated before (Basal), during (30 days and 60 days juice ingestion) and without of orange juice ingestion (Washout).

During the intake of orange juice there was an increase ($p < 0.05$) from 5.89 ± 0.90 $\log_{10}$ CFU to 7.12 ± 0.50 $\log_{10}$ CFU of *Lactobacillus* spp., population (Figure 2). There was an increase of 6.97 ± 0.84 to 6.12 ± 0.36 of $\log_{10}$ CFU for *Bifidobacterium* spp., (Figure 2). There was also a significant increase 6.18 ± 0.71 $\log_{10}$ CFU for 7.12 ± 0.50 $\log_{10}$ CFU of total anaerobic bacteria and after the period of ingestion occurred a reduction of this population 6.94 ± 0.48 $\log_{10}$ CFU, but showed no significant difference.

The counts for *Clostridium* spp., showed that there was a significant increase ($p < 0.05$) from 6.19 ±1.04 $\log_{10}$ CFU to 7.14 ± 0.59 $\log_{10}$ CFU

throughout the experimental period. The results suggest that the pasteurized orange juice may stimulate the growth of *Lactobacillus* spp., *Bifidobacterium* spp., and *Clostridium* spp., on intestinal microbiota of women.



Different letters represent significant difference ($p < 0.05$) between periods of experiment, for the same microorganisms. Basal: before juice ingestion, 30 and 60 days: juice ingestion, Washout: without juice ingestion. n=10.

Figure 2: Average population (± DP) expressed as $\log_{10}$ CFU.g^{-1} of different bacterial groups in the feces of women during the experimental protocol. n = 10.

The DGGE analysis was used to monitor qualitative changes in the composition and structure of total bacteria in the stool on four women during the all experimental protocol. A cluster analysis of the DGGE profiles showed that great similarity among the four women (73 at 93%) independent of experimental period (Figure 3).

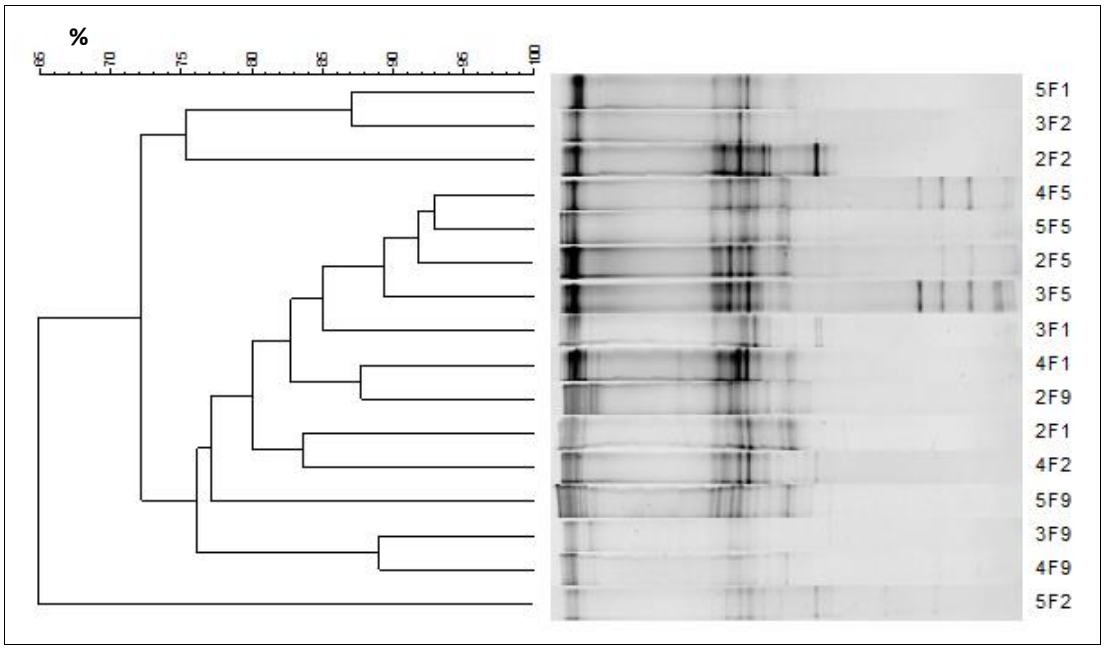
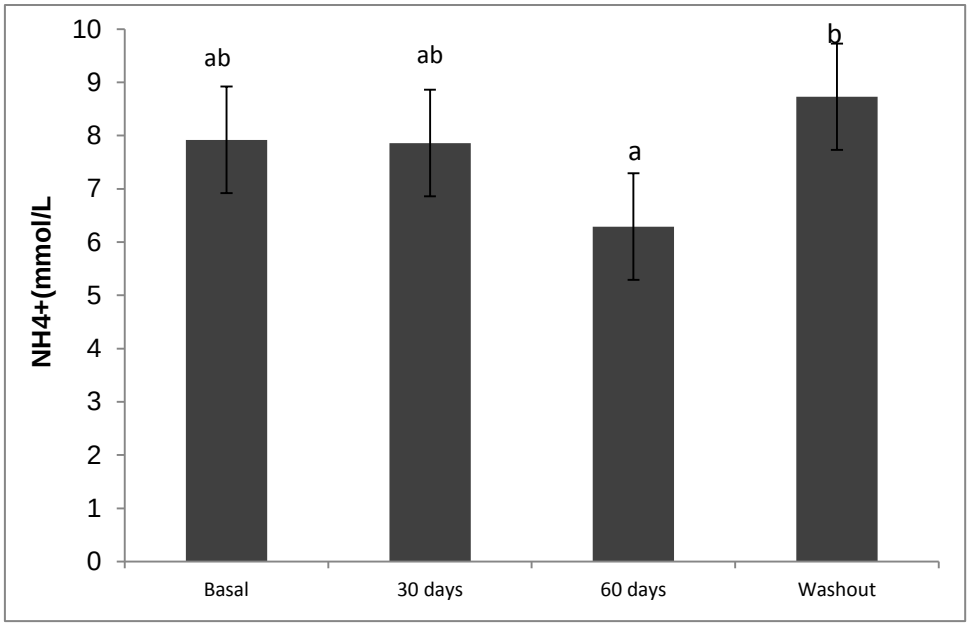


Figure 3: Analysis of denaturant gradient gel electrophoresis (DGGE) of total bacteria show the four women (F1, F2, F5 and F9), during treatment with orange juice (2 = Basal; 3 = 30 days juice ingestion; 4 = 60 days juice ingestion) and without juice ingestion (5 = Washout).

4.3 Effect of orange juice on the intestinal bacteria metabolism

Figure 4 shows a reduction of $NH4^+$ after 60 days of juice ingestion.



Different letters represent significant difference ($p < 0.05$) between the different periods of experiment, for the same microorganism. Basal: before juice ingestion, 30 and 60 days: juice ingestion, Washout: without juice ingestion. n=10.

Figure 4: Average and standard deviation of concentration of ammonium ions (mmol/L) evaluated in the feces of women during the trial period.

The production of acetic acid showed an increase ($p < 0.05$) throughout the experimental period, and increase ($p < 0.05$) of butyric and propionic acid after 30 days of orange juice intake and decrease ($p < 0.05$) after the period without juice ingestion.

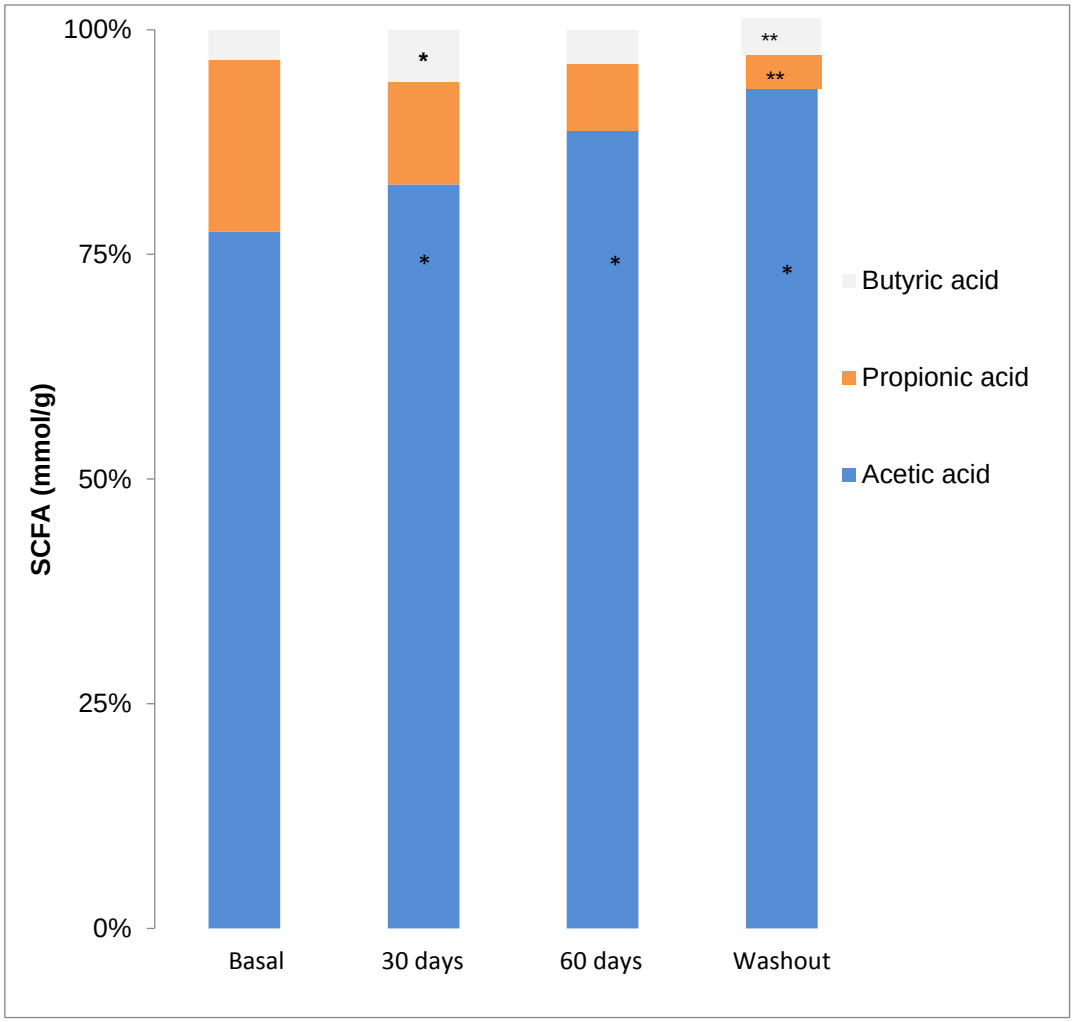
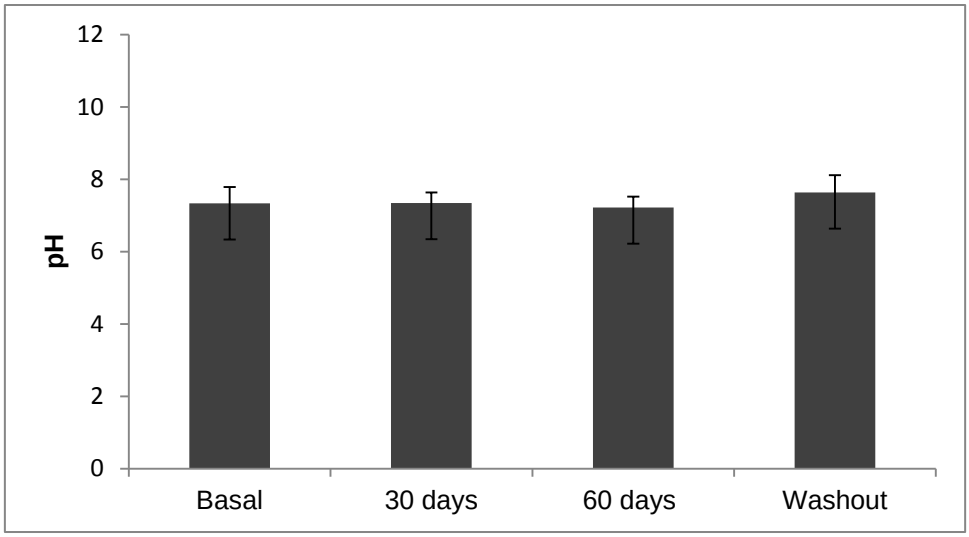


Figura 5: SCFA ratios upon feces. Absolute SCFA levels are indicated above each bar (mmol/g) (A).Significant decreases compared to the control are indicated by **, while significant increases are indicated by * ($p \leq 0.05$).Basal: before juice ingestion, 30 and 60 days: juice ingestion, Washout: without juice ingestion. n=10.

The average pH values showed showed no significant variation during the experimental period (Figure 6).



Basal: before juice ingestion, 30 and 60 days: juice ingestion, Washout: without juice ingestion. n=10.

Figure 6: Average and standard deviation of pH values evaluated in the feces of women during the trial period.

4.4 Effects of orange juice on intestinal function of women

The effect of orange juice on intestinal function of volunteers is shown in table 2. Stool consistency levels, frequency of bowel movements, effort to evacuate and painful evacuations did not present significant differences during the periods examined. It is observed that after 60 days of ingestion there was an improvement in the consistency of the stool from 0.94 ± 1.0 to 1.2 ± 0.63, the average frequency of evacuations remained in 1.4 ± 0.69, moreover, juice intake has helped in reducing the effort to evacuate of 2.4 ± 0.69 to 2.2 ± 0.41 and in the painful bowel movements of 3.5 ± 0.52 0.51 ± 0.52 ± 2.4 to 2.4 ± 0.51.

Table 2: Intestinal health of women during the trial period

	Basal	30 days	60 days	Washout
Consistency of stool	1.0 ± 0.94	0.9 ± 0.56	1.2 ± 0.63	1.2 ± 0.63
Frequency of bowel movements	1.4 ± 0.69	1.3 ± 0.48	1.4 ± 0.69	1.6 ± 0.69
Effort to evacuate	2.4± 0.69	2.1 ± 0.31	2.2 ± 0.41	2.4 ± 0.42
Painful bowel movements	3.5 ± 0.52	3.5 ± 0.51	2.4 ± 0.51	2.4 ± 0.51

Basal: before juice ingestion, 30 and 60 days: juice ingestion, Washout: without juice ingestion. n=10.

5-Discussion

There is growing scientific interest in the nutraceuticals and functional foods and their potential effects on health and more recently on gut microbiota. In this sense, orange juice contains a set of powerful bioactive molecules including flavonoids, carotenoids, vitamin C, folate, and other phytochemical compounds (21).Studies are currently showing that daily consumption of orange juice is being associated with the reduction of chronic diseases, improves the lipid profile, decreased total cholesterol, decreased systolic and diastolic (14,13). In addition, studies using colonic model have shown that orange juice has a positive effect on the intestinal microbiota, such as increased bacterial population of *Lactobacillus* spp., *Bifidobacterium* spp., and reduction of Enterobacteria. *In vitro* studies also show that juice increased the production of short chain fatty acids and decreased the content of ammonium ions (22, 23).

The present study aims to assess the effects of chronic ingestion of pasteurized orange juice (POJ) on the anthropometric measurements, intestinal microbiota, microbial metabolism and bowel function of adult women.

First was evaluated the effect of POJ on anthropometric measurements, weight and BMI eutrophic women because there is an assumption that the orange juice consumption increases body weight due to your energy content (24). The high consumption of 300 mL/day of pasteurized orange juice during 60 days in the present study did not show any weight and BMI variation during the entire period of the experimental protocol (table 1). These results suggest that POJ did not interfere in measures of weight and BMI and this can be confirmed in studies that evaluated the chronic intake of orange juice during 12 weeks and 8 weeks, in which show that the juice did not affect body weight and abdominal fat reduction of individuals who participated in the study (25, 26, 27).

Concerning on intestinal microbiota, the orange juice induced positive modulations of the microbiota composition and metabolic activity. In fact, orange juice mediated significant increases of *Lactobacillus* spp., *Bifidobacterium* spp., and total anaerobes. The lactobacilli, bifidobacterial and total anaerobes changes show that the prebiotic functionality of the orange juice was maintained during washout period. In addition, *Clostridium* spp., showed a significant increase during the experimental period. However, the elevated *Clostridium* spp., counts are not necessarily associated with negative effects on health, because, some species of the genus *Clostridium spp.,* are associated with the production of short-chain fatty acids, which are beneficial to health (28, 29). Similar results were observed by Duque et al (23), which assessed the

influence of fresh juice and pasteurized on the intestinal microbiota using colonic model (SHIME®).

Studies involving the effect of orange juice on the microbiota are still rare. Some authors suggest that a relationship between diet rich in polyphenols favors the increase of *Lactobacillus* spp., and *Bifidobacterium* spp. (30, 14, 31).

For the quantitative microbiological analysis, a conventional method of plating was used. This method includes selective medium preparation, inoculation and count of colonies and these methods time-consuming and labor-intensive. However, it has been estimated that less than one-half of the gut microbes is cultivable with the standard laboratory protocols (32, 33, 7). Therefore, a qualitative analysis of total bacteria was performed using the cultivation-independent PCR-DGGE method. PCR-DGGE analysis has the ability to monitor visually the profile and the changes that occur in the microbial community when different treatments are applied (34). PCR-DGGE analysis showed high similarity for total bacteria among the individual independently of experimental protocol period. This result indicates that an ingestion of orange juice did not affect a total bacteria community.

Gut microbiota-released metabolites, which are intermediates and/or end products of dietary constituents by commensal metabolism, may exert indispensable actions on host immunity and health. The concentration of ammonium ions (NH_4^+) in the intestine is resulting mainly from the deamination of amino acids and urea hydrolysis by intestinal bacteria (35). We observed a reduction ($p < 0.05$) of NH_4^+ after 60 days of orange juice consumption. This results are interesting, because the high concentration of ammonium can have detrimental effect to intestinal health (35), and NH_4^+ can affects the energetic

metabolism of colonic epithelial cells, inhibiting oxidation of short-chain fatty acids and repressing the cellular proliferation (36). The ingestion of prebiotics, probiotics (19, 37) and orange juice (23) has shown to be effective in reducing the concentration of NH_4^+.

Some of anaerobic gut microbes have the potential of converting dietary carbohydrates into organic acids including lactate, and short-chain fatty acids (SCFAs), the latter principally referring to acetate, propionate and butyrate (35,38). The SFCA have important role in gut physiology are recognized as primary source of energy to the enterocyte; stimulate epithelial cell proliferation; improve blood flow; increase the absorption of sodium and water, important in the cases of diarrhea; decreases the intraluminal pH and decreases the absorption of ammonia (39).

In fact, after 30 days of POJ intake showed a significant increase of butyric acid and propionic acid. Similar results were observed by studies that evaluated the influence of orange juice on the intestinal microbiota using a colonic model (23, 40). The increase of acetic acid can be attributed to flavonoids citrus. Orange juice flavanones were poorly bioavailable and 70% of ingested flavanones pass from the small to the large intestine where, in those with an intact colon, they are subjected to the action of the microbiota, which can hydrolyze flavanone glycosides (41). Pereira-Caro et al., (10) showed in vitro colonic study, that naringenin undergoes ring fission yielding 3-(4-hydroxyphenyl) propionic acid, and subsequently undergoes gut microbiota mediated dehydroxylation to produced sizable quantities of 3-(phenyl)propionic acid and smaller amounts of 4-hydroxyphenylacetic acid as result of a shortening of the side chain.

Recent studies have shown that the intestinal microbiota is capable of transforming phenolic compounds into SCFA, which can help to achieve better intestinal health (42, 43, 7).

In addition, the increase of SFCA can also be attributed to the carbon sources present in the orange juice, since carbon serves as substrate in the fermentation of intestinal bacteria and effectively participates in the production of SFCA in the colon (34). The carbon used by *Bifidobacterium* spp. and *Lactobacillus* spp. are responsible for producing large quantities of SFCA (44).

The increase in acetic acid production is beneficial in the body, because it may increase the cholesterol synthesis after absorption (45) and promotes calcium absorption in the colon (46). Butyric acid is the main energy source of the colonocytes and has anti-inflammatory and anti-carcinogenic properties and propionic acid is widely absorbed by the liver (47).

The pH of stool showed that the intake of POJ did not change the pH values during the experimental period as showed by PEREIRA-CARO et al (10) in clinical study with orange juice containing microencapsulated probiotic.

In addition the bowel habits of the participants in this study were evaluated based on the stool consistency, frequency of bowel movements, effort to evacuate and painful bowel movements. Ingestion of 60 days of pasteurized orange juice showed an improvement in the consistency of the stool as well as maintained the frequency of bowel movements, facilitated the effort to the defections and painful evacuations decreased. These results are interesting since the constipation can cause some discomfort in humans as bloating and abdominal pain, as well as headaches, dizziness and lack of appetite (48).

6. Conclusions

In general, findings have shown that continuous intake of orange juice may improve intestinal microbiota, microbial metabolism and intestinal function in adult women with marked reduction of bowel movement and painful bowel movements. Therefore, our results indicate a possible prebiotic effect of orange juice and an effective alternative for healthy eating, with effect on the modulation of the intestinal microbiota of adult women.

Acknowledgements

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CONCLUSÕES

O conjunto de resultados obtidos indica que:

1- A ingestão crônica de suco de laranja não influenciou nas medidas antropométricas dos voluntários.
2- O suco de laranja pasteurizado modificou a composição da comunidade microbiana com aumento de *Bifidobacterium* spp. e *Lactobacillus* ssp
3- O suco mostrou efeito no metabolismo microbiano como, redução da concentração de íons amônio e aumento do conteúdo de ácidos graxos de cadeia curta, indicando efeito prebiótico do suco de laranja sobre a microbiota intestinal.
4- O suco de laranja mostrou ser uma alternativa eficaz de alimentação saudável e com efeito na modulação da microbiota intestinal de indivíduos saudáveis e eutróficos.