MARLENE MARIA AMARAL SCHEID

"THE EFFECTS OF REGULAR INTAKE OF FREEZE-DRIED POWDERED YACON IN ELDERLY PEOPLE"

"AVALIAÇÃO DOS EFEITOS DO CONSUMO DE YACON LIOFILIZADO EM IDOSOS"

Campinas

2013

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ENGENHARIA DE ALIMENTOS

UNICAMP

MARLENE MARIA AMARAL SCHEID

"THE EFFECTS OF REGULAR INTAKE OF FREEZE-DRIED POWDERED YACON IN ELDERLY PEOPLE"

Orientadora: Profa. Dra. Glaucia Maria Pastore

"AVALIAÇÃO DOS EFEITOS DO CONSUMO DE YACON LIOFILIZADO EM IDOSOS"

Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Faculdade de Engenharia de Alimentos, da Universidade Estadual de Campinas para obtenção do título de Doutora em Ciência de Alimentos.

Doctorate thesis presented to the Food Science Postgraduation Programme of the School of Food Engineering of the University of Campinas to obtain the Ph.D. grade in Food Science.

Este exemplar corresponde à versão final da tese
defendida pela aluna Marlene Maria Amaral Scheid
e orientada pela Profa. Dra. Glaucia Maria Pastore

Assinatura do Orientador

_____

CAMPINAS

2013

FICHA CATALOGRÁFICA ELABORADA POR
MÁRCIA REGINA GARBELINI SEVILLANO – CRB8/3647- BIBLIOTECA DA
FACULDADE DE ENGENHARIA DE ALIMENTOS – UNICAMP

Sch25a

Scheid, Marlene Maria Amaral.
Avaliação dos efeitos do consumo de yacon liofilizado em idosos / Marlene Maria Amaral Scheid. -- Campinas, SP : [s.n.], 2013.

Orientador: Gláucia Maria Pastore.
Tese (doutorado) - Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Yacon liofilizado. 2. Idosos - Aspectos imunológicos. 3. Envelhecimento - Aspectos nutricionais. 4. Diabetes. 5. Fruto-oligossacarídeos. I. Pastore, Gláucia Maria, 1953- II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em inglês: The effects of regular intake of freeze-dried powdered yacon in elderly people
Palavras-chave em inglês:
Freeze-dried powdered yacon
Elderly - Imunological aspects
Aging - Nutritional aspects
Diabets
Fructooligosaccharides
Área de concentração: Ciência de Alimentos
Titulação: Doutora em Ciência de Alimentos
Banca examinadora:
Glaucia Maria Pastore [Orientador]
Luciana Silva Ferreira
Maria Elena Guariento
Mário Roberto Maróstica Júnior
Valéria Maria Caselato de Sousa
Data da defesa: 28-02-2013
Programa de Pós Graduação: Ciência de Alimentos

BANCA EXAMINADORA

Profa. Dra. Glaucia Maria Pastore
Faculdade de Engenharia de Alimentos – UNICAMP
Orientadora

Profa. Dra. Luciana Silva Ferreira
Escola de Nutrição - UNIRIO
Titular

Profa. Dra. Maria Elena Guariento
Faculdade de Ciências Médicas – UNICAMP
Titular

Prof. Dr. Mário Roberto Maróstica Junior
Faculdade de Engenharia de Alimentos – UNICAMP
Titular

Profa. Dra. Valéria Maria Caselato de Sousa
Escola de Nutrição – UNIRIO
Titular

Profa. Dra. Claudia Cardoso Netto
Centro de Ciências Biológicas e da Saúde – UNIRIO
Suplente

Profa. Dra. Elizabeth Aparecida Ferraz da Silva Torres
Faculdade de Saúde Pública - USP
Suplente

Prof. Dr Yong Kun Park
Faculdade de Engenharia de Alimentos _ UNICAMP
Suplente

AGRADECIMENTOS

Primeiramente agradeço a Deus, por ter sido a Luz do meu caminho, me guiando sempre com amor.

Agradeço aos meus Pais, Geraldo e Geralda, por terem me dado não somente a vida, mas também a coragem e terem me ensinado a seguir o caminho da honestidade e do amor.

Minha querida Avó, Maria Conceição, pelo exemplo de vida.

À Profa. Dra. Glaucia Maria Pastore pela confiança, apoio, amizade e competência.

Aos meus irmãos, que mesmo distantes, sempre estiveram presentes.

À Profa. Dra. Yara M. F. Moreno, por ter me mostrado a luz nas horas de dificuldades e sempre me ter recebido com palavras positivas.

À Profa. Dra. Patrícia Genaro, pelas ajudas nas análises estatísticas.

Aos bolsistas, Aline Mariano, Michelle Teixeira e Emanoel Silva: sem vocês não teria organizado todos dados.

À Comissão Examinadora, Profa. Dra. Luciana Silva Ferreira, Profa. Dra. Maria Elena Guariento, Prof. Dr Mário Roberto Maróstica Junior, Profa. Dra. Valéria Maria Caselato de Sousa, Profa. Dra. Cláudia Cardoso Netto, Profa. Dra. Elizabeth Aparecida Ferraz da Silva Torres, Pro Dr Yong Kun Park pela correção da tese com valiosas sugestões.

Às grandes amigas, Patrícia Sampaio e Iramaia Angélica Neri Numa: obrigada pelas ajudadas constantes, por terem me dado o ombro nas horas dos choros, pelas conversas, pelas risadas.

À Verônica Pavan e Dra. Ana Flávia Azevedo Carvalho, pelas análises dos carboidratos e por terem tido paciência com minha insistência.

Aos amigos do Bioaromas pela amizade, risadas e apoio constante.

Aos funcionários Nadir, Cosme Perota e Marcos Sampaio da UNICAMP e Carlos Guedes, da UNESP por estarem sempre dispostos a nos auxiliar.

Aos colegas de trabalho da UNIVAP, UNIP e do Hospital REGER por terem me apoiado durante este trabalho.

Ao Prof. José Roberto Rodrigues e Profa. Denise Nicodemo da Universidade Aberta da Terceira Idade - UNESP e Profa. Débora W.O. Guedes da Faculdade Aberta da Terceira Idade – UNIVAP por terem me aberto as portas para realizar este trabalho.

À minha amiga Lúcia Helena Ferreira pela formatação deste trabalho.

A Rita de Cássia Fonseca, meu braço direito.

À Corn products Brasil, pela doação da maltodextrina.

Por todas as amizades que nasceram nesta etapa, e a todos os amigos (as) que de uma maneira ou de outra sempre me incentivaram e contribuíram para tornar possível a realização deste trabalho.

A FAPESP pelo financiamento desta pesquisa.

MINHA ESPECIAL GRATIDÃO

- **Meu marido, Vladimir, meu eterno amor;**
- **Meus grandes tesouros, Cassimiro e Maria**;

peço muitas desculpas pelos momentos que abandonei vocês para me dedicar a este trabalho. Muito obrigada por vocês existirem.

A todos idosos da Faculdade Aberta da Terceira Idade da UNIVAP e UNESP, pela amizade, pela confiança e por terem aceitado participar desta pesquisa.

MUITO OBRIGADA

Marlene Maria Amaral Scheid

"O que é valioso neste mundo não é nossa posição, nossa educação ou nosso conhecimento, mas nossa conduta e nosso comportamento baseados em valores espirituais. A conduta e o comportamento determinam o resultado que recebemos por nossos esforços. A educação verdadeira é amor, e nada além do amor. Sem amor, a vida não é digna de ser vivida". **Sathya Sai Baba**

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

1-FEH	fructan 1- exohydrolase
1-FFT	fructan:fructan 1 fructosyl transferase
1-SST	sucrose:sucrose 1 fructosyl transferase
Alb	albumin;
BMI	body mass index
CHL	cholesterol
CRP	C-reactive protein;
DBRCT	double-blind randomized placebo trial
DCV	doenças cardiovasculares
DM	diabetes mellitus
DP	degree of polymerization
EG	experimental group
FB	fecal bifidobacterias
FDY	freeze-dried powdered yacon
FOS	fructooligosaccharides
GALT	gut-associated lymphoid tissue
GF2	1-kestose
GF3	nystose
GF4	fructosyl nystose
GIT	gastrointestinal tract

GLU	glucose
GOS	galactooligosaccharides
HA	hipertensão arterial
HDL-c	high density lipoprotein cholesterol
IBGE	Brazilian Institute of Geography and Statistics
IFN-α	interferon-α
Ig	immunoglobulin.
IgA	immunoglobulin A
IgG	immunoglobulin G
IL-1α	interleukin 1α
IL-10	interleukin 10
IL-1β	inteleukin 1β
IL-2	interleukin 2
IL-4	interleukin 4
IL-6	interleukin 6
IL-8	interleukin 8
ITAL	Institute of Food Technology
ITF	inulin-type fructans
LDL-c	low density lipoprotein cholesterol
MNA	mini nutritional assessment;
NF	nuclear factor

NK	killer cells;
OS	oligosaccharides
PC	placebo controlled
PG	placebo group;
PO	peroxidase
PPO	polyphenol oxidase
PS	prospective study
RB	rectal biopsy
SCFAs	short-chain fatty acids
TNF	tumour necrosis factor;
Trigs	triacylglycerol
VLDL-c	very low density lipoprotein cholesterol
WC	waist circumference
YL	yacon liofilizado

RESUMO

Os objetivos deste estudo foram avaliar as alterações físicas e o conteúdo de FOS e açúcares nas raízes de yacon fresco armazenadas e verificar se a ingestão diária, por nove semanas de yacon liofilizado (YL) contendo 7,4 g de FOS altera a motilidade intestinal, perfil glicêmico e lipídico, a ingestão alimentar e sistema imunológico de idosos frequentadores da Universidade Aberta da Terceira Idade.

A longevidade da população humana tem aumentado em todo mundo e está associada a doenças e alterações no sistema imunológico, no metabolismo glicêmico, lipídico e na motilidade intestinal. O uso de prebióticos tem demonstrado eficácia contra condições patológicas, comuns na população idosa, como doenças cardíacas, metabólicas e alterações intestinais. Yacon (Smallanthus sonchifolius) uma planta originária dos Andes, é reconhecida pelo potencial prebiótico devido à alta concentração de frutooligossacarídeos (FOS). Para avaliar as alterações físicas e mudanças no teor de açúcar e FOS, as raízes de yacon fresco foram armazenadas à temperatura ambiente (25ºC), e em geladeira (4ºC) durante 31 dias. Glicose, frutose, sacarose e FOS de cada amostra foram extraídas com água e analisados por cromatografia de íons em um sistema Dionex ICS-5000. Setenta e dois idosos, média de idade 67,11 ± 6,11 idade, foram estudados durante 9 semanas em um estudo duplo-cego placebo-controlado. Eles foram aleatoriamente divididos em grupo experimento, que recebeu 18g de YL contendo 7,4 g FOS e grupo placebo, que recebeu 12g de maltodextrina. O estado nutricional foi realizado antes e após 9 semanas de intervenção através da avaliação do consumo alimentar, antropométrica e bioquímica. O hábito intestinal foi avaliado antes e após a intervenção através de um questionário, contendo informações sobre frequência evacuatória, esforço para evacuar, dor à evacuação, sensação de evacuação incompleta, dores abdominais, tempo gasto para iniciar a evacuação, tipo de auxílio para evacuação, tentativas falhas/dia e duração da constipação. A avaliação da função imune, realizada antes e após 9 semanas foi feita pelas dosagens de citocinas (IL-1α, IL-1β, IL-2, IL-4,

IL6, IL-8, IL10, TNF- α, INF-ɣ). O estudo revelou que raízes de yacon têm elevado conteúdo de FOS (74% em massa seca) e durante o armazenamento o teor FOS reduz-se nas raízes armazenadas a 4°C e a 25°C, sendo menor no armazenamento em geladeira (4º C). Além disso, durante o armazenamento, tubérculos de yacon fresco se deterioram, tornando-se murchos e desidratados. A ingestão diária de YL com 7,4 g de FOS por 9 semanas diminuiu significativamente (4,6%) a glicemia sanguínea (p = 0,013), mas não reduziu a concentração de lipídios séricos em idosos. A dose dada foi limitada para melhorar o trânsito intestinal, e não causou inchaço, flatulência ou desconforto intestinal. Idosos de ambos os grupos apresentavam adequado estado nutricional e adequada função imunológica antes da intervenção. Os idosos que consumiram YL mostraram um decréscimo significativo nos níveis de IL-1α (p < 0,001) e quando ajustado para idade e massa gorda, houve redução significativa de 54,6% na IL-6 (p = 0,039) quando comparado com o grupo placebo. Nosso estudo apresentou um elevado nível de participação (97,3%). Nossos dados indicam que raízes de yacon são consideradas uma fonte potencial de FOS, é recomendável que sejam armazenado à 4º C e consumidas até o décimo sétimo dia pós-colheita. YL foi bem aceito pelos idosos, é um produto de fácil armazenamento e quando consumido diariamente melhora o perfil glicêmico e a resposta imunológica, entretanto não podemos afirmar que se associa à melhora do perfil lipídico e do hábito intestinal de idosos.

Palavras-chave: frutooligossacarídeos, yacon liofilizado, idoso, glicose, sistema imune

ABSTRACT

The aim of this study was to evaluate the physical changes and the changes of FOS and sugar content in fresh yacon roots post- harvest and to verify if the daily intake for 9 weeks of freeze-dried powdered yacon (FDY) containing 7.4 g of FOS could alter bowel habit, affect glycaemic and lipid metabolic profile, the dietary intake and the immune system of non-institutionalised elderly.

Human longevity has increased in many countries and is associated with diseases and changes in the immune system and the metabolism of glucose, lipids, and intestinal transit. The use of prebiotics has demonstrated efficacy against pathological conditions that are common in the elderly, including heart disease, inflammatory and metabolic disease and intestinal dysmotility. Yacon (Smallanthus sonchifolius) is a plant originating from the Andes, which is recognised for its prebiotic potential due to its high concentration of fructooligosaccharides (FOS). To evaluate the physical changes and changes in sugar and FOS in fresh yacon roots stored under room temperature and chilled conditions during 31 days, the glucose, fructose, sucrose and FOS in each sample were extracted with water and analysed in an Ion Chromatograph Dionex Model ICS-5000. Seventy two elderly, (mean age 67.11 ± 6.11 years), were studied for 9 weeks in a double- blind placebo-controlled experiment. They were randomly assigned to either the supplemented group, receiving 18g FDY containing 7.4 g of FOS and the placebo group, receiving 12g Maltodextrin. Anthropometric measurements, intestinal transit, blood samples, clinical analyses, dietary intake and serum cytokine levels were determined at the start and at the end of the experiment. The study revealed that yacon roots have a good content of FOS, and that the FOS content of yacon root stored at 4°C decreased, but to a lesser extent than that stored at 25°C. Besides this, during storage, fresh yacon tubers deteriorated in appearance where they became withered and dried. A daily intake of FDY containing 7.4 g FOS for 9 weeks produced a significant effect in decreasing mean serum glucose (p = 0.013) while was incapable of reducing serum lipid concentration in the elderly. The dose given was limited in order not to adversely affect intestinal transit. It did not cause bloating, flatulence or intestinal

discomfort. The elderly of both groups were well-nourished with an adequate immune function. Comparative analyses of variables between the 2 groups and the 2 time showed significant decrease on IL-1α ($p < 0,001$). When adjusted for age and fat mass the production of IL-6, in FDY-supplement group has a significant decrease of 54, 6% compared to that of the placebo group ($p = 0.039$).

Our study had a high level of participation being 97.3%. Our data indicate that yacon roots are a potential source of FOS, and that it is recommended that yacon should be stored at 4^{o}C and consumed before 17 days post-harvest. Freeze-dried powdered yacon was well accepted and when consumed daily had beneficial effects on serum glucose and on the immune system of the elderly

Keywords: fructooligosaccharides, elderly, freeze-dried powdered yacon, glucose, immune system

INTRODUÇÃO GERAL

O envelhecimento populacional ocorreu de forma lenta nos países desenvolvidos e vem acontecendo de modo acelerado nos países em desenvolvimento (GIATI & BARRETO, 2003), tornando-se alvo de intensa preocupação dos órgãos de saúde. No Brasil, a população idosa é a que mais cresce em termos proporcionais. Segundo projeções do IBGE, se o país continuar aumentando anos na vida média de sua população, alcançará em 2050 o patamar de 81,3 anos (IBGE, 2010). Com essas mudanças demográficas, aumenta a prevalência de doenças não transmissíveis e inflamatórias, que acometem, predominantemente, a população idosa. A velhice é uma etapa da vida associada ao desenvolvimento de resistência insulínica, predispondo o idoso a diabetes mellitus (DM), dislipidemia, hipertensão arterial (HA), doenças cardiovasculares (DCV) (FREITAS, 2006), assim como declínio da função imunológica e consequente aumento da frequência de infecções (ASPINAL & ANDREW, 2000). A prevalência e incidência de DM vêm aumentando com o aumento da expectativa de vida (FREITAS, 2006). A constipação intestinal em idosos ocorre, dentre outros fatores, devido à redução da motilidade intestinal, mudanças do hábito alimentar e a alteração da composição da microbiota intestinal (MURRAY & BLISS, 1991, RUSSEL, 1992).

A alimentação, desde que nutricionalmente adequada, exerce papel fundamental na promoção, manutenção e recuperação da saúde de idosos. Os alimentos funcionais são aqueles que, além das funções nutricionais básicas, exercem efeitos benéficos; metabólicos e/ou fisiológicos, à saúde (Brasil, 1999). Os carboidratos com ação prebiótica são aqueles pertencentes aos grupos: frutanos tipo inulina e galactooligosaccarídeos (GOS) (CASELATO DE SOUZA *et al*., 2011, ROBERFROID *et al*., 2010). Evidências mostram que a ingestão de certos alimentos e seus constituintes apresenta potencial para prevenir doenças crônicas (WAHLQVIST, 1997), e que a suplementação de alguns nutrientes exerce efeitos benéficos na imunidade em idosos (MARKO *et al*., 2007). Portanto,

a literatura é deficiente de informações acerca de possíveis benefícios decorrentes da ingestão de yacon sobre o metabolismo lipídico, glicêmico e imunológico em idosos.

Os prebióticos são definidos como ingredientes que estimulam seletivamente o crescimento e/ou a atividade de um, ou um número limitado, de espécie/gênero de bactérias da microbiota, conferindo benefícios à saúde e bem estar do hospedeiro (ROBERFROID *et al.*, 2010). Os prebióticos afetam o metabolismo de carboidratos, de lipídeos e de minerais, além de atuar como imunomoduladores, mas podem apresentam efeitos gastrointestinais, como flatulência, indisposição intestinal (DELZENNE *et al.*, 2002; SCHLEY & FIELD, 2002; SCHOLZ-AHRENS & SCHREZENMEIR, 2002; LOBO & FILISETTI, 2003).

Muitas espécies vegetais, como yacon, almeirão, cebola, topinambo, apresentam frutanos como carboidratos de reserva (BHATIA & RANI, 2007, CARABIN & FLAMM, 1999).

O yacon (Smallanthus sonchifolius) é uma raiz nativa dos Andes, com sabor adocicado, textura crocante, consumida crua, cozida, assada, desidratada ou como refresco (FERNÁNDEZ *et al.*, 1997). As raízes de yacon apresentam 83% a 90% de água do peso fresco, e em base seca apresentam 40 a 70% de FOS, 5 a 15% de sacarose, 5 a 15 % de frutose e menos de 5% de glicose (MANRIQUE & PARRAGA, 2005). As raízes de yacon recém-colhidas são insípidas, mas no armazenamento ocorre hidrólise dos frutanos, que leva ao aumento do teor de frutose e glicose e redução de FOS (ZARDINI, 1991). Durante o armazenamento, as raízes de yacon tornam-se murchas devido à desidratação e escuras devido ao escurecimento enzimático (VILHENA, 2001, FUKAI *et al.*, 1997). Quando consumidas como fonte de FOS, as raízes de yacon devem ser consumidas logo após a colheita ou armazenadas em condições que reduzam a conversão do FOS em glicose e frutose (GRAEF *et al.*, 2004).

Estudos clínicos em animais e humanos têm demonstrado o efeito benéfico do consumo de FOS (GIBSON & ROBERFROID, 1995, GENTA *et al.*,

2009, VALENTOVÁ *et al.*, 2008). Genta e colaboradores (2009) mostraram em estudo duplo cego controlado por placebo, que mulheres em fase pré-menopausa que consumiram xarope de yacon, durante 3 meses apresentaram redução no índice de massa corporal (IMC) e nos níveis de LDL-colesterol. Estudo conduzido por Valentová e colaboradores (2008), em adultos, mostrou que a ingestão de uma mistura de yacon e silumarin apresenta efeito hipoglicemiante em indivíduos com síndrome metabólica.

Por outro lado, estudos em animais e humanos têm investigado as mudanças na produção de citocinas associadas ao envelhecimento (BERNSTEIN & MURASKO, 1998, NAKAMURA *et al.*, 2004, VULEVIC, 2008, GUIGOZ *et al.*, 2002). Guigoz e colaboradores (2002) realizaram uma pesquisa com 19 idosos e constataram que a ingestão diária de 8g FOS por três semanas reduziu a atividade fagocitária de granulócitos e monócitos assim como a expressão de IL-6 em monócitos sanguíneos. Bunout *et al.* (2002) não encontraram nenhum efeito imunológico positivo em idosos que ingeriram uma mistura com 6g de prebióticos durante 28 semanas.

A presente tese está dividida em quatro capítulos na forma de artigos científicos. O capítulo um corresponde à revisão bibliográfica acerca dos efeitos dos prebióticos na saúde de idosos. O capítulo dois apresenta as alterações físicas e na composição de carboidratos de raízes de yacon armazenadas. O capítulo três relata o efeito do consumo de yacon liofilizado nos níveis glicêmicos e lipídicos e hábito intestinal de idosos. No capítulo quatro estão expostos os resultados do efeito do consumo diário de yacon liofilizado sobre a função imune de idosos.

Ao final apresentamos as conclusões gerais e sugestões de pesquisas futuras.

REFERÊNCIAS BIBLIOGRÁFICAS

ASPINAL, R, ANDREW, D. Immunosenescence: potential causes and strategies for reversal. **Biochemical Society Trans**, v. 34, p.512-518, 2000

BERNSTEIN, E.D., MURASKO, D.M.M., Effect of age on cytokine production in humans. **Age**, v.21, p.137-151, 1998

BHATIA, A., RANI, U. Prebiotics and health: clinical implications. Journal of Clinical **Diagnostic Research**, Dec, V.1, n.6, p. 546-554, 2007

BUNOUT, D, HIRSCH, S, PIA de la MAZA, M, MUNOZ, C, HASCKE, F, STEENHOUT, P, KLASSEN, P, BARRERA,G. *et al.*. Effects of prebiotics on the immune response to vaccination in the elderly. **Journal of Parenteral and Enteral Nutrition**, v. 26, n.6, p. 372–376, 2002

BRASIL, Ministério da Saúde. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução nº 18 de 30 de Abril de 1999. Aprova o Regulamento Técnico que estabelece as diretrizes básicas para análise e comprovação de propriedades funcionais e/ou de saúde alegadas em rotulagem de alimentos. Disponível em:http://www.anvisa.gov.br/legis/resol/18-99.htm acesso em 29 nov.2012

CASELATO DE SOUZA, V.M., SANTOS, E.F., SGARBIERI, V.C. The importance of prebiotics in functional foods and clinical. **Scientific Research**, v.2, n.2, p.133-144, 2011

CARABIN, I. G., FLAMM, W. G. Evaluation of safety of inulin and oligofructose as dietary fiber. **Regulatory Toxicology and Pharmacology**, v.30, p. 268-282, 1999

DELZENNE, N. M.; DAUBIOUL, C.; NEYRINCK, M.; LASA, M.; TAPER, H. S. Inulin and oligofructose modulate lipid metabolism in animals: review of

biochemical events and future prospects. **British Journal of Nutrition**, v. 87 (suppl. 2), p. 255-259, 2002.

FERNANDEZ, C E; LIPAVSKA, H; MILCHL, J Determination of saccharides content in different ecotypes of yacon (Polymixia sonchifolia poepp. and endlicher) cultivated under conditions of Czech Republic. **Agricultura Tropica e subtropical**. Universitas Agriculturae Praga. v. 30, p. 79-89. 1997

FREITAS, EV. Diabetes Mellitus. In: FREITAS, EV, PY L, CANÇADO FAX, DOLL J, GORZONI, ML. **Tratado de Geriatria e Gerontologia**. 2ª ed. Guanabara Koogan, Rio de Janeiro, 2006. P. 776-86

FUKAI, K; OHNO. S; GOTO, K; NANJO, F; HARA, Y. Seasonal fluctuations in fructan content and related enzyme activities in yacon (Polymnia sonchifolia). **Soil Science Plant of Nutrition,** v.43, p.171–177, 1997

GENTA, S; CABRERA, W; HABIB, N; PONS, J; CARILLO, I; GRAU, A; SÁNCHEZ, S. Yacon syrup: Beneficial effects on obesity and insulin resistence in humans, **Clinical Nutrition** v.28, n.2, p. 182-187, 2009

GIATTI, L; BARRETO, S.M. Saúde, trabalho e envelhecimento no Brasil. **Caderno de Saúde Pública**, V.19, N.3,P. 759-771. 2003

GIBSON, G. R.; ROBERFROID, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. **Journal of Nutrition**, v.125, n.6, p.1401-1412, 1995.

GRAEF, S; HERMANN, I; MANRIQUE, S; GOLOMBEK, S; BUERKERT, A. Effects of post-harvest treatments on the carbohydrate composition of yacon roots in the Peruvian Andes. **Field Crops Research**. v.86, p.157-65, 2004

GUIGOZ, Y; ROCHAT, F; PERRUISSEAU-CARRIER, G; ROCHAT, I; SCHIFFRIN, E.J. Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. **Nutrition Research**, v. 22, p.13-25, 2002

IBGE Instituto Brasileiro de Geografia e Estatística. **Estudos e pesquisas informações demográficas e socioeconômicas nº 27. Síntese de Indicadores Sociais. Uma Análise das condições de vida da população brasileira**. Rio de Janeiro: IBGE, 2010, 343 p.

LOBO, A. R.; FILISETTI, T. M. C. C. Bone mineral parameters in growing rats fed fructooligosaccharides. **Revista Brasileira Ciências Farmacêuticas**, v. 39 (supl. 2), p. 67, 2003

MANRIQUE, I & PÁRRAGA, A. Conservación y uso de la biodiversidad de raíces y tubérculos Andinos: Una década de investigación para el desarrollo (1993-2003). **Jarabe de yacón: principios y procesamiento.** Lima: Centro Internacional de La Papa, p. 40, 2005

MARKO, M.G; AHMED, T; BUNNEL, S.C; WU, D; CHUNG; H; HUBER, B.T. Age-associated decline in effective immune synapse formation of CD4(+) T cells is reversed by vitamin E supplementation. **Journal of Immunology,** v.178, n.3, p. 1443-1449, 2007

MURRAY, FE; BLISS, CM. Geriatric constipation – a brief update on a commom problem. **Geriatrics**, v. 46, n.3, p. 64-68, 1991

NAKAMURA, Y; NOSAKA, S; SUZUKI, M; NAGAFUCHI, S; TAKAHASHI, T; YAJIMA, T; TAKENOUCHI-OHKUBO, N; IWASE, T; MORO I. Dietary fructooligosaccharides up-regulate immunoglobulin A response and polymeric immunoglobulin receptor expression in intestines of infant mice. **Clinical & Experimental Immunology** v.137, p.52–58, 2004

ROBERFROID, M.B; GIBSON, G.R., *et al.*. Prebiotics effects: metabolic and health benefits. **British Journal of Nutrition**, v.104, n.2, p.1-63, 2010

RUSSEL, RM. Changes in gastrointestinal attributed to aging. **American Journal of Clinical Nutrition**, v.55, n. 6 suppl, p.1203-1207. 1992

SCHLEY, P. D.; FIELD, C. J. The immune-enhancing effects of dietary fibers and prebiotics. **British Journal of Nutrition**, v. 87 (suppl. 2), p. 221-230, 2002.

SCHOLZ-AHRENS, K. E.; SCHREZENMEIR, J. Inulin, oligofructose and mineral metabolism – experimental data and mechanism. **British Journal of Nutrition**, v. 87 (suppl. 2), p. S179-S186, 2002.

VALENTOVÁ, K; STEJSKAL, D; BARTEK, J; DVORÁCKOVÁ, S; KREN, V; ULRICHOVA, J; SIMANEK, V. Maca (Lepidium meyenii) and yacon (Smallanthus sonchifolius) in combination with silymarin as food supplements: in vivo safety assessment. **Food and Chemical Toxicology**. v. 46, p. 1006-1013, 2008

VILHENA, S.M.C. **Ciclo de cultivo e técnicas pós-colheita de yacon (Polymnia sonchifolia Poep. Endl.) em função do conteúdo de frutose total nos órgãos subterrâneos.** 2001. Tese (Doutorado em Agronomia/Horticultura) Faculdade de Ciências Agronômicas, Universidade Estadual Paulista. Brasil

VULEVIC, J; DRAKOULARAKOU, A; YAQOOB, P; TZORTZIS, G; GIBSON, G.R. Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. **American Journal of Clinical Nutrition**, v. 88, p.1438 – 1446, 2008.

WAHLQVIST, ML. Nutritional deficiencies underpin some clinical disorders, **Current Therapeutics**. v. 38, n.12, p.34-35, 1997

ZARDINI, E. Ethnobotanical notes on "yacon", Polymnia sonchifolia (Asteraceae). **Economic Botany**. , v. 45, n.1, p.72-85, 1991.

CAPÍTULO 1

EFFECT OF PREBIOTICS ON THE HEALTH OF THE ELDERLY

Marlene Maria Amaral Scheid[a], Yara Maria Franco Moreno[b], Mário Roberto Maróstica Junior [c], Glaucia Maria Pastore[a]

[a] Department of Food Science, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil (Departamento de Ciência dos Alimentos, Faculdade de Engenharia dos Alimentos, Universidade de Campinas, Campinas, São Paulo, Brasil)

[b] Department of Nutrition, Centre for Health Sciences, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil (Departamento de Nutrição, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brasil)

[c] Department of Food and Nutrition, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

This manuscript was submitted to Food Research International

ABSTRACT

The longevity of the human population has increased in many countries, due to better quality of life generated by improved diet and medical advances. Ageing is related to diseases and alterations in the intestinal microbiota that predispose to changes in the immune system and the metabolism of glucose, lipids, and minerals. The use of prebiotics has shown a positive effect on the production of short-chain fatty acids (SCFAs) by the intestinal microbiota and has demonstrated efficacy against pathological conditions that are more frequently found in the elderly, including irritable bowel syndrome, ulcerative colitis, intestinal cancer, and heart disease. However, additional intervention studies should be conducted among the elderly to prove the efficacy of prebiotics in this population. Moreover, studies that analyses the mechanisms of action of these compounds during the senescence are needed. The aim of this review is to analyses the effect of prebiotics on the health of the elderly people.

Keywords: fructans, prebiotics, health of elderly, intestinal microbiota

1. INTRODUCTION

Ageing of the population is a subject of great concern for health agencies. In developed countries, this phenomenon has occurred slowly, whereas ageing has quickly gained significance in developing countries with a sharp increase in the elderly population relative to the general population (Giatti & Barreto, 2003). The growth rate of the elderly population in Brazil has been systematic and steady. The relative number of elderly in the total population of Brazil rose from 9.15% to 11.3% in the period from 1999 to 2009. According to the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística – IBGE), this growth will increase the life expectancy at birth to 81.29 years in 2050 (IBGE, 2010).

The prevalence of chronic degenerative diseases increases with the life expectancy at birth, which predominantly affects the elderly population; thus, longevity is not necessarily related to healthy ageing (Chaimowicz, 1997). Chronic non-communicable diseases and illnesses can trigger impairments that impact daily activities, which compromise the quality of life of the elderly. Ageing is associated with histological and physiological changes in the gastrointestinal tract (GIT) that have implications in the digestion and absorption of nutrients and mucosal damage. There is a correlation among ageing, chronic diseases, and changes in the composition of the intestinal microbiota and the host immune system (Rowland & Gill, 2008). In combination with chronic diseases and cachexia, the changes of intestinal microbiota and damage of gut epithelium may contribute to inflammatory processes in the elderly (Schiffrin *et al.,* 2007, Murphy *et al.*, 2009).

Following birth, the human gastrointestinal tract is colonized by microbiota, which constitutes a population of beneficial and pathogenic microorganisms that includes approximately 10 million bacteria from 1000 different species (Wallace *et al.*, 2011). These bacteria develop close interactions with the host; thereby promote the health and wellbeing (Bäckhed *et al.*., 2005). Through fermentation of undigested dietary residues, the intestinal microbiota produce a variety of compounds that have positive and/or negative effects on the intestinal physiology

and systemic influences (Gibson and Roberfroid, 1995a), as a principal conditions, in which the compositions of potentially beneficial bacteria, especially the Bifidobacterium and Lactobacillus, are elevated and/or more active than the potentially pathogenic bacteria include proteolytic/putrefactive genera/species (Gibson *et al.*, 1995b).

The metabolic activities of the intestinal microbiota can have a variety of effects, with the potential to influence areas outside of the colon and include, among others, the growth inhibition of pathogenic microorganisms through competition for ecological niches and metabolic substrates, improved digestion of lactose in lactose-intolerant individuals, improved absorption of ions, such as calcium, magnesium, and iron, stimulated synthesis of vitamins, particularly B vitamins groups, and proteolytic enzymes, better intestinal function, modulation of the immune system, gene expression, and intestinal cell differentiation (Roberfroid, 2008), cholesterol reduction, and regulation of inflammatory bowel diseases (Wallace *et al.*, 2011).

There is a balance between the beneficial and harmful bacteria found in a healthy intestine (Wells *et al.*, 2008). When a change in the intestinal permeability occurs, the balance of the intestinal bacteria can be disrupted, which results a predominance of harmful bacteria. The organism subsequently becomes more susceptible to infections and immunological changes (Chichlowski & Hale, 2008). The increase in pathogenic bacteria enables their fixation on the intestinal epithelium, which allows the colonisation and invasion of the intestinal wall and triggers a decline in biliary function, hypochlorhydria, an inflammatory response (Dubert-Ferrandon *et al.*, 2008), and increased susceptibility to infectious and noninfectious diseases (Vulevic *et al.*, 2008). Non-sporulating anaerobic bacteria, including *Bacteroides spp., Bifidobacterium spp., Eubacterium spp., Clostridium spp., Lactobacillus spp., Fusobacterium sp*p., and several gram-positive cocci, predominate in the adult intestine. *Enterococcus spp., Enterobacteriaceae, methanogens*, and sulphate-reducing bacteria are found in lower numbers (Wallace *et al.*, 2011).

Under normal conditions, the total number of anaerobic bacteria seems to remain relatively constant in older people, but the composition of the microbiota does change with age. Recent studies suggest that the change in microbiota by the aging may be attributed to various factors, including lifestyle (free living or nursing home), nutritional habits, fraily score, nationality (Biagi *et al.*, 2011), medication and gastrointestinal infections(Tiihonen *et al.*, 2010, Tuohy & Gibson, 2007). Several human studies have examined the composition and changes in the intestinal microbiota during aging and have shown a decrease in the number of *bifidobacteria and Bacteroides* and an increase in the levels of *enterobacteria lactobacilli*, and some species of *Clostridium* (Hopkins *et al.*., 2001, Gavini *et al.*, 2001). The decrease in the number of bifidobacteria and others beneficial colonic bacteria in the elderly may have metabolic and health consequences because these bacteria and the equilibrium among them are able to exert the responsiveness of the intestinal immune system, production of SCFA and in resistance to gastrointestinal infections, which have consequences for the host, including malnutrition and intestinal dysmotility (Murphy *et al.*, 2009). The SCFA produced in the gut are the preferred energy substrate of the colonic epithelial cells, besides SCFAs have important effects on the gut physiology, in particular, butyrate affecting epithelial proliferation and differentiation (O´Keefe, 2008). The decrease in *Bacteriodes* numbers may change the productions of short chain fatty acid and may affect other bacterial species that depend nutritionally on polysaccharide digestion.

The human immune system undergoes morphological and functional changes that peak during puberty and gradually decline with ageing (Ewers *et al.*, 2008); these changes predispose the elderly to infectious and non-infectious diseases (Aspinal & Andrew, 2000). The immune function can be affected by nutrition. Probiotics, prebiotics, and symbiotic are among the ingredients responsible for the improvement of the immune response. Studies using prebiotics, inulin, oligofructose (OF), and galacto-oligosaccharides (GOS) in animal models and human trials have shown positive effects for the reduction of colon cancer and

inflammatory bowel disease and protection against metabolic disease (Langlands *et al.*, 2004, Pool-Zobel, 2005, Tuohy, 2009)

Functional foods, as the fermented foods red wine, yogurt and tempeh, should be included in the diet because they feature, in addition to traditional nutrients, specific (bioactive) compounds that promote health, because they may provide specific nutritional and metabolic benefits that help placebo and decrease the risk of diseases (Roberfroid, 2002). Probiotics and prebiotics stand out among the bioactive components that are found in functional foods. This review aims to analyse the effect of prebiotics on the health of the elderly.

2. PREBIOTICS

The prebiotic concept has been known for over 100 years, but recent studies have shown its scientific and beneficial effects on health and the prevention and treatment of diseases.

Prebiotics are defined as ingredients that selectively stimulate the growth and/or activity of one or a limited number of species/genera of bacteria in the microbiota, thereby conferring benefits to the health and wellbeing of the host (Roberfroid *et al.*, 2010). Prebiotics have attracted the interest of researchers and the food industry due to their nutritional and economic benefits, and they are used in food, particularly in the production of functional foods (Macfarlane & Cummings, 2006, Bossher, 2009). A food component must meet the following requirements to be considered prebiotic: be resistant to salivary, pancreatic, and intestinal enzymes; be fermentable by the intestinal microbiota; and selectively stimulate the growth and/or activity of intestinal bacteria to contribute to health and wellbeing (Gibson, 2004). A recent study showed that inulin-type fructans (ITF), such as inulin and FOS, and GOS exhibit prebiotic effects (Roberfroid *et al.*, 2010).

Many plant species, such as chicory, onion, Jerusalem artichoke and yacon, contain fructans as reserve carbohydrates (Figureueiredo-Ribeiro, 1993, Carabin & Flamm, 1999, Bhatia & Rani, 2007). Fructans are carbohydrates that consist of one or more fructose units linked or not linked to a terminal sucrose molecule, which may have a linear or ramified structure, with molecules united by β (2-6) or β (2-1)-type fructosyl-fructose bonds. According to their degree of polymerisation (DP), ITF are divided into inulin, with a DP from 10 to 60 units of monosaccharides and related compounds, and FOS, with a DP lower than 10 (Roberfroid & Slaving, 2001).

3. FUNCTIONAL EFFECTS OF PREBIOTICS ON THE HEALTH OF THE ELDERLY

The health-promoting effects of prebiotics include benefits to host nutrition, the growth inhibition of pathogens, and the promotion of beneficial microbiota (Choque-Delgado *et al*., 2011). Ageing is characterised by physiological changes in the gastrointestinal tract with a consequent imbalance of the intestinal microbiota. The changes in the structure and composition of the intestinal microbiota may be related to conditions that are common in the elderly, such as immunosenescence, metabolic syndrome, diabetes, and sarcopenia (Biagi *et al*., 2012). During ageing, intestinal changes may compromise the health of the elderly. Hypochlorhydria resulting from gastric atrophy is common in the elderly and is responsible for the decreased absorption of calcium, iron, and vitamin B12 (Elphick *et al*., 2006). The decrease in food intake (Murphy *et al*., 2009) in combination with the slowing of the intestinal motility results in reduced faecal weight (Woodmansey, 2007) and constipation, which lead to reduced excretion of bacterial metabolites. The increased retention time of the stool is related to a greater fermentation of proteins and a consequent increase in the levels of ammonia and phenols produced by intestinal putrefaction, which may favor the onset of gastrointestinal diseases (Murphy *et al*., 2009). The amount of Bacteroides declines with ageing

(Woodmansey, 2007), which impairs the degradation of residual polysaccharides from diet (Macfarlane & Gibson, 1991) and increases the number of Clostridia (Hopkins & Macfarlane, 2002). The resulting dysbiosis may be among the causes of increased GIT infections in the elderly and appears to correlate with the increase of pro-inflammatory cytokines (IL-6 and IL-8) (Biagi *et al*., 2012) and with augmented intestinal permeability, which may exacerbate the inflammatory process (Ohland & MacNaughton, 2010). Malnutrition in the elderly, which can be caused by various factors, including a decrease in the amount and type of food consumed, due to a decline in taste and smell and a decreased ability to masticate and decreased intestinal motility, may alter the intestinal epithelium and function, thereby resulting in decreased intestinal immune homeostasis and absorption of nutrients (Murphy *et al*., 2009), which leads to loss of appetite, reduced food intake, and worsening of malnutrition.

Human ageing is characterised by a progressive loss of bone mass in terms of both density and quality, which leads to osteoporosis and subsequent circulatory, respiratory, and thrombo-skeletal complications (Lobo, 2004). Figure 1 shows some physiological changes that affect the elderly and the possible sites of action of prebiotics. The significance of intestinal microbiota homeostasis for host health has attracted the interest of researchers regarding the development of prebiotic and probiotic preparations for the elderly. Food is one of the main factors that can affect the immune system in the GIT (Roller *et al*., 2004) in addition to the composition of the intestinal microbiota and its metabolites. The presence of dietary compounds in the small intestine can benefit the functioning and development of the intestinal immune system (GALT – gut-associated lymphoid tissue) and the systemic immune system (Seifert & Watzl, 2007).

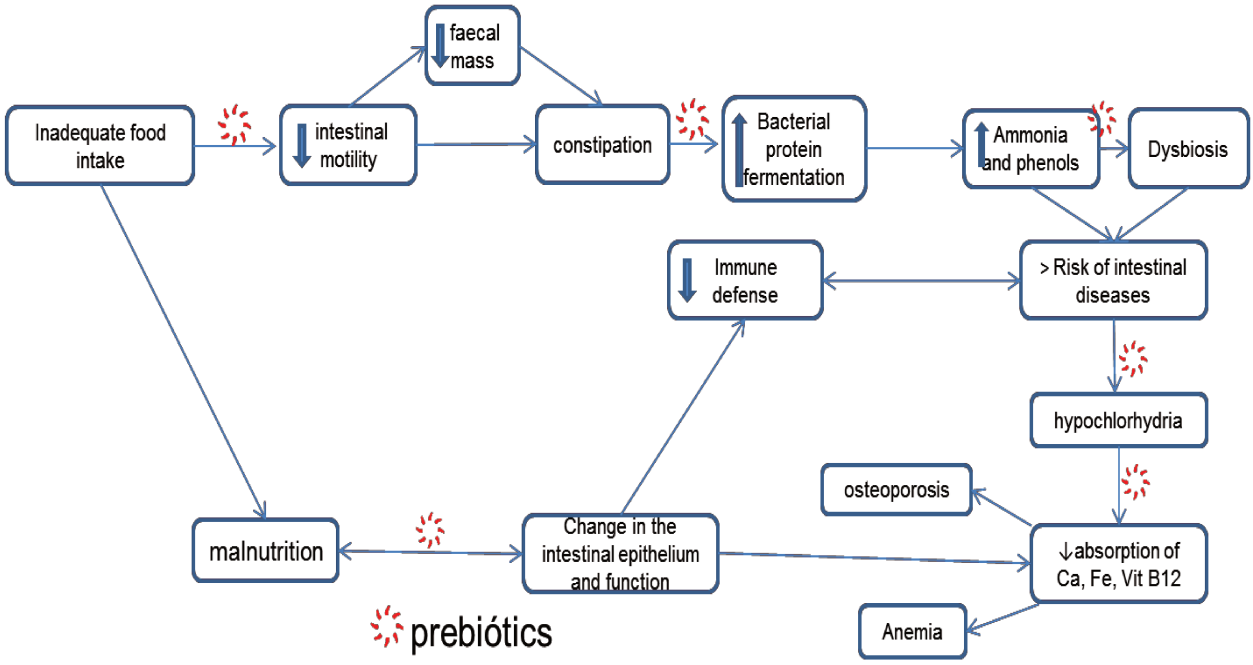

FIGURE 1: Physiological changes in the elderly and possible sites of action of prebiotics (source: author)

The stimulatory effect of prebiotics is more evident when the number of intestinal bifidobacteria is low (Tuohy *et al.*, 2001), which suggests that prebiotics are most effective in the elderly. Roberfroid *et al.* (1998) suggest that the increase in bifidobacterial numbers depend more on the initial number of bifidocaterial population levels in the gut microflora, irrespective of the dose of the FOS. According to this argument, the prebiotic effect is influenced by the starting number of bifidobacteria in the subjects prior to administration of the prebiotic, which means that the larger the number of initial fecal bifidobacteria present in an individual, the greater the potential for a bifidogenic effect (Davis *et al.*, 2010). Rowland and Gill (2008) argue that the intestinal microbiota balance may be partly restored through dietary supplementation with prebiotics that selectively stimulate the growth of intestinal beneficial bacteria.

Mammals lack prebiotic-hydrolysing enzymes. The principle of action consists in prebiotics reaching the large intestine intact. There, prebiotics are

fermented by the intestinal microbiota to produce primary metabolites, H2, CO2, and CH4 gases, and SCFAs, especially acetate, propionate, and butyrate (Bosscher, 2009). The decrease in pH due to SCFA production inhibits the growth of potentially pathogenic bacteria, including the *Clostridium spp* and *Bacteroides spp*, and promotes the growth of non-pathogenic bacteria, including *Bifidobacterium spp* and *Lactobacillus-Enterococcus spp* (Kolida *et al.*, 2002), which has a positive effect on the immune system, intestinal motility, plasma glucose and lipid placebo, calcium bioavailability, malnutrition, and intestinal diseases (Kaur & Gupta, 2002, Passos & Park; 2003; Kruger *et al.*, 2003). Figure 2 shows the possible mechanisms of action of prebiotics on in the elderly.

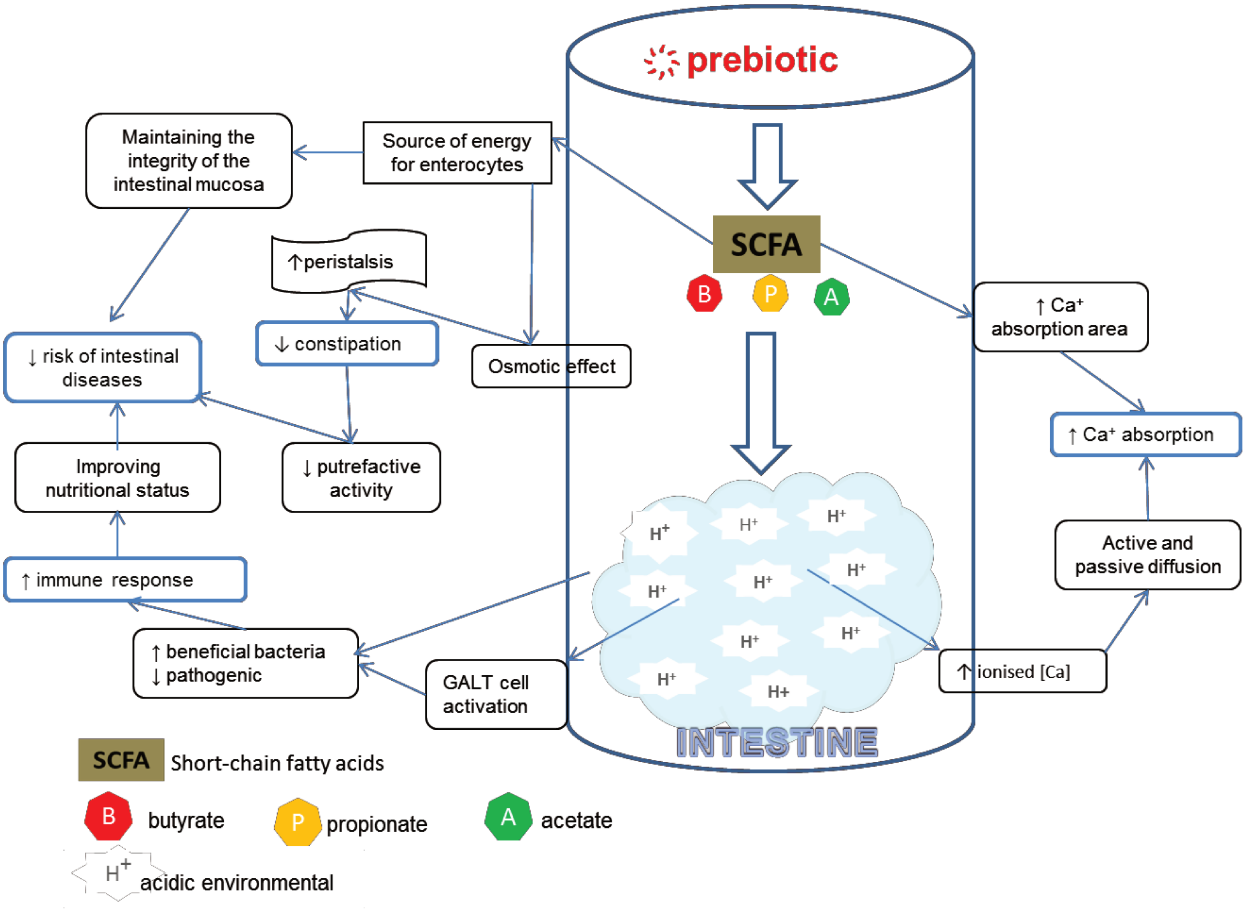

FIGURE 2: Possible mechanisms of action of prebiotics on the elderly (source: author)

The principles of action of prebiotics on the immune system and inflammatory processes can be explained by the enhanced production of SCFAs, which are used by the microbiota or absorbed through the intestinal mucosa to be used as an energy source by host cells. Butyrate may reduce inflammation by inhibiting the activation of NF-k (nuclear factor) and increasing the cytoplasmatic inhibitor of NF-kB, which inhibits the production of pro-inflammatory cytokines (IFN-ɣ and IL-2) (Delzenne, 2003, Segain *et al.*, 2000), whereas acetate and propionate stimulate the production of immunomodulatory IL-10 and IgA and natural killer (NK) cell activity (Roberfroid *et al.*, 2010). Immune cells use glutamine as an energy source, and the increased production of butyrate may reduce the demands of epithelial cells for glutamine, which preserves this amino acid for immune cells (Jenkins *et al.*, 1999). In addition, propionate- and butyrate-induced mucin production contributes to the maintenance of the mucosal layer, which enables epithelial protection (Pullan *et al.*, 1994). Prebiotics improve the intestinal barrier function by increasing the production of mucus and IgA in the mucus, which prevents epithelial apoptosis and stimulates defensin production (Wallace, 2011), and activating the production of cytoprotective molecules. The improvement in the structure and function of intestinal cells activates the immune system, thereby reducing the risk of malnutrition in the elderly due to a greater intestinal absorption capacity.

The SCFAs that are formed by the intestinal fermentation of prebiotics have an osmotic effect in the colon. The influx of water into the intestinal lumen promotes peristalsis and intestinal motility (Hamilton-Miller, 2004, Ohland & MacNaughton, 2010), as well as the improvement of constipation symptoms in the elderly.

The bifidogenic effect of prebiotics is attributed to non-digestibily and selective fermentation in the colon by beneficial bacteria and is associated with decrease in pH and changes in the SCFA pattern (Reyed, 2007). These changes favour the growth of bifidobacteria and *lactobacilli* and simultaneously attenuates the activity of putrefactive bacteria, such as *Escherichia coli* and *Clostridium*

perfingens. This mechanism inhibits the formation of toxic metabolites, such as ammonia and phenols, and consequently protects against intestinal diseases in the elderly. SCFAs, particularly butyrate, are the main energy source for enterocytes and stimulate cell proliferation to ensure the integrity of the intestinal mucosa and reduce the risk of intestinal diseases (Roberfroid *et al*., 2010).

Prebiotics may increase the absorption of minerals due to their osmotic effect, which transfers water into the intestine and thereby facilitate mineral solubilisation. Products from prebiotic fermentation acidify the intestinal lumen by increasing the concentration of ionised minerals, which is a condition that promotes the passive diffusion of calcium (Demigné *et al*., 1995, Bouglé *et al*., 2002). SCFAs may have a direct effect on the transcellular calcium absorption by increasing surface area in the large intestine, which results from the greater number of crypts and epithelial cells per crypt (Scholz-Ahrens *et al*., 2001), as well as by enhancing active transport of calcium and magnesium associated with decrease in pH (Lobo, 2004). These mechanisms provide a rationale for the possible use of prebiotics for the prevention of osteoporosis.

Prebiotics can modulate various physiological processes, such as mineral absorption, lipid metabolism, modification of intestinal microbiota (Puthanapura *et al*., 2011, Roberfroid, 2005) through their positive effect on the immune system, enhancing resistance against infections and microbiological activity as well as decrease of allergic reactions in experimental models (Choque-Delgado *et al*., 2010). Studies have shown that the number of *Clostridium spp*. and *Bacteroides spp* increases and the number of enterobacteria did not change in the elderly following the ingestion of FOS (Bouhnik *et al*., 2007). Another study showed that GOS administration to healthy elderly led to increase in beneficial bacteria, *Biffidobacterium spp., Lactobacillus-Enterococcus spp., Clostridium coccoidesT-Eubacterium rectale*, and decrease in *Bacteroides spp, Clostridium hystolyticum group* and *Escherichia coli*, and had a positive immunomodulatory response effects (Vulevic *et al*., 2008).

Experimental data suggest that prebiotics, such as inulin and FOS, alter the balance of intestinal bacteria in a beneficial way and stimulate GALT cells, which affect the immune system through the modulation of cytokines, the production secretory IgA antibodies, improved SCFA binding to G-protein-coupled receptors, and greater interaction of prebiotics with the carbohydrate receptors on leukocytes (Seifert, 2007).

Evidence shows that the intake of prebiotics affects the cholesterol metabolism of experimental animals and humans (Delzenne *et al.*, 2002, Pereira e Gibson, 2002). SCFA productions, butyrate may inhibit liver cholesterol synthesis and provide energy for human colon epithel cells, propionate may inhibit the synthesis of fatt acids in the liver, thereby decreasing the rates of triacylglycerol secretion (Scholz-Ahrens *et al.*, 2001, Gibson, 1999c). Dyslipidaemia is directly correlated to risk of atherosclerotic cardiovascular disease. The regular intake of prebiotics may produce benefits related with either the reduction or prevention of cardiovascular disease (Roberfroid, 2000).

A study of hospitalised elderly patients who ingested 4 g of FOS twice a day for 3 weeks showed an increase in the amount of faecal bifidobacteria. In addition, the percentage of T lymphocytes, including CD3, CD4 and CD8 cells, was higher compared with the placebo group. In contrast, the phagocytic activity of the peripheral blood granulocytes and monocytes and the IL-6 mRNA expression were lower in the FOS-treated group compared with the placebo group. The FOS supplementation accounts for these results (Guigoz *et al.*, 2002).

Research studies conducted with non-hospitalised elderly patients that were administered 6 g oligofructans for 28 weeks examined the immune response to vaccination against influenza and pneumococcus. In the study, the administration of the prebiotic following vaccination had no immune effect, i.e., there was no difference in the serum antibodies between the placebo and placebo groups (Bunout *et al.*, 2002). To evaluate the effect of lactose or inulin on the bowel habit in constipated elderly, Kleessen *et al.* (1997) assayed faecal changes

after 19 days of intervention, with a dose of 20 g/d from days 1 to 8, and gradually increased to 40 g/d from days 9 to 11. The results showed an increase in the faecal bifidobacteria, a decrease in enterococci and enterobacteria in the inulin intake group, and no change in the content of faecal SCFAs and lactase in the lactose-intake group. Inulin exhibited a better laxative effect than lactose in this study.

In another study, which was aimed at assessing the effect of a diet supplemented with 6 g FOS and *L. paracasei* on the immune response and the response to influenza and pneumococcus vaccination in 60 elderly subjects, Bunout *et al.* (2004) reported an increase in the NK cell activity in the supplemented group after 4 months of intervention. In addition, the IL-2 production by the peripheral blood mononuclear cells (PBMC) and the proportion of T cell and NK cell activity decreased in the placebo group and did not change in the supplemented group. The elderly subjects who were administered the supplement reported a reduction in infections. The authors concluded that the supplementation increased the inactive immunity and provided protection against infections in the elderly.

An intervention performed by Schiffrin *et al*.. (2007) concluded that the administration of liquid supplement containing 1.3 g OS/250 ml to malnourished elderly subjects had a positive effect on inflammation in this population. A study conducted with 12 healthy elderly subjects showed that the intake of 8 g FOS per day for 4 weeks increased faecal bacteria and the excretion of cholesterol (Bouhnik *et al*.., 2007). The oral administration of 5 g GOS/d to the elderly for 10 weeks exhibited a positive effect on both the composition of the microbiota and the immune response. The supplementation significantly increased the number of beneficial bacteria, NK cell activity, and the production of anti-inflammatory cytokines and decreased the production of pro-inflammatory cytokines (e.g., IL-6 and IL-1β) and tumour necrosis factor-α (Vulevic *et al*.., 2008).

As shown in Table 1, several human studies that investigated the effects of prebiotics on health suggest that the oral intake of prebiotics may have significant health benefits for the elderly. These effects are particularly observed in the anti-inflammatory properties and the effects on the human immune system. However, additional studies are needed to clarify these effects.

In humans, the clinical manifestations of age-related immune dysfunction increase the susceptibility to certain infections and the incidence of certain autoimmune disorders and certain types of cancers (Aspinal & Andrew, 2000). Prebiotics appear to play a prominent role not only in the modulation of the immune system but also in the placebo of the frequency of bowel movements, the metabolism of minerals, and cell differentiation. In addition, prebiotics participate in the prevention and treatment of disorders that affect the elderly.

Table 1: Functional effects of prebiotics on the health of the elderly

Author, year	Population (N, age)	Study design	Intervention (dose and type of prebiotic, duration)	endl (N)	Assessed parameters	Results
Guigoz Y, 2002	19 (77 - 97 years)	Pre- and post-test	8 g/d FOS, 3 weeks	9	MNA, FB, immunological (IL-6 and CRP) and biochemical (serum alb and α-glycoprotein) analysis	↑ FB, total lymphocytes, CD4, and CD8, ↓ phagocytic activity of monocytes and IL-6 mRNA expression in monocytes
Schiffrin, 2007	74 (> 70 years)	DBRCT	1.3 g FOS/250 ml drink, 12 weeks	4	Blood (CD3+, CD4+, CD8+, CD19+, CD16+, CD56+, CD4/CD8, alb, pre-alb, α-glycoprotein, PCR, GLU), plasma (IL-2R, TNF, soluble-IL-6R, soluble-CD14), FB	No change circulating cytokine levels and FB, ↓ IL-6 and TNF mRNA in the EG, Non-significant CD14↓ in the EG
Furrie *et al*.., 2005	18 (24 - 67 years)	DB, PC, clinical study, DBRCT	6 g mixture FOS/inulin + *B. longum* 2x/d, 4 weeks	16	RB, CRP, TNF, IL-1α	↓ sigmoidoscopy markers, ↓ mRNA levels of β-defensins in the EG, ↓ TNFα and IL1α
Bunout *et al*.., 2004	60 (> 70 years)	DB	6 g/d FOS + lactobacillus, 12 months	0	Serum dosage (NK activity, cytokines, lymphocytes)	↑ NK activity in the EG, ↓IL-2, ↓proportion of T and NK cells in the CG, ↓infections in the CG
Bouhnik *et al*.., 2007	12 (69 years)	3 study periods	8 g FOS/d, 10 weeks	2	CT, BA, FB, IT	↑FB, CT and production of gases, ↓ faecal pH, no change in IT time.
Kleessen *et al*.., 1997	35 (68 – 89 years)	DBRCT	20 g/d inulin or lactose for 8 days and 40 g/d inulin or lactose for 11 days	5	SCFA, lactate, FB	Significant ↑ bifidobacteria and ↓ enterococcus in the EG, no change in faecal SCFA and lactate concentrations, laxative effect in the EG
Bunout *et al*.., 2002	66 (>70 years)	PC, R, DB DBRCT	6 g prebiotic mixture (raftiline and raftilose) for 28 weeks; vaccination against influenza and pnemococcus at week 2	3	Proteins, alb, serum Ig, saliva IgA, antibodies against influenza and pneumococcus, IL-4	No change in protein, alb, serum Ig, secreting IgA, and IL-4 and interferon-gamma secretion, ↑ antibodies against influenza B and pneumococcus
Vulevic *et al*.., 2008	44 (64-79 years)	DB, PC, R DBRCT	5.5 g GOS/d for 10 weeks	1	FB, NK activity, cytokines, cholesterol, serum HDL-CT	Significant ↑ bifidobacteria in the EG, NK activity, and IL-10, ↓ IL-6, IL-1β and FNT-α, no change in CT and HDL-CT

FOS=Fructooligosaccharides; **GOS**=glucooligosaccharides; **MNA**=mini nutritional assessment; **CRP**=C-reactive protein; **RB**=rectal biopsy; **GLU**=glucose; **TNF**=tumour necrosis factor; **SCFA**=short-chain fatty acids; **PC**=placebo controlled; **PS**=prospective study; **DBRCT**=double-blind randomized placebo trial; **FB** = fecal bifidobacteria; **EG**= experimental group; **CG**= control group; **CT**= cholesterol; **IT** = intestinal transit; **NK**= killer cells

4. CONCLUSIONS

The incidence of chronic diseases has grown with the increase in life expectancy and the number of elderly people and has had an economic impact on the healthcare system and the quality of life. Changes occur in the intestinal microbiota with ageing; these changes affect the immune function, diet, and lifestyle, and predispose the elderly to illness. The effect of prebiotics on the quality and number of intestinal microbiota during ageing has been investigated. The use of prebiotics in food is an effective option for preserving colonic health and the health and welfare of the host because these reduce the risk of chronic diseases in the elderly.

Fructans are short-chain carbohydrates that are able to benefit host health. The dietary strategy of ingesting prebiotics enables a change in the microbiota. The functional properties of the prebiotics have encouraged the development of products and the use of foods fortified with this nutrient to inhibit the growth of pathogenic bacteria and stimulate the development of beneficial intestinal bacteria, which promote a reduction in diseases among the elderly population. In vitro and in vivo studies have shown the favourable effect of prebiotics on the gastrointestinal tract, immune system, and metabolism of minerals. The mechanisms of action of prebiotics in the aged human body are not yet fully explained. Therefore, new and broader interventions in humans are necessary to determine the effectiveness of prebiotics in the elderly, especially studies with specific methodologies that clarify the metabolism and possible mechanisms and sites of action of these nutrients in the elderly.

Acknowledgements

The authors thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - São Paulo Research Foundation - Research Project 2010/50689-7) for the financial support

REFERENCES

Aspinal, R., Andrew, D. (2000) Immunosenescence: potential causes and strategies for reversal.*Biochemical Society Transactions*, 28, 250-254

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., Gordon JI. (2005). Host-bacterial mutualism in the human intestine. *Science*,307(5717),1915–1920.

Bhatia, A., Rani, U. (2007). Prebiotics and health: Clinical implications. *Journal of Clinical Diagnostic Research*, Dec,1(6), 546-554

Biagi, E., Candela, M., Fairweather-Tait, S., Franceschi,C., Brigidi, P. (2012). Ageing of the human metaorganism: the microbial counterpart. *Age*, February, 34(1), 247-267

Biagi,E., Candela,M., Franceschi,C., Brigidi, P. (2011) The aging gut microbiota: New perspectives.Ageing *Research Reviews*,10,428-429

Bosscher, D. (2009). Fructan prebiotics derived from inulin. In D. Charalampopoulos & R.A. Rastall (Eds.), Prebiotics and probiotics Science and technology. (pp. 163-205). UK: Springer

Bouglé, D., Vaghefi-Vaezzadeh, N., Roland, N., Bouvard, G., Arhan, P., Buréau, F., Neuville, D., Maubois, J. L. (2002). Influence of short chain fatty acids on iron absorption by proximal colon. *Scandinavian Journal of Gastroenterology*, 37(9), 1008-1011

Bouhnik, Y., Achour, L., Paineau, D., Riottot, M., Attar, A., Bornet, F., (2007). Four-week short chain fructo-oligosaccharides ingestion leads to increasing fecal bifidobacteria and cholesterol excretion in healthy elderly volunteers. *Journal of Nutrition*, Dec 5, 6:42

Bunout, D., Barrera, G., Hirsch, S., Gattas, V., de la Maza, M.P., Haschke, F., Steenhout, P., Klassen, P., Hager, C. (2004). Effects of a nutritional supplement on the immune response and cytokine production in free-living Chilean elderly. *Journal of Parenteral and Enteral Nutrition*, 28(5), 348–354.

Bunout, D., Hirsch, S., la Maza, M.P, Munoz, C., Haschke, F., Steenhout, P., Klassen, P., Barrera, G. *et al*... (2002). Effects of prebiotics on the immune response to vaccination in the elderly. *Journal of Parenteral and Enteral Nutrition*, 26(6), 372–376,

Carabin, I. G., Flamm, W. G. (1999). Evaluation of safety of inulin and oligofructose as dietary fiber. *Regulatory Toxicology and Pharmacology*, 30(3), 268-282.

Chaimowicz, F. (1997) A saúde dos idosos brasileiros às vésperas do século XXI: problemas, projeções e alternativas. *Revista de Saúde Pública*. 31, 184-200

Chichlowski M, Hale LP. (2008). Bacterial-mucosal interactions in inflammatory bowel disease—an alliance gone bad. *American Journal of Physiology Gastrointestinal and Liver Physiology,* 295,1139–1149.

Choque-Delgado, G.T., Tamashiro, W.M.S.C., Maróstica-Júnior, M.R., Moreno, Y.M.F., Pastore, G.M. (2011). The putative effects of prebiotics as immunomodulatory agents. *Food Research International*. 43,1231-1236

Choque-Delgado, G.T., Tamashiro, W.M.S.C., Pastore, G.M. (2010). Immunomodulatory effects of fructans. *Food Research Internationa*l, 43,1231-1236

Davis,L.M.G., Martínez,I., Walter, J., Hutkins, R.(2010). A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy 35 adults. *International Journal of Food Microbiology*. 144(2),285-292

Delzenne, N.M. (2003) Oligosaccharides: state of the art. *Proceedings of the Nutrition Society*, 62(1), 177–182.

Delzenne,N.M., Daubiol,C., Neyrinck,A., Lasa,M, & Taper,H.S. (2002). Inulin and oligofructose modulate lipid metabolism animals. Review of biochemical events and future prospects. *British Journal of Nutrition*,87(2),255-259

Demigné, C., Levrat, M. A., Younes, H., Rémésy, C.(1995). Interactions between large intestine fermentation and dietary calcium. *European Journal of Clinical Nutrition*. 49 (suppl.) (3), 235-238

Dubert-Ferrandon, A., Newburg, D.S., Walker, A.W.(2008). Immune functions and mechanisms in the gastrointestinal tract. In G.R. Gibson & M.B. Roberfroid (Eds.), *Handbook of prebiotics* (pp. 115-141). New York:CRC Press

Elphick, H.L., Elphick, D.A., Sanders, D.S., 2006. Small bowel bacterial overgrowth. An underrecognized cause of malnutrition in older adults. *Geriatrics* 61 (9), 21–26.

Ewers, I., Rizzo, L.V., Kalil Filho, J., (2008) Imunologia e envelhecimento. *Einstein*; 6 (Supl 1),13-20

Figureueiredo_Ribeiro,R.C. (1993). Distribuição, aspectos estruturais e funcionais dos frutanos, com ênfase em plantas herbáceas do cerrado. *Revista Brasileira de Fisiologia Vegetal*, 5(2), 203-208.

Gavini, F., Cayuela, C., Antoine, J.M., Lecoq, C., Lefebvre, B., Membre, J.M. *et al.*. (2001). Differences in the spatial distribution of bifidobacterial and enterobacterial species in human faecal microflora of three different (children, adults, elderly) age groups. *Microbial Ecology in Health and Disease* 13, 40–45.

Giatti., L., Barreto, S. M. (2003). Saúde, trabalho e envelhecimento no Brasil. *Caderno de Saúde Pública*, 19(3), 759-771.

Gibson, G.R., Roberfroid, M.B. (1995a). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, 125(6), 1401–1412.

Gibson, G.R., Beatty, E.R., Wang, X., Cummings, J.H. (1995b). Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology*, 108(4), 975-982

Gibson, G. (1999c).Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *Journal of Nutrition*, 129,1438-1441

Gibson, G.R., Probert, H.M., Van Loo, J.A.E., Roberfroid, M.B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*,17(2), 257–259.

Guigoz, Y., Rochat, F., Perruisseau-Carrier, G., Rochat, I., Schiffrin, E.J. (2002). Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. *Nutrition Research,* 22(1-2), 13–25.

Hamilton-Miller, J.,M. (2004). Probiotics and prebiotics in the elderly. *Postgraduate Medical Journal*, 80, 447–451

Hopkins, M.J., Sharp, R., Macfarlane, G.T.(2001) Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance and community cellular fatty acid profiles. *Gut*, 48(2), 198-205

Hopkins, M.J. and Macfarlane, G.T. (2002) Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection. *Journal of Medicin and Microbiology*, 51(5), 448–454.

IBGE – Instituto Brasileiro de Geografia e Estatística. Síntese de indicadores. Estudos e pesquisas informações demográficas e socioeconômicas nº 27. *Síntese de Indicadores Sociais. Uma Análise das condições de vida da população brasileira* 2010. Rio de Janeiro: IBGE

Jenkins, D.J.A., Kendal, C.W.C. and Vuksan, V. (1999). Inulin, oligofructose and intestinal function. *Journal of Nutrition*, 129(7), 1431– 1433.

Kaur, N., Gupta, A.K. (2002). Applications of inulin and oligofructose in health and nutrition. *Journal of Biosciences*, 27(7), 703-714.

Kleessen, B., Sykura, B., Zunft, H.J., Blaut, M. (1997). Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. *American Journal of Clinical Nutrition*, 65(5) 1397–1402.

Kolida, S., Tuohy, K., Gibson, G.R. (2002). Prebiotic effects of inulin and oligofructose. *British Journal of Nutrition*, 87(suppl2), 193-197

Kruger, M., Brown, K., Collet, G., Layton, L., Schollum, L. (2003) The effect of fructooligosaccharides with various degrees of polymerization on calcium

biovailability in the growing rat. *Experimental Biology and Medicine*, 228(6), 683-688

Langlands, S.J., Hopkins, M.J., Coleman, N., Cummings, J.H. (2004). Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. *Gut*, 53, 1610–1616.

Lobo, A. R. (2004). *Efeito dos frutanos (frutooligossacarídeos) na biodisponibilidade de cálcio e magnésio em ratos*, São Paulo, Dissertação (mestre), Departamento de Ciências Farmacêuticas, Universidade de São Paulo (USP).

Macfarlane, G.T., Macfarlane,S., Cummings, J.H. (2006). Review article: prebiotics in the gastrointestinal tract. *Alimentary Pharmacology &Therapeutics*, 24(5), 701-714

Macfarlane, G.T. and Gibson, G.R. (1991). Co-utilization of polymerized carbon sources by bacteroides ovatus grown in a two-stage continuous culture system. *Applied and Environmental Microbiology*, 57(1), 1-6

Murphy, E., Murphy, C., O´Mahony L. (2009). Influence of the gut microbiota. In: Percival, S.L. *Microbiology and Aging: clinical manifestation*, (pp. 153-173). Springer

Ohland,C.L., MacNaughton,W.K. (2010). Probiotic bacteria and intestinal epithelial barrier function. *American Journal of Physiology and Gastrointestinal Liver Physiology*, 298, 807–819

O'Keefe SJ.(2008).Nutrition and colonic health: the critical role of the microbiota. Current Opinion in Gastroenterology,24, 51–58.

Passos, L.M.L., Park, Y.K. (2003). Frutooligossacarídeos: Implicações na saúde humana e utilização em alimentos. *Ciência Rural*, 33(2), 385-390

Pereira DI, Gibson GR. (2002).Effects of Consumption of Probiotics and Prebiotics on Serum Lipid Levels in Humans. *Critical Reviews in Biochemistry and Molecular Biology*, 37(4),259–281.

Pool-Zobel, B.L. (2005). Inulin-type fructans and reduction in colon cancer risk: review of experimental and data. *British Journal of Nutrition*, 93(1), 73–90

Pullan, R.D., Thomas, G.A., Rhodes, M., Newcombe,R.G.,Willians,G.T., Allen,A., Rhodos, J. (1994) Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. *Gut*, 35, 353–359.

Puthanapura, M. C, Keelara, V. H. P., Yeldur, P.V. (2011). Isolation, structural elucidation and immunomodulatory activity of fructans from aged garlic extract. *Phytochemistry*,72, 255–264

Reyed M. (2007).The Role of bifidobacteria in health. *Research Journal of Medicine and Medical Sciences*; 2(1): 14-24.

Roberfroid,M.B.; Van Loo,J.A.E.; Gibson, G.R. (1998). The bifidogenic nature of chicory inulin and its hydrolysis products. *Journal of Nutrition*, 128,11-19

Roberfroid, M.B. (2000). Prebiotics and probiotics: Are they functional foods? *American Journal of Clinical Nutrition*, 71, 1682-1687

Roberfroid, M.B.; Slavin, J. L. (2001). Resistant oligosaccharides. In: S.S. Choo, M.L. Dreher(Eds.),

Handbook of Dietary Fiber.(pp. 125-145). New York: Marcel Dekker

Roberfroid, M.B. (2002). Functional food concept and its application to prebiotics. *Digestion Liver Disease*. 34(Suppl. 2), 105-110.

Roberfroid,M.B. (2005).Introduction inulin-type fructans.*British Journal*,93(1),13-25

Roberfroid, M.B. (2008). General introduction: prebiotics in nutrition. In: G.R. Gibson, M.B. Roberfroid (Eds.), *Handbook of prebiotics*. (pp. 01-11). New York: CRC Press

Roberfroid, M.B., Gibson, G.R., Hoyles, L., McCartney, A.L., Rastall, R., Rowland, I., Wolvers, D., *et al*.. (2010). Prebiotics effects: metabolic and health benefits. *British Journal of Nutrition*, 104(2),1-63

Roller, M., Rechkemmer, G., Watzl, B., (2004). Prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis modulates intestinal immune functions in rats. *Journal of Nutrition*, 134(1), 153–156.

Rowland, I., Gill, C. (2008) Prebiotics and nutritions in the elderly: The concept of healthy ageing. In:G. R. Gibson & M. B. Roberfroid, (Eds.). *Handbook of prebiotics*. (pp. 405 – 419). New York:CRC Press.

Schiffrin, E.J., Thomas, D.R., Kumar, V.B., Brown, C., Hager, C., Van´t Hof, M.A., Morely, J.E., Guigoz, Y. (2007). Systemic inflammatory markers in older persons: the effect of oral nutritional supplementation with prebiotics. *Journal of Nutrition Health and Aging*, 11(6), 475 -479

Scholz-Ahrens, K. E., Schaafsma, G., Van Der Heuvel, E. G. H. M., Schrezenmeir, J. (2001). Effects of prebiotics on mineral metabolism. *American Journal of Clinical Nutrition,* 73 (suppl.), 459 - 464

Segain, J.P., Raingeard de la, B.D., Bourreille, A., *et al*... (2000). Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut*, 47(3), 397– 403.

Seifert,S., Watzl, B (2007). Inulin and oligofrutose: Review of experimental data on immune modulation. *Journal of Nutrition*, 137(11), 2563-2567

Tiihonen, K., Ouwehand, A.C., Rautonen, N. (2010). Human intestinal microbiota and healthy ageing. *Ageing Research Reviews*, 9(2), 107-116

Tuohy, K.M., Kolida, S., Lustenberger, A.M., Gibson, G.R. (2001). The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study. *British Journal of Nutrition*, 86(3), 341 - 348.

Tuohy, M.K., Gibson, G.R. (2007). Functions of the human intestinal flora: the use of probiotics and prebiotics. In A. Crozier, M. N. Clifford, H. Ashihara (Eds.), Plant

Secondary Metabolites: Occurrence, Structure and Role in the Human Diet, (pp. 174 – 207). UK:Blackwell Publishing

Tuohy, K.M. (2009). Commentary on prebiotics, immune function, infection and inflammation: a review of the evidence. *British Journal of Nutrition*.101, 631-632

Vulevic, J., Drakoularakou, A., Yaqoob, P., Tzortzis, G., Gibson, G.R. (2008). Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. *American Journal of Clinical Nutrition*, 88, 1438 – 1446.

Wallace, T.C , Guarner, F., Madsen, K., Cabana, M.D., Gibson, G., Hentges, E., Sanders, M.E (2011). Human gut microbiota and its relationship to health and disease, *Nutrition Reviews*, 69(7), 392 – 403

Wells, A.L., Saulnier, D.M.A., Gibson, G.R. (2008). Gastrointestinal microflora and interactions with gut mucosa. In G. R. Gibson, M. B. Roberfroid (Eds.), Handbook *of prebiotics*. (pp. 405 – 419). New York: AC Press

Woodmansey, E.J. (2007). Intestinal bacteria and ageing. *Journal of Applied Microbiology*, 102, 1178–1186

CAPÍTULO 2:

CHANGES IN PHYSICAL CHARACTERISTICS AND CARBOHYDRATE COMPOSITION OF FRESH YACON (*Smallanthus sonchifolius*) ROOTS DURING STORAGE

Scheid, M. M. A.[a], Silva, E. P. O.[b], Maróstica Junior, M. R. [c], and Pastore, G. M.[a]

[a] Department of Food Science, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

[b] UNIVAP (University of Vale of Paraíba), Avenue Shishima Hifumi 2911, 12244-000, São José dos Campos, São Paulo, Brazil

[c] Department of Food and Nutrition, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

This manuscript was submitted to Food Chemistry

ABSTRACT

Yacon (Smallanthus sonchifolius) is a plant with high concentration of FOS. Changes in the concentration of FOS and sensory characteristics occur during the storage of roots. Our aim was to evaluate the physical changes and the changes of FOS and sugar content in fresh roots stored under 25^{o}C, 4^{o}C and cooked roots 3, 10, 17, 24 and 31 days post-harvest. Sugar and FOS in roots stored at 25^{o}C and 4^{o}C were extracted with water and analyzed in Ion Chromatograph Dionex Model ICS-5000. Results indicate high variability in the FOS content and a reverse relationship between FOS and glucose and fructose. The FOS content of yacon storage at 4°C also decreased but in lesser proportion than at 25°C. Fresh yacon tubers deteriorate in appearance where they become withered and dried. It is recommended that yacon be stored at 4^{o}C and consumed before 17 days post-harvest.

Keywords: yacon, physical characteristics, carbohydrate compositions, post-harvest, fructooligosaccharides (FOS)

1. INTRODUCTION

Yacon (*Smallanthus sonchifolius*) is a tuberous plant from the South American Andes, which has been used for centuries as a staple food among the Andean population and has spread from the 1990's to several countries outside South America, such as New Zealand, Europe, USA and Japan. The commercial cultivation of yacon in Brazil commenced in 1991, in Capão Bonito (SP) and in Itajaí (RS) (Kakihara *et al.*, 1996). It is reported that yacon tubers contain a high concentration of oligofructans and polyphenols (Ohyama *et al.* 1990), including potential health benefits.

A major portion of yacon root biomass is composed of water usually exceeding 70% of fresh weight. Around 90% of the dry weight (DW) is carbohydrate. Even though the proportion of each sugar varies, the following composition on a dry basis can be considered: 40 -70% FOS, 5 -15% sucrose, 5 -15% fructose, less than 5% glucose and low content of fibre, vitamins, minerals and protein in the roots (Manrique *et al.* 2005).

Yacon tuberous roots accumulate inulin-type oligofructans, FOS, which are linear polymers consisting of fructose monomers linked together by β-(2→1) bonds with a polymerization degree of 3–10 fructans (Asami *et al.*, 1991). The FOS is a mixture of kestose (GF2), nystose (GF3), and furanosylnystose (GF4) oligosaccharides (Lewis, 1993). Fructooligosaccharides (FOS) are considered to be beneficial for humans, as an intake of FOS selectively stimulates the specific growth and metabolic activity of beneficial bacteria in the colon, thereby contributing to the host´s health. On consumption, the FOS reaches the colon intact and are fermented by gut micro flora, which produce short-chain fatty acids (SCFA) especially acetate, propionate, and butyrate , and the gases, H2, CO2, and CH4 (Roberfroid *et al.*, 1993). FOS play an important role in health as a bifidogenic agent, reducing blood lipid and glucose levels in diabetic subjects (Hsiao-Ling *et al.*, 2000), placeboling constipation (Beylot, 2005), increasing

mineral absorption and modulating the immune system (Guigoz *et al.*, 2002, Hosono *et al.*, 2003).

The majority of studies concerning prebiotics have focused on inulin, FOS and galactooligosaccharides (GOS). As a source of fructooligosaccharides, which are classified as prebiotics, yacon may have several functions in the food and dietary supplement industry as a functional food.

Yacon roots have a sweet taste and crisp texture and can be consumed raw, boiled, baked, or used for making drinks. For consumption, yacon roots are traditionally eaten raw or dried after exposure to the sun for several days ('sunning' or 'soleado' in Spanish), a treatment known to increase the roots' sweetness (Grau & Rea, 1997). Yacon can also be dehydrated and processed into attractive convenience products. Fructans are synthesised by the conversion of two molecules of sucrose into kestose and glucose by sucrose:sucrose 1-fructosyl transferase (1-SST), and by fructan:fructan 1-fructosyl tranferase (1-FFT), the enzyme responsible for the chain elongation (Luescher *et al.*, 1996). The fructan depolymerisation occurs by the action of fructan 1-exohydrolase (1-FEH), which catalyses the release of free fructose at the end of the growing season and during storage (De Roover *et al.,* 1999). Yacon roots are quite susceptible to dehydration when directly exposed to the sun. As a result of dehydration, the roots lose weight and develop a rough appearance, making them less attractive to the consumer. Following harvest, prolonged storage at higher temperatures cause major changes in the chemical composition of the tubers as well as affecting their physical and sensory characteristics.

Graefe *et al.* (2004) analysed the concentration of FOS in yacon dehydrated 6 days post-harvest and found a considerable decrease of FOS in the dry mass (DM) of the roots. During storage at low temperatures (5^{o} to 10^{o} C) and high humidity, the FOS content is maintained, but cannot completely suppress FOS reduction (Asami, *et al.*, 1991). Narai-Kanayama *et al.* (2007) demonstrated a decrease in FOS in yacon root during storage under conditions of around 90%

relative humidity at 8° C. It is important to be aware of the changes in the composition of yacon tubers during storage incurred from harvest to processing or consumption, in order to standardise yacon quality.

As yacon is a seasonal plant, it is important to use specific technological treatments in order to preserve and make it available for consumption throughout the whole year. It is also necessary to develop products which preserve their nutritional content and maintain their sensory characteristics.

The aim of this research was to evaluate the physical changes and the changes of FOS and sugar content in fresh yacon roots stored under ambient temperature (25°C), chilled temperature (4°C) and cooked roots 3, 10, 17, 24 and 31 days post-harvest.

2. MATERIAL AND METHODS

2.1. Plant material

The yacon (*Smallanthus sonchifolius*) tubers used were cultivated and obtained from a field near the town of Piedade – São Paulo, Brazil. The plants were harvested and separated into two samples. At the onset of the experiment (3 day *post-harvest*) samples of yacon roots were stored at room temperature (25°C) (A) and in a refrigerator at 4°C (F). The sugar (glucose, fructose, sucrose) and fructooligosaccharides (FOS), ash and water content were determined 3, 10, 17, 24 and 31 days *post-harvest*. The physical changes of the fresh roots were also analysed on the same days.

2.2. Physical changes of fresh yacon

The physical changes of fresh yacon were documented photographically and assessed based on a subjective evaluation of samples of fresh yacon stored at

25°C and at 4°C after 3, 10, 17, 24 and 31 days post-harvest. The durability from the start to the end of storage was determined by a score ranging from 0 to 5, according Campos (1987).

0 = 0% of wrinkling and 0% browning;

1 = 12.5% of wrinkling (mainly, on the side where the plant roots were fixated) and 25% browning;

2 = 25% of wrinkling and 50% browning;

3 = 50% wrinkling and 75% browning;

4 = 75% wrinkling and 100% browning;

5 = 100% wrinkling.

2.3. Extraction of carbohydrates

Sugars (glucose, fructose and sucrose) and fructans in each sample were extracted by the method used by Oliveira, Nishimoto, (2004) and by an adaptation of the method used by Pereira, (2009). 100g of peeled yacon tubers stored under room temperature (25°C) and chilled (4°C) conditions were weighed and transferred into a blender reservoir. 100 ml of water at 90° C was added to the blender. The mixture was liquidized for 2 minutes in order to produce a homogeneous product. The homogenate was filtered through a Buchener funnel containing a No.1 filter paper. The filtrate was heated at 90°C for 10 minutes and filtered. The filtrate was measured and stored at – 20°C. Samples of roots (100g) stored at 4°C were cooked (4°C + cooked) and the sugars and fructans extracted as previously described.

2.4. Determination of carbohydrates

The sugars (glucose, fructose, sucrose) and FOS content were determined by high performance anion exchange chromatography (HPLC-PAD) on a Dionex ICS 5000 chromatograph (Dionex, Sunnyvale, CA) equipped with a Pulsed Ampometric Detector (PA). 25 µL of the sample was injected into a Dionex ICS 5000 (Sunnyvale, CA EUA). BioLC HPLC fitted with a CarboPac PA-100 (250 x 4 mm, P/N 043055) analytical column and a CarboPac PA-100 (50 x 4 mm) guard column. The degassed mobile phase consisting of 97% NaOH and 3% NaAC was initially run for 10 minutes. From 10.0 to 25.0 min, the proportion of NaOH and NaOAc was altered linearly to a final ratio of 62% NaOH and 38% NaOAc, which was maintained from 25.0 to 30.0 minutes to clean the column. The eluents were changed to a concentration of 20% NaOH and 80% NaOAc. The column then was re-equilibrated for 10.0 minutes with 97% NaOH and 3% NaOAc. All eluents were prepared with deionized water. Flow rate was constant at 1.0 mL/min, and elution was conducted at room temperature. Chromatographic peaks were identified by comparing sample retention times (tR) to those of known standard mixtures. All samples were analysed in triplicate in our laboratory.

2.5. Water Content and ash determination

To determine root dry matter, the procedure was repeated four times. 50g of root samples were cut into slices and placed in Petri dishes previously weighed and dried at 105 ° C for 48 hours. The plates were then removed from the stove, placed in desiccators to cool at room temperature. They were then weighed to constant weight, thereby obtaining the dry weight. Data was presented as percentages (Araujo *et al*., 2006). Ash content was determined as previously described by Leonoel & Cereda (2002). A 5g dry root sample was cut into slices, put in a porcelain crucible previously weighed and placed in an oven at 550°C for 2 hours. Later, the plates were removed from the stove, placed in desiccators to cool

at room temperature which were then weighed to constant weight, thereby obtaining the ash weight. Data was again presented as percentages.

2.6. Statistical analysis

Data for the analytical results were subjected to analysis of variance (ANOVA) to compare the time of storage, temperature of storage and interaction temperature x time followed by Tukey's multiple comparison test. Analyses were conducted with software SAS System for Windows (Statistical Analysis System), vs. 9.1.3. (SAS Institute Inc, 2002-2003, Cary, NC, USA). One-sided p values < 0.05 were considered statistically significant.

3. RESULTS

Samples of roots were observed and photographed weekly. At the initial stage (3 days post-harvest) the roots had a good appearance and deep yellow colour. From the observation, (Table 1), (Figure 1) and (Figure 2), it is clear that physical changes in general appearance and changes in colour of the roots stored at 25^{o} C and at 4^{o} C occurred over time.

In tubers stored at 25^{o}C, no change was observed in general appearance or colour during 10 days of storage. The skin of roots stored at 25^{o} C became a little loose and shriveled after 10 days. On the 17th day of storage at 25^{o} C the roots became 75% wrinkled and 0% browned. After 24 days of storage, the roots were 100% wrinkled and 50% browned. Following 24 days of storage at 25^{o}C the yacon tubers began to develop fungi on the skin of the roots. The appearance and colour of the roots stored at 4^{o}C began to change after 17 and 24 days respectively. On the 17th day of storage at 4^{o}C, the roots were 12.5% wrinkled. Only on the 31st day post- harvest, the roots became 50% wrinkled and the colour

of yacon tubers stored at 4° C changed and became 25% browned. No fungi growth was observed in the roots stored at 4°C.

TABLE 1: Change in colour and general appearance of yacon roots stored at 25° C and at 4° C during 31 days, according to Campos (1987)

	WRINKLING		**BROWNING**	
	25°C	**4°C**	**25°C**	**4°C**
3 days	0	0	0	0
10 days	1	0	0	0
17 days	4	1	0	0
24 days	5	2	1	0
31 days	5	3	2	1

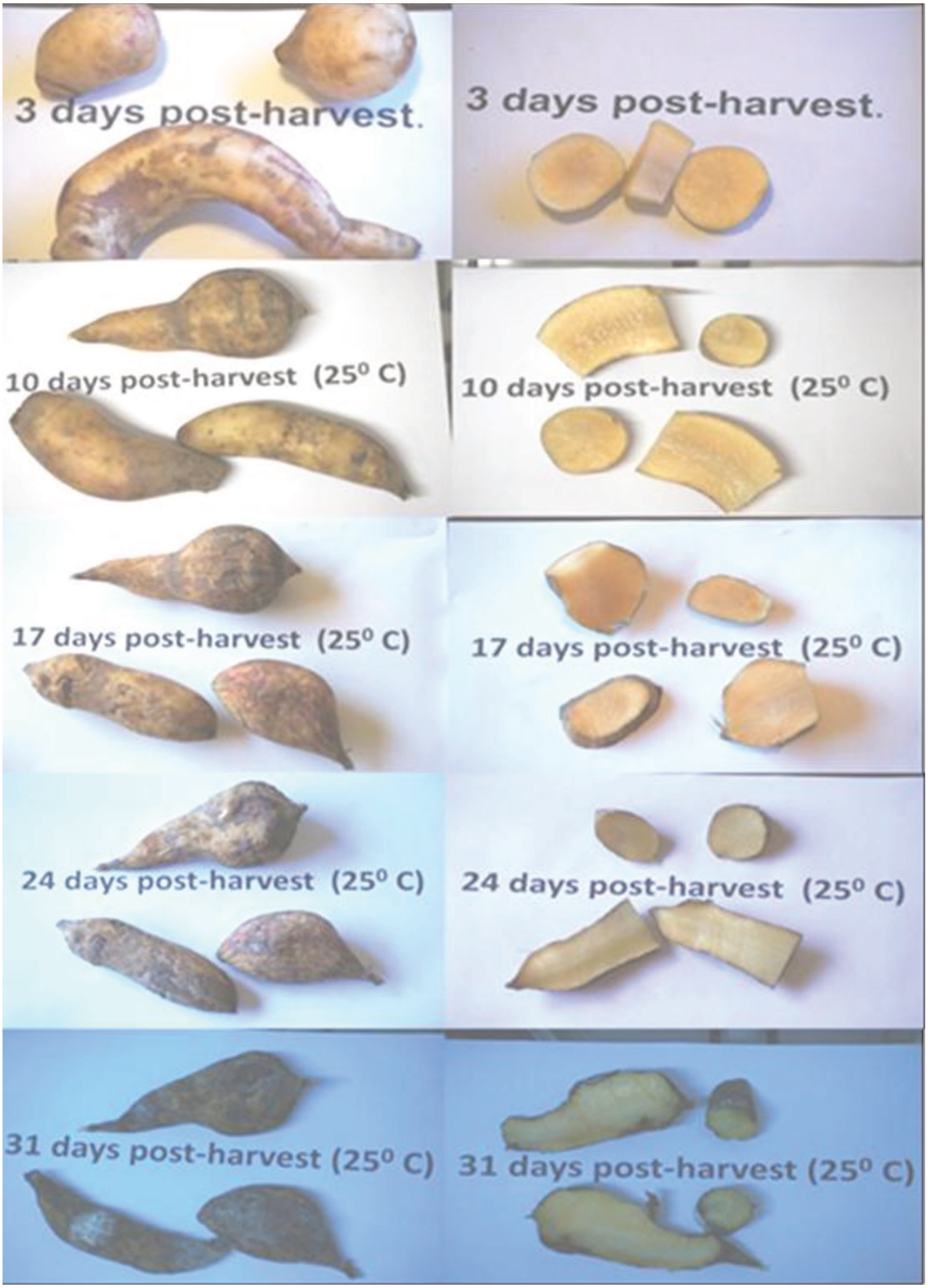

FIGURE 1: Physical changes in fresh yacon roots stored at 25°C during 31 days post-harvest.

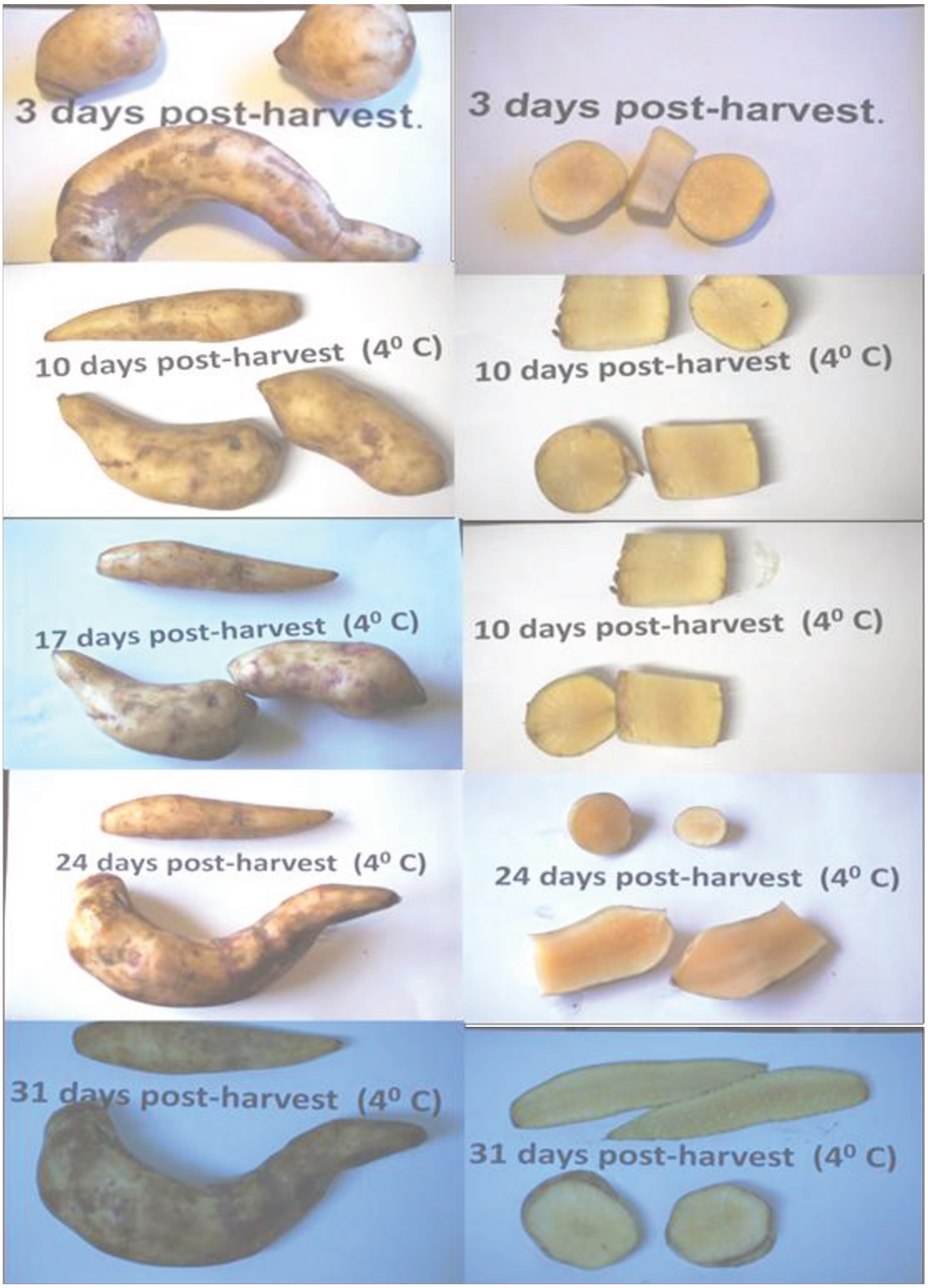

FIGURE 2: Physical changes in fresh yacon roots stored at 4°C during 31 days post-harvest documented photographically

The results from the chemical analyses of fresh yacon roots revealed a content of 2.43 ± 0.14% ash and 86.15 ± 1.25% humidity.

From the extracted carbohydrates (Figure 3), the Yacon roots were found to be composed of monosaccharides, such as glucose and fructose, disaccharides, such as sucrose, and FOS (kestose – GF2, nystose – GF3, and fructofuranosylnystose – GF4). The composition profile during storage varied depending on storage temperature (25°C and 4°C) and treatment conditions (4°C and 4°C + cooked).

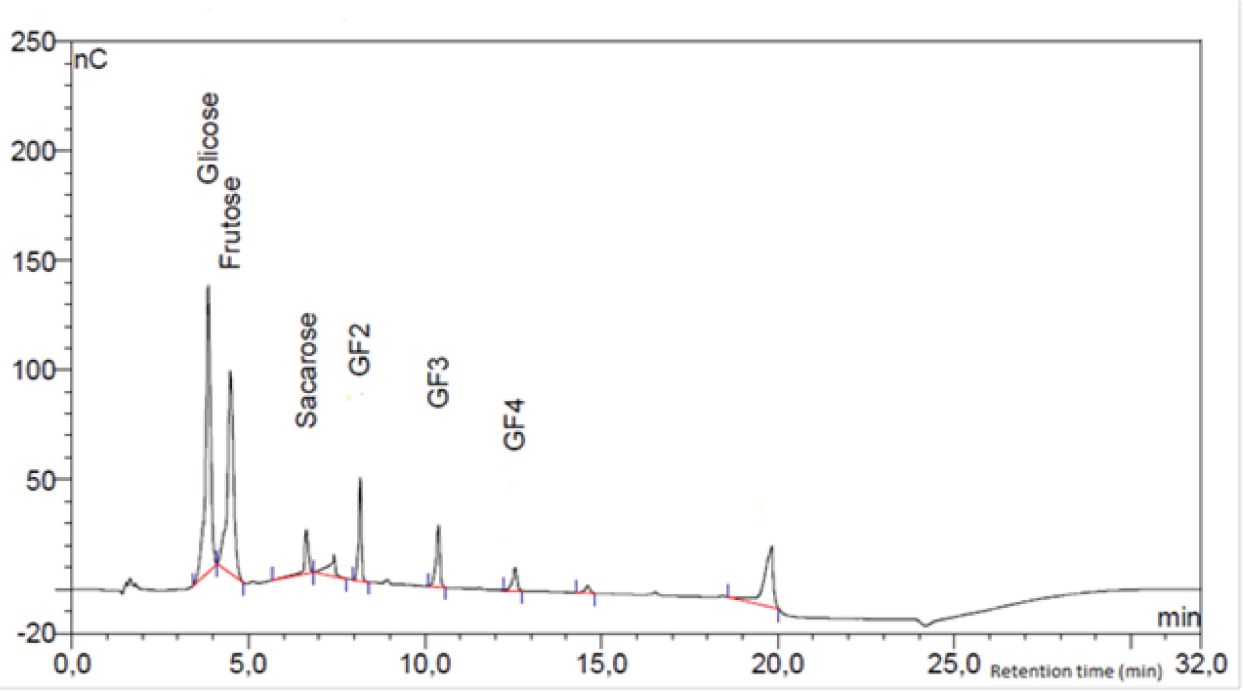

FIGURE 3: Chromatogram of glucose, fructose, sucrose and FOS of fresh yacon roots by high performance anion exchange chromatography (HPLC-PAD) on a Dionex ICS 5000 (Dionex, Sunnyvale, CA) chromatograph

During the 31day storage period, the FOS concentration, which accounted for 74.5% of root dry matter at the onset of the experiment, decreased significantly (p = 0,006), by about one-third in roots stored at 25° C (Table 2). At 25° C the FOS content further decreased following the 10th day of storage. The FOS content of roots stored at 4° C increased, not significantly, by 6.6 percentage points (PP)

during the first 17 days of storage and decreased significantly, 21.8 PP ($p = 0.004$) during the last 14 days. The FOS concentration increased by 19 PP during the first week of storage in the cooked sample and decreased 24.17 PP ($p = 0.019$) by the end of the experiment, therefore, the FOS content decreased, significantly, 5 PP during 31 days of storage. After 31 days of storage, roots stored at 25°C had the least amount of FOS; however this was not significantly different from the FOS content of tubers stored at 4° C. During 31 days of storage FOS concentration in root dry matter (DM) decreased significantly in all samples. The changes in FOS content was not significantly different ($p = 0.054$) or temperature dependent, but there were significant differences between temperature x time specific dependence (P = 0,009) in the speed of FOS conversion (Figure. 4).

The fructose concentration increased significantly since the onset of the experiment, irrespective of storage or treatment conditions (A, F, C). During the course of the experiment, fructose increased significantly around 48 PP, 42 PP and 27PP during the 31 days of storage in A ($p < 0.001$), F ($p = 0.021$) and C ($p < 0.001$), respectively. The highest fructose concentration (60.4 % DM) was on the 31st day in the roots stored at room temperature. Fructose concentration increased significantly in all samples (Figure 4). There were significant differences in temperature-specific ($p < 0.001$), time-specific ($p < 0.001$) and differences in temperature x time specific dependence ($p = 0.008$). In samples stored at 25°C and 4°C, the fructose concentration ranged significantly in the same proportion as the FOS during the 31 days of the experiment. At 25°C the range of fructose and FOS was 48 PP and at 4° C was 43 PP. In the cooked samples, the FOS content decreased (5 PP) and was less than the increase of fructose content (27 PP). Both ranges were significant

TABLE 2: Changes in sugar composition of fresh yacon root during storage at 25°C (A), 4°C (F) and of cooked root (C) (in % dry matter).

Storage and treatment conditions	Days of storage	Fuctooligosaccharides $\bar{x}$ (sd)	Fructose $\bar{x}$ (sd)	Sucrose $\bar{x}$ (sd)	Glucose $\bar{x}$ (sd)
	03	74.46 (8.98)	12.45 (3.63)	32.04 (4.33)	9.08 (0.53)
	10	69.48 (16.94)	27.95 (10.35)	27.10 (6.57)	11.90 (4.65)
25° C	17	49.07 (7.19) [a]	47.16 (5.60) [a]	30.71 (1.32)	17.65 (0.69) [a]
(A)	24	36.08 (22.37)	55.41 (4.81)	21.92 (9.47)	16.01 (13.79)
	31	25.89 (3.87)	60.43 (8.68)	26.93 (3.23)	22.60 (4.37)
	03	74.46 (8.98)	12.45 (3.63)	32.04 (4.33)	9.08 (0.53)
	10	76.45 (22.67)	32.16 (5.59)	22.15 (5.22)	10.98 (1.18)
4° C	17	81.09 (8.26)	30.72 (5.10)	26.50 (2.91)	10.59 (1.48)
(F)	24	43.58 (8.70)	46.91 (9.84)	15.05 (3.87)	16.45 (3.68)
	31	30.81 (6.16)	54.81 (11.26)	13.58 (1.85)	20.75 (3.07)
	03	58.12 (3.18)	15.60 (3.16)	29.34 (2.24)	8.46 (2.47)
4º C cooked	10	77.26 (11.91)	16.61 (1.97)	21.25 (2.13)	6.90 (0.32)
(C)	17	51.40 (8.51)	29.32 (2.69)	21.60 (4.33)	10.18 (1.24)
	24	55.45 (9.99)	29.21 (5.35)	20.63 (3.72)	10.43 (2.96)
	31	53.09 (3.51)	42.6 (1.18)	18.08 (1.52)	14.85 (0.71)

Each value is the mean of three replicate samples (sd). The data is presented as % of root dry matter.

In all samples, sucrose concentration accounted for 29 – 32% of root DM at the onset of the experiment, however showed changes during storage. The sucrose content did not significantly decrease (5 PP) in roots stored at 25° C, however decreased significantly by 18 PP in tubers stored at 4° C and 11 PP in

cooked roots after 31 days of storage. The reduction of sucrose content was greater after 17 days of storage in A and F (Table 2).

There were large significant differences, temperature-specific (p = 0.001) and time-specific (p < 0.001) in the concentration of sucrose over time, but not significant differences in temperature x time (p = 0.192). In roots stored at 25°C (A) sucrose content decreased, however not significantly by 0.22 PP (p = 0.173) daily during storage. In roots stored at 4°C (F), sucrose decreased significantly by 0.63 (p < 0,001) daily. Sucrose content in C shows a significant change (p = 0.01) during storage (Figure 4). The decrease in sucrose was greater in F (K = 0.63) and lower in A (k = 0.22) on each day of storage (Figure. 4).

Glucose, the sugar with the lowest concentration in the yacon root, increased during the 31 days of the experiment by 13.6 PP, 11.6 PP and 6.4 PP in A, F and C, respectively (Table 2). The variation in glucose was greater before 17 days of storage in A, but greater after 17 days of storage in F. Glucose concentration increased significantly in all samples, A, C, and F during the 31 days of the experiment. There was not a significant temperature x time specific dependence on the speed of glucose conversion (P = 0.707). Glucose increased significantly by 0.45 PP (p =0.017), 0.23 PP (p = 0.001) and 0.41 PP (p < 0.001) on each day of storage in A, C and F respectively. For A, the increase in glucose was greater than that for F. Glucose concentration in the dry matter of C increased by about 6 PP during the 31 days of storage (Figure. 4).

During 31 days of storage, FOS and sucrose decreased while monosaccharides, fructose and glucose increased in tubers stored at 25°C, at 4°C and in the cooked samples.

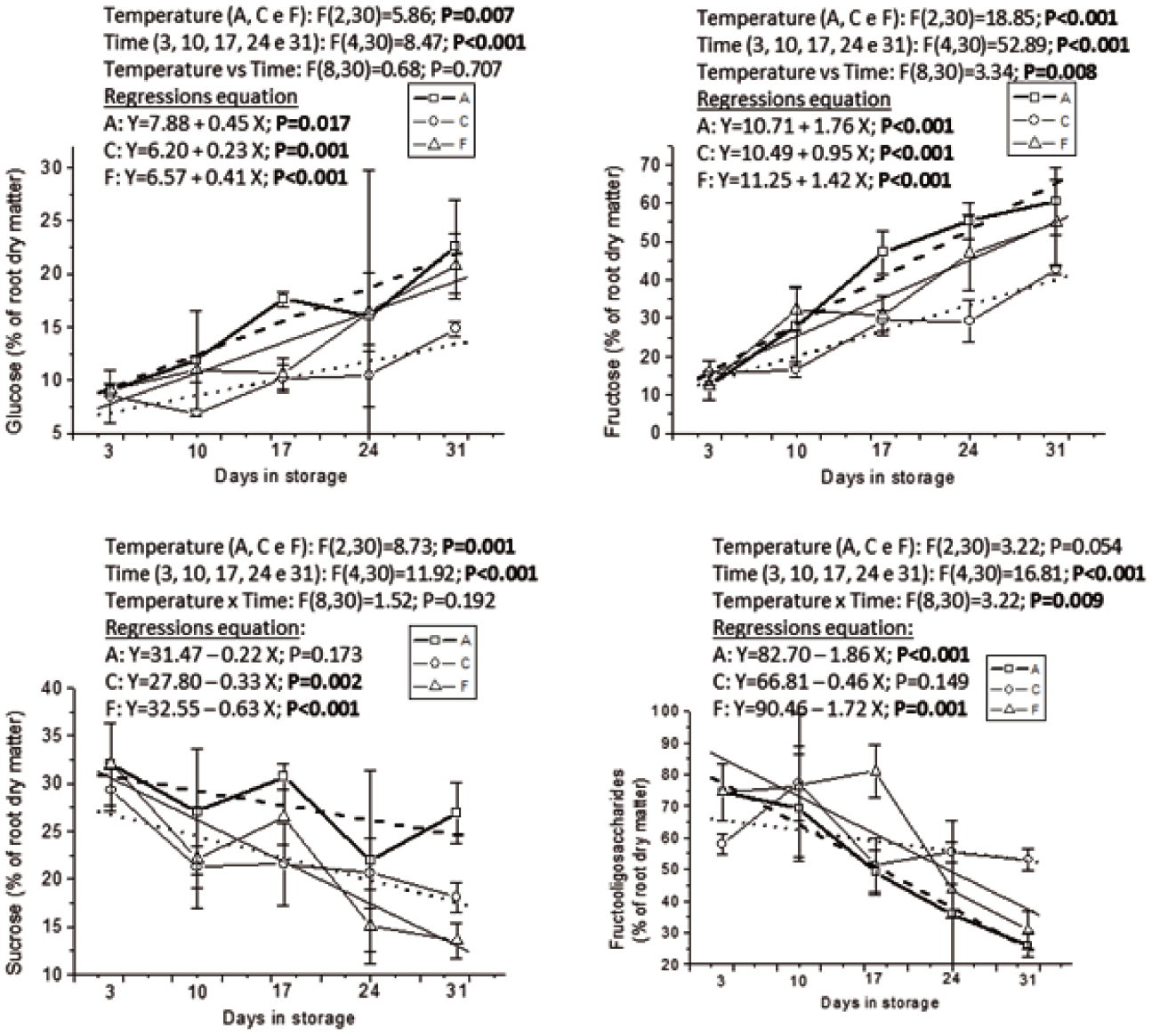

FIGURE 4: Comparative analysis and linear regression of carbohydrate content (glucose, fructose, sucrose and FOS) of fresh yacon stored at room temperature, at a refrigerated temperature and of cooked yacon (A = storage at 25°C, F = storage at 4°C, C = storage at 4°C and cooked).

4. DISCUSSION

In our study a physical change (wrinkling and colour) during storage was noted. The wrinkling of skin and browning in yacon samples stored at 25^{o}C and at 4^{o}C was observed. As in other studies, the wrinkling and browning was greater in roots stored at 25^{o}C. At low temperatures, the durability of yacon roots is greater than at ambient temperature. The scores for wrinkling and browning at low temperature storage was less than those stored at room temperature. As in our study, Vilhena, (2001) showed that the durability of yacon roots in a cold chamber was greater than that observed at room temperature. In our study, loss of mass was not measured.

Plants respiration results in the oxidation of carbohydrates (glucose) contained in the cells of the tuber, which is converted into water, carbon dioxide and heat energy. Once harvested, tubers are removed from their source of water, minerals and sustenance. Root tissue continues to respire, using available and stored sugars and organic acids, so they begin to senesce rapidly. Respiration results in water loss and consequently quality loss *post-harvest* depending on storage conditions, especially temperature (Watada *et al.*, 1996). The characteristics imparting the most important quality factors may be described by several different attributes, such as colour, texture, nutritional value, aroma and taste. Consumers generally evaluate a product based on four attributes (visual cues, aroma, taste and texture), primarily visual cues (Beaulieu, 2011).

Several studies have showed changes in colour, appearance and carbohydrate content of yacon roots stored at different temperatures. Vilhena, (2001) observed a change in colour and appearance of fresh yacon roots after 15 days of storage at ambient and cool temperature and showed that loss of mass of yacon stored at room temperature was greater than that when stored under chilled conditions. The decrease in mass was significant under both storage conditions. Sun-exposed yacon roots incur high mass losses which increases their sweet

flavor. Vilhena (1997) observed mass losses, on average between 8 and 43% following 1 and 8 days of exposure to the Sun, respectively.

Other studies have showed similar changes with other tubers. If the humidity is low, sweet potatoes loses weight as moisture evaporates from the surface of the roots. This results in weight loss and may cause the skin to shrivel, especially at root ends. Minor losses of fibre mass during storage at low temperatures have been found in other tuberous roots like Arracacia xanthorrhiza (Avelar Filho, 1997). In roots of Pachyrhizus erosus stored at 13^{o}C and 10^{o}C, a mass loss of 8 and 12% was observed after three weeks (Cantwell *et al.*, 2002).

The cause for browning in yacon roots is the presence of polyphenol oxidases (PPO) and peroxidase (PO), which catalyse the oxidation of phenolics to quinones (Fukai *et al.*, 1997). The placebo of enzymatic darkening is limited to the inhibition of the enzymes PPO and PO. Storage at low temperatures inactivates the enzymes and consequently inhibits browning. Chilling removes excess heat and oxygen from the roots, which eliminates weight loss and inactivates the browning of yacon roots and consequently maintains the quality of the product.

As observed in our study, post-harvest yacon root contained reduced quantities of glucose and fructose and a high level of FOS, although a compositional change of the root during storage occurred. Recent studies have shown a variation in carbohydrate content of yacon root during storage (Asami *et al.*, 1991, Cisnero-Zevallos *et al.*, 2002, Graefe, 2002, Graefe *et al.*, 2004,). Following harvest, some biochemical reactions take place which are important in the production of energy during the process of root respiration. The breakdown of FOS into simple sugars is catalyzed by fructan-hydrolase (FH), which releases terminal fructose molecules (Fukai *et al.*, 1997) resulting in fructose and sucrose as end products. The reduction of FOS in the sample stored at 25^{o} C observed during the 17 days of storage may at be partly due to the temperature favouring enzymatic activity. The variation of FOS observed in samples stored at 4^{o}C during

the first two weeks (day 17) may be due to the lower temperature delaying the onset of FOS conversion.

Graefe *et al.* (2004) showed a decrease by about one-third in FOS concentration during a12 day storage period at different altitudes and that following 7 days of storage at room temperature, 30 – 40% of FOS was converted into simple sugar. Asami *et al.* (1991) showed an acceleration of FOS conversion and an increased amount of glucose, fructose and sucrose following a rise in temperature in yacon during weeks of storage. A reduction in FOS in yacon tubers roots during storage at 8^{o}C was demonstrated by Narai-Kanayama *et al.* (2007). As in our study, a high variability in FOS content and also an inverse relationship between FOS and reducing sugars was observed in yacon samples stored at room and refrigerated temperature. Similarly in our study, Cisnero *et al.* (2002) observed that the FOS content in yacon stored at 4^{o} C decreased but to a lesser amount than that in roots stored at 25^{o}C.

Variation of simple sugar content during storage has been observed in other roots. Imahori *et al.* (2010) revealed that there was an increase in FOS content in Burdock roots during storage under low temperatures. An increased amount of simple sugar during storage is common in Chicory roots (*Cichorium intybus* L.) and Jerusalem artichokes (*Helianthus tuberosus* L.) (Edmunds *et al.*, 1994).

Our results showed that in yacon roots samples stored at 25^{o}C and 4^{o}C, the fructose concentration ranged significantly in the same proportion as that of FOS, while in the former sample a reduction of sucrose was smaller compared to the roots stored at 4^{o} C, whereas glucose increased in the same proportion in both samples. In yacon stored at 25^{o}C, the storage conditions most probably favoured the breakdown of FOS into its component fructose, glucose and sucrose. In yacon roots stored at 4^{o} C, the reduction in sucrose was greater. When yacon was cooked, the variation of carbohydrates (FOS, glucose, sucrose, and fructose) was smaller. The modified Arrhrenius equation uses the current understanding of

relationships between temperature and chemical reactions. The greatest values of *K* in yacon stored at 25^{o} C showed that the variation of carbohydrate is dependent on the temperature. Increased temperatures accelerate enzyme activity and chemical reactions. It has been suggested that low temperatures change plant physiological responses leading to different mechanisms of FOS depolymerisation (Porters *et al*, 2008).

The cooking of yacon roots may have affected the regulation of enzymes involved in carbohydrate metabolism as well as the function of enzymes in the cell compartments. The lower variations of fructose, glucose, sucrose and FOS content in cooked roots after 31 days might be due to a reduced activity in the enzymes responsible for FOS depolymerisation. A variation in appearance, colour and FOS content, as well as an increase in glucose and fructose was observed in yacon stored during 31 days. Less variation occurred in roots stored at lower temperatures.

5. CONCLUSION

The study revealed that yacon roots have a high content of FOS and the FOS content of yacon root storage at 4°C also decreased but in less proportion than at 25°C. Besides this, during storage, fresh yacon tubers deteriorate in appearance where they become withered and dried. During storage, yacon roots become sweeter due to dehydration and an increase in glucose and fructose content, consequently unsuitable for diabetics. For beneficial effects of yacon in glycemic placebo, it is recommended that yacon be stored at 4^{o}C and consumed before 17 days post-harvest. The results showed that it is essential that appropriate post-harvest handling methods be developed for Yacon roots, which are considered a potential source of FOS. It is also recommended to develop a yacon product with a greater content of FOS (such as freeze-dried powdered yacon) which can be well accepted by the general public.

Acknowledgements

The authors thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - São Paulo Research Foundation - Research Project 2010/50689-7) for the financial support

REFERENCES

Araujo, A. A. S.,Mercurill, L.P., Stuckert-Seixas, .S.R., StorpirtisI,S. Mato, J.R. (2006). Determinação dos teores de umidade e cinzas de amostras comerciais de guaraná utilizando métodos convencionais e análise térmica. *Revista Brasileira de Ciências Farmacêutica,* 42 (2), 269-277

Asami, T., Minamisawa, K.; Tsuchiya, T.; Kano, K.; Hori, I.; Ohyama, T.; Kubota, M.; Tsukihashi, T.. (1991). Flutuation of oligofructan content in tubers of yacon (Polymnia sonchifolia) during growth and storage. Ibaraki, Japão. *Japanese Journal of Soil Science Plant Nutrition*, 62. 621-627

Avelar Filho, J.A. (1997). Manejo pós-colheita da mandioquinha-salsa. *Informe Agropecuário*. Belo Horizonte, 19(190), 55-56

Beaulieu, J. (2011). Factors affecting sensory quality of fresch-cut produce. In: Martin-Belloso, O., Soliva-Fortuny, R. *Food preservation technology series Advances in fresh-cut fruits and Vegetables processing*, CRC Press, USA

Beylot M.2005. Effects of inulin-type fructans on lipid metabolism in man and in animal models. *British Journal of Nutrition*, 93(Suppl. 1), 163–168

Campos, A.D. Modificações após colheita no grau de deterioração fisiológica e composição química de raízes de três cultivares de mandioca (Manihot esculenta Crantz). Lavras, 1987. 80p. Tese (Doutorado). Escola Superior de Agricultura de Lavras.

Cantwell, M.I., Peiser, G., Mercado-Silva, E. (2002). Induction of chilling injury in jicama (*Pachyrhizus erosus*) roots: changes in texture, color and phenolics, *Postharvest Biology and Technology*, 25, 311-320

Cisneros-Zevallos, L., Núnez, R., Campos, D., *et al.*. (2002). Characterization and evaluation of fructooligosaccharides on yacon roots (Smallantus sonchifolia Poepp. & End) during storage*. Annual Meeting and Food Expo* - Anaheim, California

De Roover, J., Van Laere, A. & Van Den Ende, W. (1999). Effect of defoliation on fructan pattern and fructan metabolizing enzymes in young chicory plants (Cichorium intybus). *Physiologia Plantarum* 106, 158-163.

Edmunds, B., Boyette, M., Clark, C., Ferrin, D., Smith, T., Holmes, G. (1994) Postharvest handling of sweet potatoes. *College of agriculture & life sciences* pg.56

Fukai K, Ohno S, Goto K, Nanjo F, Hara Y. (1997). Seasonal fluctuations in fructan content and related enzyme activities in yacon (Polymnia sonchifolia). *Soil Science Plant of Nutrition*, 43, 171–177.

Graefe, S. (2002). Post-harvest compositional changes of yacon roots (Smallanthus sonchifolius Poepp. & Endl.) as affected by storage conditions and cultivar. M.Sc. Thesis. University of Kassel, Germany.

Graefe S., Hermann I., Manrique, S., Golombek, S., Buerkert, A. (2004). Effects of post-harvest treatments on the carbohydrate composition of yacon roots in the Peruvian Andes. *Field Crops Research*, 86, 157-165

Grau, A., Rea, J., (1997). Yacon. Smallanthus sonchifolius (Poepp. & Endl.) H. Robinson. In: Hermann, M., Heller, J. (Eds.), *Andean Roots and Tubers*: Ahipa, Arracacha, Maca and Yacon. *Promoting the Conservation and Use of Underutilized Crops*. IPK, Gatersleben/IPGRI,(pp.199-242). Rome, Italy.

Guigoz, Y.; Rochat,F.; Perruisseau-Carrier, G. ; Rochat, I.; Schiffrin, E.J. (2002). Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. *Nutrition Research*. 22,13-25

Hosono A, Ozawa A, Kato R, Ohnishi Y, Nakanishi Y, Kimura T, Nakamura R. (2003). Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cells. *Bioscience of Biotechnology and Biochemy,67,758*–764.

Hsiao-Ling Chen RD, Yu-Ho Lu RN, Jiun-Jr Lin MD, Lie-Yon Ko MD. (2000). Effects of fructooligosaccharide on bowel function and indicators of nutritional status in constipated elderly men. *Nutrition Research*,20(12), 1725–1733

Imahori, Y., Kitamura, N., Kobayashi, S., Takihara, T., Ose, K., Ueda, Y. (2010) Changes in fructooligosaccharides composition and related enzyme activities of burdock root during low-temperature storage. *Postharvest biology and technology*, 55, 15-20

Kakihara, T. S.; Câmara, F. L. A.; Vilhema, S. M. C. *et al*... (1996). Cultivo e industrialização de yacon *(Polymnia sonchifolia)*: uma experiência brasileira. In: *I Congresso Latino Americano de Raízes Tropicais e IX Congresso Brasileiro de Mandioca*, São Pedro, SP, resumo n. 148, 1996.

Leonel, M.; Cereda, M. P. (2002). Características físico-químicas de algumas tuberosas amiláceas. *Ciência e Tecnologia de Alimentos*, 22 (1), 65-69

Lewis, D. H. (1993). Nomenclature and Diagrammatic Representation of Oligomeric Fructans as Paper for Discussion. *New Phytologist*, 124, 583-594.

Luescher, M., Erdin, C., Sprenger, N., Hochstrasser, U., Boller, T. & Wiemken, A. (1996). Inulin synthesis by a combination of purified fructosyl transferases from tubers of *Helianthus tuberosus*. *FEBS Letter*, 385, 39-42

Manrique I, Parraga A, Hermann M. (2005). Yacon syrup: principles and processing. In: Series: Conservacio´n y uso de la biodiversidad de raı´ces y

tube´rculos andinos: uma de´cada de investigacio´n para el desarrollo (1993–2003), N8 8B. Lima, Peru´ : International Potato Center, Universidad Nacional Daniel Alcides Carrio´ n, Erbacher Foundation Swiss Agency for Development and Cooperation;. 31 pp.

Narai-Kanayama, A., Tokita N., Aso, K. (2007). Dependence of fructooligosaccharide contente on activity of fructooligosaccharide metabolizing enzymes in yacon (Smallanthus sonchifolius) tuberous roots during storage. *Journal Food Science*, 72(6), 381-387

Ohyama T., Itoa, O., Yasuyoshia, S., Ikarashia, T., Minamisawa, K., Kubot, M., Tsukihashia, T., & Asamia, T. (1990). Composition of storage carbohydrate in tubers of yacon (Polymnia sonchifolia). *Japan Journal Soil Science Plant Nutrition,* 36 1, 167-171.

Oliveira, M.A., Nishimoto,E.K. (2004). Avaliação e desenvolvimento de plantas de yacon (Polymnia sonchifolia) e caracterização dos carboidratos de reservas em HPLC. *Brazilian Journal of Food Technology*, 7(2), 215-220.

Pereira, R.A.C.B. (2009). *Extração e utilização de frutanos de yacon na funcionalização de alimentos*, (tese de doutorado). Botucatu (SP): Universidade Estadual Paulista, Faculdade de Ciências Agronômicas.

Porters, M.T., Figueriredo-Ribeiro, R.C.L., Carvalho, M.A.M., (2008). Low temperature and defoliation affect fructan-metabolizing enzymes in different regions of the rhizophores of Vernonia herbacea. *Journal of. Plant Physiology*. 165, 1572–1581.

Roberfroid, M. Gibson, G.R., Delzenne, N. (1993). The biochemistry of oligofructose, a nondigestible fiber: An approach to calculate its caloric value. *Nutrition Research*, 51(5), 137-146

Vilhena, S.M.C. Efeitos da exposição ao sol e do armazenamento sobre o conteúdo e a composição dos carboidratos de reserva em raízes tuberosas

de “yacon” (Polymnia sonchifolia Poep. Endl.). Botucatu, 1997. 63p. Dissertação (Mestrado em Agronomia/Horticultura) – Faculdade de Ciências Agronômicas, Universidade Estadual Paulista

Vilhena, S.M.C. Ciclo de cultivo e técnicas pós-colheita de yacon (Polymnia sonchifolia Poep. Endl.) em função do conteúdo de frutose total nos órgãos subterrâneos. Botucatu, 2001. 73p. Tese (Doutorado em Agronomia/Horticultura) – Faculdade de Ciências Agronômicas, Universidade Estadual Paulista.

Watada, A.E., N.P. Ko and D.A. Minott. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology*. 9:115-125

CAPÍTULO 3:

FREEZE-DRIED POWDERED YACON: EFFECTS OF FOS ON SERUM GLUCOSE, LIPIDS AND INTESTINAL TRANSIT IN THE ELDERLY PEOPLE

Scheid, M.M.A[a], Genaro, P.S.[b], Pastore, G.M.[a]

[a] Department of Food Science, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil (Departamento de Ciência dos Alimentos, Faculdade de Engenharia dos Alimentos, Universidade de Campinas, Campinas, São Paulo, Brasil)

[b] University of Vale do Paraíba, Brazil (UNIVAP)

This paper was submitted to European Journal of Nutrition

ABSTRACT

Background & aims: Freeze-dried powdered yacon (FDY) could be considered as a nutraceutical product due to its fructooligosaccharide (FOS) content. The effect of a daily intake of 18g FDY containing 7.4 g of FOS for 9 weeks was investigated regarding dietary intake, glucose and lipid metabolism and intestinal transit within a group of free-living elderly..

Methods: 72 elderly, (mean age 67.11 ± 6.11) were studied for 9 weeks in a double blind placebo-controlled experiment. They were randomly assigned into the supplement group (which received 7.4 g of FOS as FDY) and a placebo group. At the beginning and at the end of the study, anthropometric measurements, blood sampling, clinical analyses and dietary intake were assessed.

Results: A daily intake of FDY containing 7.4 g of FOS for 9 weeks caused a significantly decrease in serum glucose (p = 0.013) however was incapable of reducing serum lipid concentration in the elderly. The administered dose of FOS did not adversely affect intestinal transit as it did not cause bloating, flatulence or intestinal discomfort and was able to increase the fiber intake in both experiment groups.

Conclusion: FDY is a source of FOS and its daily consumption can have a favourable effect on serum glucose in the elderly. It is also practical, easy to use and safe to store.

Keywords: Freeze-dried powdered yacon, elderly, serum glucose, serum lipids, intestinal transit, fructooligosaccharide

1. INTRODUCTION

Worldwide the population is aging, which has led to increased attention to the elderly's physiological and health requirements. Aging and inadequate eating habits are associated with a reduction in overall health and an increase in degenerative, chronic diseases such as diabetes, cardiovascular problems and obesity. The excessive intake of fat, salt, sugar and low consumption of fibre leads to an increased incidence of chronic, degenerative diseases [1].

There is a pressing need to implement nutritional strategies in order to prevent or ameliorate these problems and also improve the quality of food. Lifestyle changes are advised, such as the intake of prebiotics and probiotics, which appear to have an important role in the retardation of diseases, such as Type 2 diabetes, dyslipidaemias and metabolic syndrome development [2]. Probiotics and prebiotics are live microorganisms and a selectively fermented ingredients, respectively, which provide health benefits to the host when administered in adequate amounts [3].

Some experimental models [4,5] and human studies [6,7] have shown positive effects of food with prebiotic properties on energy homeostasis, satiety regulation, modulation of metabolic processes associated with metabolic syndrome, such as obesity and Type 2 diabetes [8,9]. Recent studies have shown the prebiotic effects of inulin-type fructans (ITF), inulin, fructooligosaccharide (FOS), and galactooligosaccharides (GOS) due to their ability to modify gut flora composition after a short feeding period at reasonably low doses [10]. In the small intestine, FOS are metabolised by colonic microflora where gases, lactate and short-chain fatty acids are produced as end products of fermentation [11]. Short-chain fatty acids, acetate and propionate enter the portal blood and may influence systemic carbohydrate and lipid metabolism [12] in experimental models and in diabetic subjects [13-15]. Yamashita *et al.* [16] showed that an intake of 8g of FOS per day for 2 weeks significantly decreased blood glucose, serum total cholesterol and LDL-cholesterol in diabetic subjects. Luo *et al.* [17] showed that the

consumption of 20g FOS for 4 weeks increased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism or serum lipids. However, Alles *et al.* [11] concluded that supplementation with 15g FOS for 20 days had no statistical effect on blood glucose or serum lipids.

Inulin-type fructans (ITF) are naturally occurring oligosaccharides which are found in several foods such as yacon, leeks, asparagus, chicory, onions, wheat, bananas, and oats [18].

Yacon is an Andean root [19] known as a medicinal plant which has received much attention due to its abundant content of FOS and phenolic compounds [20,21]. Several studies have shown that yacon is a source of FOS and its regular consumption confers beneficial health effects, including the inhibition of the proliferation of potential pathogens and the promotion of beneficial microbiota [2, 22, 23]. Besides this, yacon is well tolerated and produces no adverse reactions, is non toxic and does not cause any negative nutritional effects.

Studies concerning the effects of FOS of yacon on glucose and lipid metabolism in elderly people are not conclusive; therefore additional research is required to establish a clear relationship between them.

The aim of the present study was to investigate the effect of a daily intake of 18g FDY containing 7.4 g of FOS for 9 weeks on dietary intake, glucose and lipid metabolism and intestinal transit in free-living elderly people.

2. MATERIAL AND METHODS

2.1. Characterisation of the study products – Freeze Dried Powdered Yacon (FDY)

The Smallanthus sonchifolius (yacon) roots for the experiment were cultivated and obtained from a field near the town of Piedade – São Paulo, Brazil. The fresh yacon was cleaned, peeled, freeze-dried and powdered in order to

obtain the freeze-dried powdered yacon (FDY) (Liofoods in the city of Araras, São Paulo, Brazil). The chemical composition (total carbohydrate, protein, lipid and FOS content) of FDY was determined at the Institute of Food Technology (ITAL) Campinas, SP using official methods of analysis of AOAC International (Association of Official Agricultural Chemistry, Maryland (US) [24]. The FDY contained 88,6% carbohydrates, 41,2% FOS, 4,7% protein and 0,3% lipids. The placebo was a maltodextrin GLOBE-A 1910 of MorRex, Corn Products Brasil. Both products were packaged in individual sachets of 18g FDY and 12 g maltodextrin.

2.2. Subjects

From February 2011 to March 2011, 84 elderly subjects (> 60 years old) from the University for the Third Age of the State University of São Paulo - UNESP and University of Vale do Paraíba - UNIVAP were selected for this study. The exclusion criteria were the use of antibiotics and smokers. Elderly with severe chronic disease such as cancer, colorectal and other gastrointestinal diseases and those with dietary habits, daily consumption of probiotics or other supplement with prebiotics, vegans, which might interfere with the assessment of the study were also excluded. **Figure** 1 shows the CONSORT flow diagram.

Eighty four elderly were evaluated for the study. Five subjects did not complete the evaluation due to illness, two could not be contacted and three subjects dropped out for personal reasons. A total of 74 elderly were selected to participate in a double-blind, randomised, placebo controlled study for 9 weeks. 37 elderly assigned to receive FDY and 37 elderly the placebo and 72 completed the study.

The Ethics Committee of the School of Medicine of the University of Campinas (UNICAMP, Campinas) approved the study (protocol number 949/2009) (ANNEX 2). Informed consent was obtained from all participating individuals. The protocol, the aims and risks of the study were fully explained to the participants.

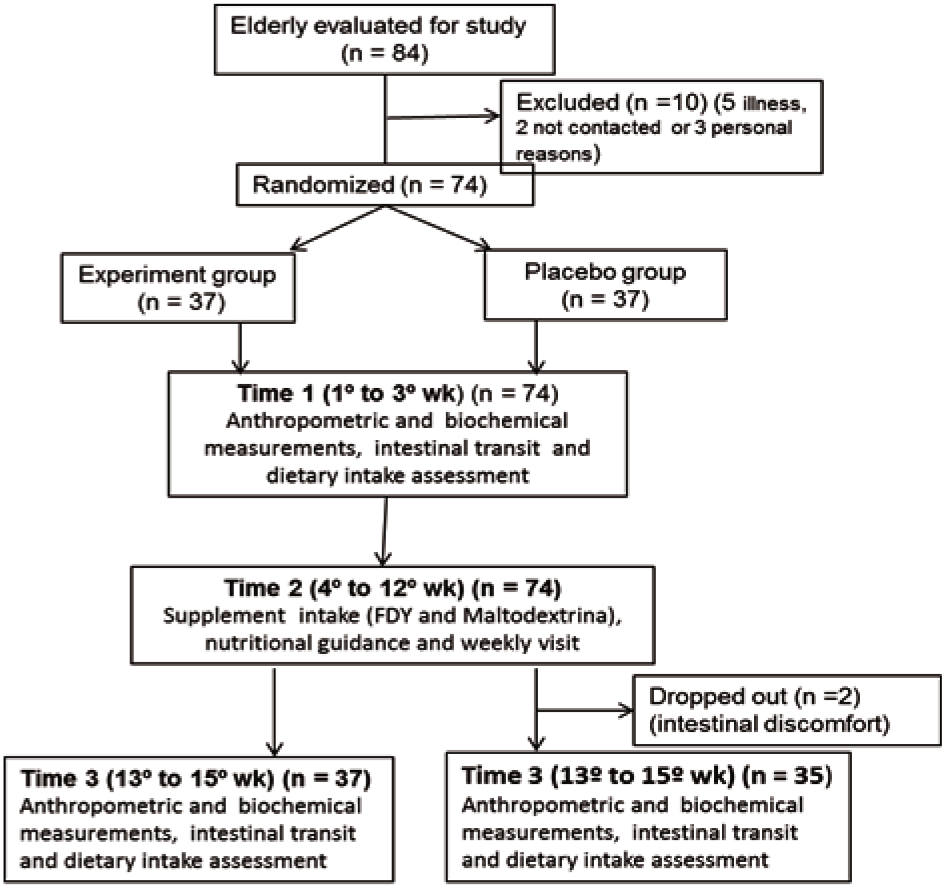

FIGURE 1: Consort schematic overview of a double-blind, randomized, placebo-controlled study during 9 weeks with elderly people

2.3. Study design and supplements

The study was designed as a double-blind, randomised, placebo-controlled study to assess the beneficial effects of a daily consumption of 7.4 g of fructooligosaccharides (FOS) of FDY on glycaemic and lipid metabolism and intestinal transit. Treatment duration was 9 weeks with follow-up visits at weekly intervals to ask whether there were any problems with the products. The supplement group received a dose of 18 g of freeze-dried powdered yacon (FDY) containing 7.4 g of FOS. The dose was chosen to avoid side effects, why FOS can cause bloating, flatulence, and intestinal discomfort, especially when taken at

doses of 15 g or higher daily. The placebo group was given a dose of 12 g maltodextrin. To ensure the daily consumptions, the elderly were advised to reconstitute the supplements with milk or juice and consume it every day during breakfast. The treatment time was according others studies (17, 23).

During the experiment, the elderly were questioned about their degree of tolerance of the supplements. Biochemical markers of glucose and lipid metabolism were analyzed, dietary data was collected and body composition measurements were taken at baseline and 9 weeks after the intervention period. All evaluations were done at baseline to identify differences between groups and effect of FOS.

2.4. Anthropometric parameters

Anthropometric measurements were taken by trained personnel in triplicate. Height was measured to the nearest 0.01 m with subjects standing barefoot with their back to a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg with calibrated scales Body mass index (BMI) was calculated as weight (kg) divided by height (m^2) [25]. Nutrition Screening Initiative (NSI) scores were used to measure the risk of undernourishment and obesity [26]. Waist circumference (WC), was measured with an inelastic measuring tape. Skinfold measurements of triceps (TSF), biceps (BSF), subscapular (SSF), suprailiac (SISF) were measured with Lange skinfold caliper® (Beta Tecnology Corporated - Santa Cruz, California, USA) with a constant pressure of 10g/ mm^2. WHO (32) scores were used to measure the cardiovascular risk. Fat mass (%FM) was calculated using Durnin & Womersley (33). All anthropometric measurements were taken at the beginning and at the end of the study.

2.5. Intestinal transit

Intestinal transit was assessed at baseline and after 9 weeks based on a questionnaire [29], with a score ranging from 0 to 30, where 0 indicated normal intestinal transit and 30 indicated severe constipation. The standardised questionnaire included 8 items (ANNEX 1).

2.6. Dietary and supplement intake

Food intake was assessed before and after 9 weeks of intervention to placebo the effect of FOS. Two-days of dietary records were undertaken to estimate mean energy (Kcal), protein (g), carbohydrate (g), fat (g) and fibre (g)**.** Dietary intake was analysed using the VirtualNutri Programme of Public Health (University of São Paulo, Brazil) [30]. Adequacy of nutrient intake was assessed using the Institut of Medicine [31].

2.7. Biochemical measurements

At the beginning of the study and after 9 weeks, fasting (12h) blood samples were taken. Serum glucose, insulin, total lipids, and lipoproteins, C reactive protein (CRP) and the homeostatic model assessment for insulin resistance (HOMA IR), were determined at the beginning of the study and after 9 weeks. Laboratorial examinations were measured by automated standard laboratory methods. Glucose levels, (Advia 1200 Siemens, USA) were measured by the hexokinase method, serum insulin by chemiluminescence. Enzymatic processes were used to determine serum levels of triglycerides, cholesterol and lipoproteins (Advia 1200 Siemens, USA). C-reactive protein was measured by immunoturbidimetry (Advia Centaur Siemens, USA). All biochemical assessments were made in the Laboratorio Quaglia, São José dos Campos – Brazil.

2.8. Statistical analysis

The descriptive data was expressed as means and standard deviations. The Mann-Whitney test was used to compare the continuous variables in the group, as the variables did not have a normal distribution. For comparison of numeric variables (glucose, insulin, total-cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triacylglycerol, CRP) between the two groups and between pre-treatment and after 9 weeks of the experimental period, analyses of variance (ANCOVA for repeated measures), followed by the Tukey multiple comparison test was used to compare the groups at every moment and adjusted for age and fat mass. Genta *et al.* [2] suggest that FOS could play a role in the management of obesity, metabolic syndrome and diabetes. The test profile by contrasts was also used to compare the parameters between the times in each group. Analyses were conducted with the SAS System for Windows (Statistical Analysis System) software, version 9.1.3. (SAS Institute Inc, 2002-2003, Cary, NC, USA). One-sided P values at < 0.05 were considered significant.

3. RESULTS

Baseline characteristics of both elderly groups are given in (Table 1). There were not statistically significant differences at baseline in anthropometric characteristics or intestinal transit between the placebo group and the supplemented group. Body Mass Index (BMI) of elderly in both groups were higher than the normal range (22 – 27 Kg/m2), according to NSI (1992). In our study, 52% (N = 39) of the elderly presented a BMI slightly above 27 Kg/m^2 and 62% (N = 46) of the subjects had a waist circumference above the desirable range.

Intestinal transit did not change throughout the study. All elderly had a score below 15, indicating normal intestinal transit.

TABLE 1: Baseline characteristics of elderly people according to treatment group. Brazil, 2012

Characteristics	Experiment (N = 37) $\bar{x}$ (SD)	Placebo (N = 37) $\bar{x}$ (SD)	*p*
Age (years)	67.11 (6.12)	67.11 (5.53)	0.782
Body weight (kg)	70.13 (13.58)	67.27 (12.61)	0.476
Height (m)	1.58 (0.08)	1.56 (0.06)	0.498
BMI (kg/m^2)	28.14 (5.05)	27.65 (4.90)	0.701
(WC) (cm)	95.78 (10.88)	91.21 (10.90)	0.131
Intestinal transit	2.81 (3.61)	3.00 (4.39)	0.872

Independent Mann-Whitney Test ($p < 0.05$). BMI = Body Mass Index; WC = Waist circumference

The intervention results for nutrient intake are shown in (Table 2). The baseline intakes did not differ between groups. In both groups the mean daily intakes of fibre, at baseline and post-intervention, was below the recommended nutrient intake of 30 g/day for fibre, according to the IOM [31]. Before and after intervention period, daily intakes of protein was higher than the recommendation of 56g/d for men and 46 g/d for women and carbohydrate intake was higher than the recommendation of 130g/d, in both groups, , according the Institut of Medicine [31]. Considering all participants at baseline and after 9 weeks, 91.9%, and 83.78%, respectively, of the elderly presented fiber intakes below the recommended intake.

No change was observed in the placebo group for nutrient intakes after the intervention period and in the experimental group was observed a significant decrease in protein (p = 0.014), and fat intake (p = 0.025) and no significant change in carbohydrate intake after 9 weeks intervention. As expected, the supplement use led to a significant increase in fibre intake (p = 0.002) in the experimental group and a significant decrease in fibre intake (p = 0.002) in the placebo group. However, during the 9 week intervention period, the mean daily

intake of fibre was below the recommended intake for both groups. In both groups HOMA RI and CRP change not significantly during supplement period.

TABLE 2: Nutrient intake by elderly people according to treatment group at baseline and after 9 weeks of intervention. Brazil, 2012

Nutrient intake	Experiment		Placebo	
	Baseline (N = 37) $\bar{x}$ (SD)	9 weeks (N = 37) $\bar{x}$ (SD)	Baseline (N = 37) $\bar{x}$ (SD)	9 weeks (N = 35) $\bar{x}$ (SD)
Energy (Kcal/d)	1670.4 (433.07)	1410.3 (555.42)*	1602.8 (443.44)	1398.2 (491.61)
Carbohydrate (g/d)	217.93 (51.23)	206.69 (71.17)	226.40 (80.23)	209.30 (90.63)
Fat (g/d)	60.49 (33.57)	50.09 (30.29) *	49.08 (18.74)	45.32 (19.53)
Protein (g/d)	70.37 (21.79)	59.65 (35.57) *	68.98 (22.75)	62.38 (21.96)
Fibre (g/d)	20.57 (14.32)	21.15 (7.13) *	19.39 (8.83)	14.87 (5.75) *

*$p < 0.05$ ANOVAs repeated measures

Results of the biochemical markers for glucose and lipid metabolism at baseline and post-intervention for both groups are summarized in (Table 3). All elderly have abnormal HOMA-IR in baseline and after 9 weeks. After 9 weeks of FDY treatment, HOMA-IR values decreased not significantly compared to pre-treatment value.

At the baseline there was no significant difference in any laboratory variable between the placebo and the experimental group. Serum levels of insulin, total-CHL, HDL-CHL, VLDL-CHL and Triacylglycerol decreased within the normal ranges for both groups. Taking all participants into consideration at baseline, 70.3% (N = 52) of the participants presented an LDL-CHL concentration above 100 mg/dL and 39.2% (N = 29) of the participants presented a serum glucose concentration above 99 mg/dL.

In the supplement group, the concentration of serum glucose decreased from 103.4 to 97.3 g/dl significantly after 9 weeks of intervention ($p = 0.013$). This

concentration was above the normal range and decreased to a normal range after 9 weeks of intervention. The placebo group did not change significantly its mean glucose concentration from baseline. Over this same period, LDL-CHL and Trigs concentration decreased in the supplement group; however, it was not significant ($p = 0.722$ and $p = 0.390$ respectively). **Figure** 2 represents the percentage change of the biochemical parameters after intervention. During this period, the percentage change of glucose concentration in the experimental group, when adjusted to age and fat mass, decreased, however, not significant ($p = 0.192$).

TABLE 3: Biochemical parameters of elderly people according to treatment group at baseline and after 9 weeks of intervention. Brazil, 2012

Biochemical exams	Experiment group		Placebo group	
	Baseline (N = 37) x (SD)	9 weeks (N = 37) x (SD)	Baseline (N = 37) x (SD)	9 weeks (N = 35) x (SD)
Glucose (g/dl)	103.38 (20.53)	97.35 (19.02)[a]	95.37 (9.90)	92.11 (10.59)
Insulin	13.72 (13.88)	16.25 (19.73)	11.69 (15.31)	13.91 (18.72)
HOMA IR	4.03 (5.83)	3.98 (4.37)	2.95 (3.23)	3.00 (2.98)
Total -CHL	196.43 (37.37)	192.54 (34.84)	196.79 (35.26)	193.42 (29.56)
HDL-CHL	50.70 (12.46)	51.97 (13.27)	50.11 (8.28)	50.66 (8.18)
LDL-CHL	119.05 (32.79)	113.35 (31.27)	120.26 (30.97)	117.38 (25.97)
VLDL-CHL	25.99 (9.37)	26.46 (13.86)	26.34 (9.83)	24.00 (8.71)
Triacylglycerol	133.46 (47.69)	132.57 (69.41)	131.89 (48.94)	131.08 (82.19)
(CRP)	3.66 (5.03)	3.78 (4.46)	2.03 (2.85)	2.99 (3.26)

[a] Analyses were adjusted for age and fat mass. *$P<0.05$ ANCOVA repeated measures. CRP = C-reactive protein

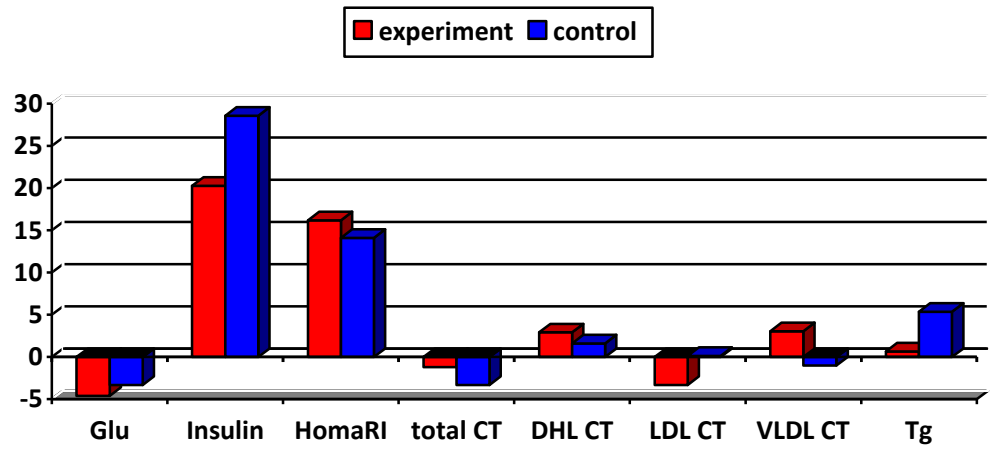

FIGURE 2: Mean percentage change from baseline for biochemical parameters, according to placebo and supplement groups. Analyses were adjusted for season of year of blood collection, age and fat mass. Glu = glucose, CT = cholesterol, Tg = tryacilglycerol

4. DISCUSSION

The present study demonstrated that the daily supplementation of FDY with 7.4g FOS for 9 weeks is enough to decrease serum glucose levels in elderly. In addition, the supplementation of 7.4 g FOS in FDY did not significantly affect their blood lipid concentration or their intestinal transit.

FDY is a novel product which contains approximately 41% of FOS obtained by freeze-drying tuberous fresh roots of yacon. It is practical, easy to store and use. Contrary to Genta´s study [2], which had a participation of only 63%, we had a 97.3% participation rate. This could be because FDY is easy to use and does not cause bowel discomfort. Dietary intervention is important for the prevention and control of diabetes, dyslipidaemia and also plays an essential role in delaying the development of metabolic complications . Yacon roots and FDY are natural products rich in FOS. It has been shown that dietary FOS is able to modify glucose and lipid serum concentration [2,32] and intestinal function [6].

There are arguments to propose that short chain fatty acids (SCFA) are beneficial in the placebo of obesity and related diseases. The SCFA, propionate

inhibits hepatic gluconeogenesis from lactate in hepatocytes, which improves insulin sensitivity [33]. A non-digestible oligosaccharide, FOS, is fermented in the colon and produces large amounts of SCFAs, has been proposed to reduce glycaemia [34]. The hypoglycemic effect of FDY, in elderly, suggests that these samples may contain compounds which probably perform by stimulating the use of glucose in peripheral tissues or may produce amounts of SCFAs which reduce glycaemia.

Our study showed a reduction in intake of energy, protein and fat intake in the experimental group. Appetite is regulated by complex mechanisms, including hormones, which inform the brain of the state of feeding. Increased hormone levels, of cholecystokinin (CCK), PP-fold peptide (PYY) and glucagon-like peptides-1 (GLP-1), in blood are associated with reduced hunger and food intake. An animal study demonstrated that oligofructan intake enhances the GLP-1 in the colon and in portal blood, resulting in a decrease of food and energy intake [7, 35,36]. However, a daily FOS intake can lead to a decrease in energy intake and a decrease in body weight and metabolic complications such as diabetes and dyslipidemias in the long term. Genta *et al.*, [2] showed a significant decrease in body weight and BMI following a daily intake of yacon syrup containing FOS.

In our study was not monitored the hormone level during the experiment, therefore our results lead us to suggest the hypothesis that the intake of FDY with 7.4 g FOS could play a role in a reduction of energy and nutrient intake through their capacity to promote the secretion of hunger hormones, and decreasing blood glucose levels. A study conducted by Valentová *et al.* [37] showed a positive effect on triglyceride and /or glucose level in human adults suffering from the metabolic syndrome using 0.8 g Silymarin with 2.4 g of yacon per day for 90 days. Only few studies have reported the influence of prebiotics on glucose homeostasis in diabetic individuals. Alles and colleagues [11], in a randomized, single-blind, crossover design, concluded that the intake of 15g per day of FOS had no major effect on blood glucose or serum lipids in patients with Type 2 diabetes.

Animal studies suggest that FOS, GOS, and inulin can significantly improve cholesterol profile; however, study outcomes in humans have been inconsistent [38-40]. In our study, no significant differences in serum lipid levels or anthropometric parameters were found. The reason for this might be because the elderly had normal serum cholesterol concentrations before the commencement of the experiment. Genta *et al.* [2] demonstrated that the intake of yacon syrup with FOS did not produce a significant decrease in fasting serum insulin, an increase in defecation frequency and satiety or affect fasting glucose and serum lipids, however did affect serum LDL-CHL levels. Contrary to Genta *et al.* [2], which had a positive effect of daily intake of yacon syrup, our results showed that a daily dose of FDY of 7.4g FOS was not able to decrease the HOMA-IR.

In studies of the effects of carbohydrates on faecal bulking, it has been shown that FOS has a laxative effect on bowel habit [6,11]. FOS appears to be generally safe. However, they can cause bloating, flatulence, and intestinal discomfort, especially when taken at daily doses of 15 g or more [40]. In the present study, the daily intake of FDY with 7.4 g of FOS did not have intestinal side effects or a laxative effect in the elderly. None of the elderly in the supplemented group reported any intestinal discomfort or flatulence.

Limitations of the study could be the duration of supplementation (9 weeks) and the daily doses. It takes around 90 days for serum mean glucose and lipid concentration to achieve a plateau after any nutritional and/or medical intervention. For that reason, studies should evaluate FOS supplementation for a minimum of 15 weeks and/other with greater dose.

In conclusion, a daily supplement dose of 7.4 g FOS of FDY for 9 weeks significantly caused a decrease in mean serum glucose, however was incapable of reducing serum lipid concentration in the elderly. In addition, the dose of FDY did not cause bloating, flatulence or intestinal discomfort. FDY is a practical product, easy to use and safe to store. FDY is a natural product, rich in FOS which could be termed as a nutraceutical as the present results demonstrate its beneficial effects

on serum glucose in the elderly while a daily dose greater than 7.4 g FOS of FDY for a minimum of 15 weeks could be capable of reducing serum lipid level in elderly.

Acknowledgements

The authors would like to thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - São Paulo Research Foundation - (Research Project 2010/50689-7) for financial support and Corn Products Brazil for the supply of maltodextrin.

REFERENCES

1. ORGANIZACIÓN PANAMERICANA DE LA SALUD (OPS) (2007) Salud en las Américas. Volumen I-Regional

2. Genta S, Cabrera W, Habib N, Pons J, Carillo I, Grau A, Sánchez S. (2009). Yacon syrup: Beneficial effects on obesity and insulin resistence in humans, Clin Nutr :28(2):182-7

3. Roberfroid, M. (2007) Prebiotics: the concept revisited. J. Nutr. 137:830-837 March.

4. Reimer RA & Russell JC (2008) Glucose tolerance, lipids, and GLP-1 secretion in JCR:LA-cp rats fed a high protein fiber diet. Obesity (Silver Spring) 16, 40–46.

5. Maurer AD, Chen Q, McPherson C, *et al.*. (2009) Changes in satiety hormones and expression of genes involved in glucose and lipid metabolism in rats weaned onto diets high in fibre or protein reflect susceptibility to increased fat mass in adulthood. J Physiol 587: 679–691.

6. Gibson GR, Roberfroid MB. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 125:1401-1412.

7. Cani PD, Neyrinck AM, Maton N, Delzenne NM. (2005) Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. Obes Res 13:1000-1007.

8. Franck, A, Alexiou, H (2009) Inulin and Oligofructose. In: Jardine, S. Ingredients handbook prebiotics and probiotics. Oxford, 2nd Ed. Wilei-Blackwell, pg 3-30

9. Roberfroid, MB, Gibson, GR, Hoyles, L, McCartney, AL, Rastall, R, Rowland, I, Wolvers, D, *et al*... (2010). Prebiotics effects: metabolic and health benefits. Brit J Nutr 104(2):1-63

10. Kolida, S, Gibson, GR (2008) The prebiotic effect: a review of experimental and human data. In G.R. Gibson & M.B. Roberfroid (Eds.), Handbook of prebiotics (pp. 405-419). New York: CRC Press

11. Alles, MS, Roos, N.M, Baks, J.C., Lisdonk, E., Zock, P.L., Hautvast, J.G.A.J (1999). Consumption of fructooligosaccharides does not favorably affect blood glucose and serum lipid concentration in patients with type 2 diabetes. Am. J. Clin. Nutr;69:64-69

12. Cummings, JH, Pomare, EW, Branch, WJ, Naylor, CPE, MacFarlane, GT (1987) Short chain fatty acids in human large intertie, portal, hepatic and venous blood. GUT 28:1221-1227

13. Moore, N, Chao, C, Yang, L, Storm, H, Oliver-Hemker, M, Saavedra, JM (2003) Effects of fructooligosaccharide-supplement infant cereal: a double-blind, randomized trial, Brit J Nutrition. 90:581-587

14. Yang Y, Tsai CE. (1993) Effects of biosynthetic indigestible carbohydrates on digestion and lipid metabolism in rats. Food Sci 20:215–28.

15. Beylot M (2005) Effects of inulin-type fructans on lipid metabolism in man and in animal models. Br J Nutr 93(Suppl. 1):163–168.

16. Yamashita K., Kawai K., Itakura, M. (1984) Effect of fructooligosaccharides on blood glucose and sérum lipids in diabetic subjets. Nutr Res. 4:961-966

17. Luo,J, Rizkalla, SW, Alamowitch, C, *et al*... (1996) Chronic consumption of short-chain fructooligosaccharides by health subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. Am J. Cli. Nutr. 63:939-945

18. Bosscher, D (2009). Fructan Prebiotics derived from inulin. In: Charalampopoulos, d, Rastall, R.A. Prebiotics and probiotics Science and technology. 1, Springer pp163-205

19. Asami T, Minamisawa K, Tsuchiya t, Kano K, Hori I, Ohyama T, Kubota M & T Tsukihashi. (1991) Fluctuation of oligofructan contens in tubers of yacòn (Polymnia sonchifolia) during growth and storage. Soil Science and Plant Nutrition 62(6): 621-627.

20. Ohyama, T, Itoa, O, Yasuyoshia, S, Ikarashia, T, Minamisawa, K, Kubot, M, Tsukihashia, T, & Asamia, T (1990). Composition of storage carbohydrate in tubers of yacon (Polymnia sonchifolia). Jap. J Soil Sc Plant Nutrition 36 1: 167-171.

21. Hondo, M, Nakano, A, Okumura, Y, Yamaki, T (2000). Effects of activated carbon powder treatment on clarification, decolorization, deodorization and fructooligosaccharide content of yacon juice. Nippon Shokuhin Kagaku Kogaku Kaishi 47: 148–154.

22. Bunout D, Barrera G, Hircg S, Gattas V, de la Maza MP, Haschke F, Steenhout P, Klassen P, Hager C. (2004). Effects of a nutritional supplement on the immune response and cytokine production in free-living Chilean elderly. JPEN J Parenter Enteral Nutr.28:348–54.

23. Guigoz, Y, Rochat F, Perruisseau-Carrier G, Rochat I, Schiffrin EJ. (2002) Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. Nut Res. 22:13-25

24. Horwitz ,W.(Ed.). Official methods of analysis of the Association of Official Analytical Chemists. 18th ed. Gaithersburg, Maryland: AOAC, 2005. cap.45, met.45.4.06B, p.92-94. Meagazyme-Fructan HK Procedure for the measurement of Fructo-oligosacharides and Fructan Polysacahariде – A modification of AOAC Method 999.03

25. World Health Organization. Physical Status: the use and interpretation of anthropometry. Report. Geneva; 1995. (WHO Technical Report Series, 854).

26. Nutrition Screening Initiative (NSI). Nutrition interventions manual for professionals caring for older Americans. Washington DC: Nutrition Screening Initiative, 1992

27. World Health Organization. Defining the problem of overweight and obesity. In: World Health Organization. Obesity: preventing and managing the global epidemic: report of a Who Consultation. Geneva; 2000. p. 241-3. (WHO Technical Report Series, 894).

28. Durnin, JVGA, Womersley, J. (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutrition, 32: 77-97.

29. Agachan F, Chent T, Pfeifer J, Reisseman P, Wexner DAS. (1996). Constipation scoring system to simplify evaluation and management of constipated patients. Dis Colon Rectum.39(6):681-5

30. Philippi, ST, Szarfac, SC, Latterza, AR (1996) Virtual nutri software. [programa de computador]. Versão 1.0. São Paulo, Disquete 31/2pol.

31. Institute of Medicine (IOM). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington (DC): National Academy Press; 2005.

32. Habib, NC, Honoré, SM, Genta, SB, Sánchez, SS (2011) Hypolipidemic effect of Smallanthus sonchifolius (yacon) roots on diabetic rats Biochemical approach. Chem Biol Interat. 194:31-39

33. Al-Lahham SH, Peppelenbosch MP, Roelofsen H, Vonk RJ, Venema K. (2010) Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochim Biophys Acta 1801(11): 1175-1183

34. Neyrinck AM, Delzenne NM. (2010). Potential interest of gut microbial changes induced by non-digestible carbohydrates of wheat in the management of obesity and related disorders.. Curr Opin Clin Nutr Metab Care. Nov13(6):722-8.

35. Daubioul, C, Rousseau, N, Demeure, R, Gallez, Taper, H, Declerck, B, Delzenne N (2002) Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker *fa/fa* rats.J Nutr 132:967-973

36. Cani, PD, Dewever, C, Delzenne, NM (2004) Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like-peptide-1 and ghrelin) in rats. Brit J Nutr 92: 521-526

37. Valentová, K, Stejskal, D, Bartek, J, Dvorácková,S, Kren, V, Ulrichova, J, Simanek, V. (2008) Maca (Lepidium meyenii) and yacon (Smallanthus sonchifolius) in combination with silymarin as food supplements: in vivo safety assessment. Food Chem Toxicol 46: 1006-1013

38. Giacco R, Clemente G, Luongo D, *et al*.. (2004) Effects of short-chain fructo-oligosaccharides on glucose and lipid metabolism in mild hypercholesterolaemic individuals. Clin Nutr 23, 331–340.

39. Schaafsma, G, Meuling, WJ, Dokkum, W, Bouley, C (1998) Effects of milk product, fermented by *Lactobacillus acidophilus* and with fructooligosaccharides added, on bloo lipids in male volunteers. Eur J Clin Nutr 54:436-440

40. Van Dokkum W, Wezendonk, B, Srikumar, TS, Van den Heuvel, EG (1999) Effect of nondigestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young health male subjects. Eur J Clin Nutr 53:1-7

CAPÍTULO 4:

EFFECTS OF FREEZE-DRIED POWDERED YACON ON THE IMMUNE SYSTEM OF ELDERLY

Marlene M Amaral Scheid[a], Glaucia M Pastore[a]

[a] Department of Food Science, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil (Departamento de Ciência dos Alimentos, Faculdade de Engenharia dos Alimentos, Universidade de Campinas, Campinas, São Paulo, Brasil)

This paper will be submitted to **Nutrition Research**.

ABSTRACT

Objective: Immune response of the elderly is impaired. Freeze-dried powdered yacon (FDY) could be considered as an immunomodulatory product due to its fructooligosaccharide (FOS) content. This study aims to assess the effect of FDY with FOS on immune system in non-institutionalised elderly people.

Methods: Seventy-two elderly, (mean age 67.11 ± 6.11) were enrolled in a double-blind placebo-controlled clinical trial for 9 weeks. They were randomly assigned into a FDY-supplemented group (receiving 7.4 g of FOS as FDY) and placebo group. At the beginning and at the end of the study, anthropometric measurements, clinical analyses, dietary intake, biochemical and immunological parameters were measured.

Results: The anthropometric data showed that the elderly people included in the study were well nourished. There were no significantly variations in anthropometric values during the study both groups. A comparative analyse of variables between the 2 groups and the 2 time showed significant decrease on IL-1α in supplement group. When adjusted for age and fat mass the production of IL-6, in supplement group has a significant decrease of 54,6% compared to that of the placebo group ($p = 0.039$).

Conclusion*:* Our results show that a daily supplementation with FDY containing 7.4 g FOS for 9 weeks caused a significant decrease of IL-1α and when adjusted for age and fat mass decreased IL-6 in not institutionalized elderly people, suggesting a possible immunomodulation in inflammatory response

Keywords: fructooligosaccharides, yacon, elderly, immune system, cytokines

1. INTRODUCTION

Progressive aging of the world's population poses new challenges to healthcare systems. Aging also leads to a marked decline in immune functions, immunosenescence, which can cause a predisposition to infectious and noninfectious diseases (1). An interrelationship between nutrition and immune function is recognized (2, 3). However, well-nourished people sometimes can present some diseases, like atherosclerosis, cancer, Crohn's disease, myasthenia gravis, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus and food allergies, which have a strong immunological component (4).

The immune system has an innate first line of defense and comprises physical barriers, such as cells in blood and tissue, phagocytes and cytokines and an acquired immune system, consisting of T and B lymphocytes, which enable the specific recognition of, and response to, invaders (5, 6).

Immunosenescence is a progressive alteration and gradual deterioration on the immune system that develop with aging (7) and it is characterized by low levels of IgG or IgA blood levels (8), as well as increased production of inteleukin-6 (IL-6), IL-1β and tumor necrosis factor-α (TNF-α) by peripheral blood mononuclear cells (PBMC) (9,10). In addition, serum C reactive protein level positively correlates with circulating levels of IL-6 (11).

The immune system can be modulated by nutrition which improves the resistance to infection (12, 13). Essential nutrients, such as non-digestible carbohydrates, such as inulin (IN) and oligofructose (OF), may alter the gut-associated lymphoid tissue (GALT), as well as the systemic immune system by modulation of cytokines. The production of IgA antibody, improves short chain fatty acids (SCFA) to bind to G-protein-coupled receptors, which increases the interaction of prebiotics with the carbohydrate receptors on leukocytes (14).

Prebiotic consumption in adequate amounts could have an immunomodulatory effect in elderly people 15). Alterations in immune

responsiveness are among the most important age-related disabilities. Some population studies have been shown positive effects of the consumption of prebiotics on immune functions in elderly (16, 17, 18). In fact, the benefits are related to the SCFA produced in the fermentation process. These SCFA can inhibit proliferation and induce differentiation 19), it can be used as an energy source (20), can modulate the synthesis of cholesterol (21), and the production of cytokines (22).

Fructooligosaccharides (FOS) is a prebiotics that occurs naturally in several foods as yacon, leek, asparagus, chicory, banana and oats (23). Yacon (*Smallanthus sonchifolius*) is a tuber (24) from the South American Andes, and as source of FOS. Due to this, it is possible that an intake of yacon could benefit the development of colonic microbiota by its potential bifidogenic effect.

Some studies (17, 24, 26) concerning the effect of FOS on the immune system in elderly people have been undertaken, however further research is required to establish how this nutrient can affect this system. The aim of this study was to assess the effect of a freeze-dried powdered yacon (FDY) on the immune function of elderly people

2. MATERIAL AND METHODS

2.1. Study design

This was a randomised, double-blind, single centre, placebo-controlled study. It was conducted at the Third Age of the State University of São Paulo (UNESP) and University of Vale do Paraíba (UNIVAP), São José dos Campos, SP.

2.2. Subjects

From February 2011 to March 2011, 84 elderly subjects (> 60 years old) from the University for the Third Age of the State University of São Paulo (UNESP) and University of Vale do Paraíba (UNIVAP) were enrolled for this study. The exclusion criteria were the use of antibiotics and smokers. Elderly with severe chronic disease such as cancer, colorectal and other gastrointestinal diseases and those with dietary habits, daily consumption of probiotics or other supplement with prebiotics, vegans, which might interfere with the assessment of the study, were also excluded. From 84 elderly enrolled for the study, five did not complete the protocol due to illness, two could not be contacted and three subjects dropped out for personal reasons. A total of 74 were selected to participate and 72 elderly completed a double-blind, randomised, placebo controlled study for 9 weeks. Figure 1 shows the experimental design. The Ethics Committee of the School of Medicinal Sciences of the University of Campinas (UNICAMP, Campinas, Brazil) approved the study (protocol number 949/2009) (ANNEX 2). Following a full explanation of the protocol, the aims and risks of the study, informed consent was obtained from all participating individuals.

2.2.1. Study design and supplements.

The study was designed as a double-blind, randomised, placebo-controlled study. Treatment duration was 9 weeks with follow-up visits at weekly intervals to ask whether there were any problems with the products. The supplement group received a dose of 18 g of freeze-dried powdered yacon (FDY) containing 7.4 g of FOS. The dose was chosen to avoid side effects, why FOS can cause bloating, flatulence, and intestinal discomfort, especially when taken at doses of 15 g or higher daily. The placebo group was given a dose of 12 g maltodextrin. To ensure the daily consumptions, the elderly were advised to reconstitute the supplements with milk or juice and consume it every day during breakfast. The treatment time was according others studies (17, 23).

During the experiment, the elderly were questioned about their degree of tolerance of the supplements. Biochemical markers of glucose and lipid metabolism were analyzed, dietary data was collected and body composition measurements were taken at baseline and 9 weeks after the intervention period. All evaluations were done at baseline to identify differences between groups.

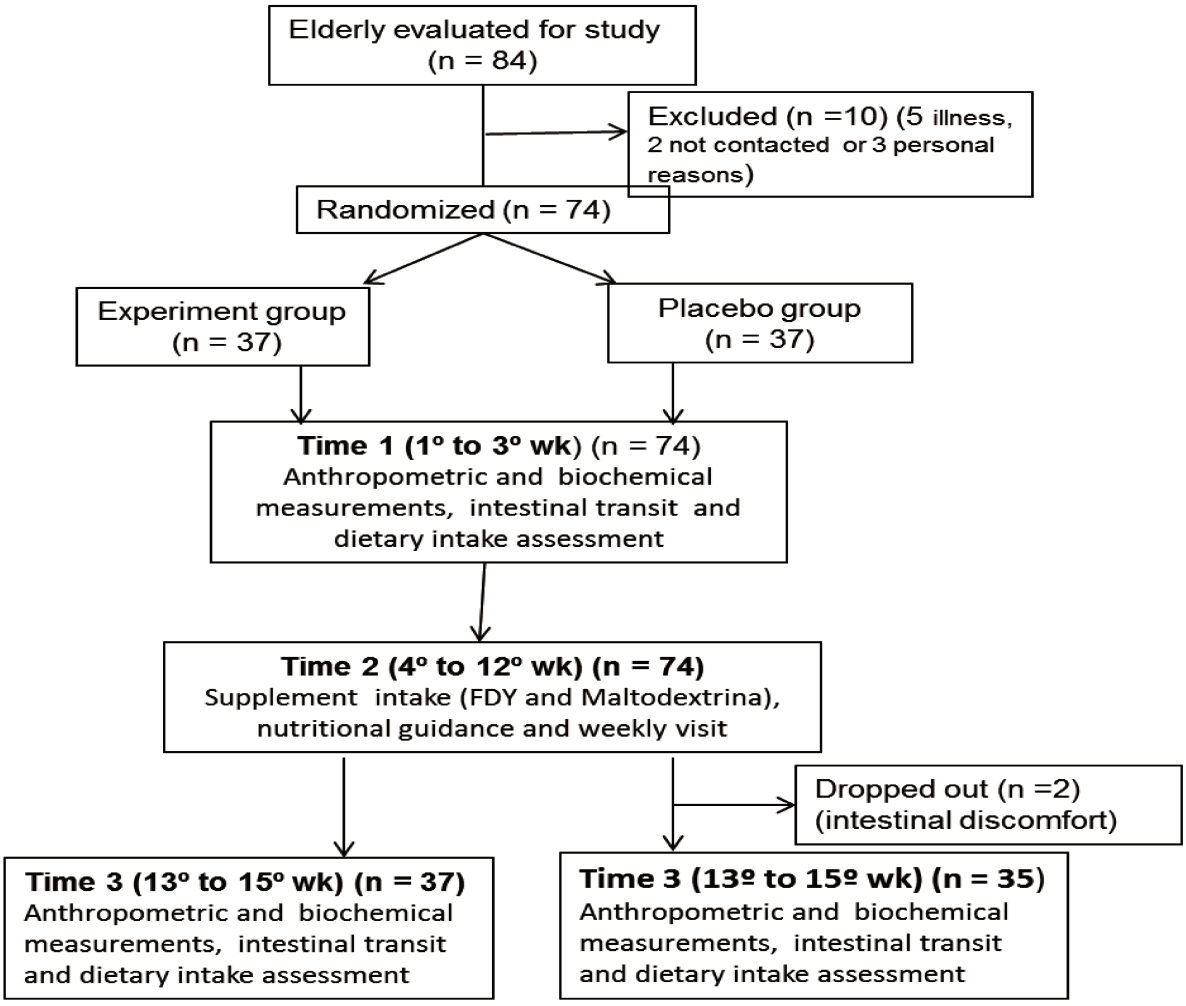

FIGURE 1: Consort schematic overview of a double-blind, randomized, placebo-controlled study during 9 weeks with elderly people

2.3. Characterization of the study products

The *S. sonchifolius* (yacon) roots were cultivated in a field near the town of Piedade, São Paulo, Brazil. The fresh yacon was cleaned, peeled, freeze-dried and powdered to obtain the freeze-dried powder yacon (FDY) (Liofoods, Araras, São Paulo, Brazil). The chemical composition of FDY was determined at the Institute of Food Technology (ITAL, Campinas, SP). Protein was determined according to the Kjeldahl method (29), and the moisture, ash (27) and lipid contents (28) also determined. Maltodextrin (GLOBE-A 1910 MorRex, Corn Products, Local, Brasil) was used as a placebo. The FDY contained 88,6% carbohydrates, 41,2% FOS, 4,7% protein and 0,3% lipids. The FDY and maltodextrin were both packaged in individual sachets containing 18g and 12 g each, respectively.

2.4. Anthropometric parameters

Height was measured to the nearest 0.01 m with subjects standing barefoot with their back to a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg with calibrated scales Body mass index (BMI) was calculated as weight (kg) divided by height (m^2) (30). Nutrition Screening Initiative (NSI) scores were used to measure the risk of undernourishment and obesity (31). Waist circumference (WC), circumferences of hip (HC), arm (AC), and calf (CC) were measured with an inelastic measuring tape. Skinfold measurements of triceps (TSF), biceps (BSF), subscapular (SSF), suprailiac (SISF) were measured with Lange skinfold caliper® (Beta Tecnology Corporated - Santa Cruz, California, USA) with a constant pressure of 10g/ mm^2. WHO (32) scores were used to measure the cardiovascular risk. Fat mass (%FM) was calculated using Durnin & Womersley (33). All anthropometric measurements were taken at the beginning and at the end of the study. (Albers, 2005, Aspinal, 2000) emphasise that there are reciprocal interactions between the immune system on one hand and obesity and nutrition on the other.

2.5. Dietary and supplement intake

Food intake was assessed before and after 9 weeks of intervention. Two-days of dietary records were undertaken to estimate mean energy (Kcal), protein (g), carbohydrate (g), fat (g) and fibre (g intake. It was used the VirtualNutri Programme of Public Health (University of São Paulo, Brazil) to analyze these data. Adequacy of nutrient intake was assessed using the Institut of Medicine (34).

2.6. Biochemical and immunological measurements

At the beginning and at the end of the study, blood samples were collected and centrifuged for 10 min at 1600 rpm. Serum was removed and storaged at - 20 °C until analysis. Analysis of IgA, IgG and C-reactive protein were evaluated by imunoturbidimetry. Laboratorial examinations were measured by automated standard laboratory methods. Enzymatic processes (hexokinase methods) were used to determine levels of triglycerides, cholesterol and lipoprotein. Cytokines (INF-ɣ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10 and TNF-α) were measured by multiplexed bead-based immunoenzymetric assay Luminex (BioSource Europe S.A. – Belgium).

2.7. Statistical analysis

The descriptive data is expressed as means and standard deviations. Mann-Whitney test was used to compare the continuous variables among the group, as the variables did not present normal distribution. For comparison of numeric variables between the two groups and between pre-treatment and following 9 weeks of intervention, analyses of variance (ANCOVA for repeated measures) was used, followed by Tukey multiple comparison test to compare the groups at every moment. Normal ageing and obesity might affect immunosenescence positively or negatively (1,4). The test profile by contrasts was

also used to compare the parameters between the times in each group. Analyses were undertaken using the SAS System for Windows (Statistical Analysis System) software, version 9.1.3. (SAS Institute Inc, 2002-2003, Cary, NC, USA). One-sided P values at < 0.05 were considered significant.

3. RESULTS

A total of 72 elderly, with means age of 67.11 ± 5.79 years were selected to participate in this study. They were randomly assigned to placebo or FDY-supplemented groups. There were not observed differences between the groups in age, height, body weight and body mass index at baseline (Table 1). Only 2.7% (2/72) of the elderly presented BMI slightly below 21 kg/m^2, and 54.0% (N = 39/72) of the elderly presented a BMI slightly above the 27 kg/m^2. There were also no variations in anthropometric values during the study between the groups.

TABLE 1: Baseline characteristics of the elderly according to treatment group

Characteristics	FDY-supplement (N = 37) $\bar{x}$ (SD)	Placebo (N = 37) $\bar{x}$ (SD)	p^b
Age (year)	67.11 (6.12)	67.11 (5.53)	0.782
Weight (kg)	70.13 (13.58)	67.27 (12.61)	0.476
Height (m)	1.58 (0,08)	1.56 (0.06)	0.498
BMI (kg/m^2)	28.14 (5.05)	27.65 (4.90)	0.701
Waist circumference (WC) (cm)	95.78 (10.88)	3.00 (4.39)	0.131

[a]independent Mann-Whitney Test ($p < 0.05$) , WC=waist circumference, BMI= body mass index

Hemoglobin (p = 0.019) and hematocrit (p = 0.039) levels showed difference between the groups in the baseline, however both parameters were in a

normal range (hemoglobin 12.0 – 15.5 g/dL and hematocrit 35.0 – 45.0%). These results confirm the absence of anaemia within the patients. C-reactive protein indicated no presence of inflammatory process (normal range > 10). There was no variation in total, HDL, LDL and VLDL-CHL between the groups. However the patients being well nourished presented LDL-cholesterol levels slightly above the normal range in both groups, increasing their risk of chronic diseases such as cardiovascular disease (Table 2).

Basal values for serum IgA, IgG, lymphocytes, monocytes and leucocytes did not differ between the groups and did not change significantly during the study (Table 2).

TABLE 2: Biochemical parameters according to treatment group at baseline and after 9 weeks

Biochemical parameters	FDY-supplemented		Placebo		*p*
	Baseline (N = 37) $\bar{x}$ (SD)	**9 weeks (N = 37)** $\bar{x}$ (SD)	**Baseline (N = 37)** $\bar{x}$ (SD)	**9 weeks (N = 35)** $\bar{x}$ (SD)	
Hemoglobin (*g/dL*)	14.26 (1.13)	14,38 (1.16)	13.61 (1.11)	13.72 (1.10)	0.019[a]
Hematocrit (%)	42.44 (3.27)	42.90 (3.49)	40.65 (3.36)	41.11 (3.01)	0.039 [a]
Total CHL (mg/dL)	196.43 (37.37)	192.54 (34.84)	196.79 (35.26)	193.42 (29.56)	0.812
HDL-CHL (mg/dL)	50.70 (12.46)	51.97 (13.27)	50.11 (8.28)	50.66 (8.18)	0.783
LDL-CHL (mg/dL)	119.05 (32.79)	113.35 (31.27)	120.26 (30.97)	117.38 (25.97)	0.664
VLDL-CHL (mg/dL)	25.99 (9.37)	26.46 (13.86)	26.34 (9.83)	24.00 (8.71)	0.738
Triacylglycerol (mg/dL)	133.46 (47.69)	132.57 (69.41)	131.89 (48.94)	131.08 (82.19)	0.962
C-reactive protein (CRP)	3.66 (5.03)	3.78 (4.46)	(2.03 (2.85))	2.99 (3.26)	0.285
IgA (mg/dL)	272.92 (109.88)	273.65 (113.26)	255.55 (153.38)	246.47 (103.26)	0.175
IgG (mg/dL)	1028.4 (247.74)	1087.5 (292.07)	1041.1 (326.08)	1091.3 (260.74)	0.966
Leucocytes (absolut/mm^3)	5773.0 (1460.7)	5973.0 (1321.3)	5747.4 (1175.4)	6092.1 (1237.8)	0.779
Lymphocytes (absolut/mm3)	1772.9 (426.45)	1824.3 (464.05)	1727.3 (444.84)	1822.4 (507.11)	0.824
Monocytes (absolut/mm3)	433.92 (114.17)	424.19 (136.30)	411.79 (141.48)	414.39 (116.38)	0.131

P<0.05 ANOVA repeated measures comparison between groups. [a] significantly different at baseline.

Results of the cytokines concentrations by comparative and evolutionary analyses of variables between the 2 groups and between the 2 times are

presented in (Table 3). At baseline no significant difference in cytokine concentration between the placebo and supplemented group was observed.

TABLE 3: Immunological parameters according to treatment group at baseline and after 9 weeks of intervention

Cytokines	**FDY-supplemented**		**Placebo**		
	Baseline (N = 37)	9 weeks (N = 37)	Baseline (N = 37)	9 weeks (N = 35)	*p*
IL-1α (pg/mL)	19.92 (79.02)	11.22 (26.89)	34.42 (94.50)	61.33 (185.80)	< 0.001 [b]
IL1-β (*pg/mL*)	1.08 (3.46)	1.18 (3.29)	1.71 (5.41)	1.52 (4.59)	< 0.001 [a]
IL-2 (*pg/mL*)	1.01 (2.67)	0.50 (1.62)	2.17 (7.27)	2.42 (8.77)	< 0.001 [a]
IL-4 (*pg/mL*)	4.80 (15.91)	21.82 (107.86)	5.06 (15.36)	8.27 (28.49)	= 0.001 [a]
IL-6 (*pg/mL*)	13.25 (37.06)	3.97 (7.32)*	5.41 (13.06)	8.19 (31.16)	0.995
IL-8 (*pg/mL*)	36.86 (52.78)	23.56 (30.55)	29.80 (17.41)	22.30 (20.91)	< 0.001 [a]
IL-10 (*pg/mL*)	3.67 (7.93)	3.01 (8.42)	6.76 (18.72)	3.44 (9.03)	< 0.001 [a]
TNFα (*pg/mL*)	17.68 (9.97)	11.61 (4.14)	17.62 (13.10)	16.02 (22.12)	< 0.001[a]
INFɣ (*pg/mL*)	9.88 (20.03)	11.22 (34.77)	29.72 (100.34)	18.51 (68.95)	< 0.002 [d]

$\bar{x}$ (SD); Evolutionary and comparative ANOVA analyses between the groups and time ($p < 0,005$) [a] Significant differences between times - t1 ≠t2 to exper and placebo, [b] Significant differences between times - t1 ≠ t2 to experiment group [d] Significant differences between times - t1 ≠ t2 to placebo group

A comparative and evolutionary analyses of variables between the 2 groups and the 2 time showed significant decrease on IL-1α,IL-2, IL-8, TNF-α and IL-10 and significant increase on IL-1β, IL-4 and INF-ɣ in supplement group. No significant decrease in IL-6 was observed in supplement group. In placebo group was observed significant decrease on IL-1β, IL-8, IL-10, TNF-α and INF-ɣ and significant increase on IL-2 and IL-4.

Figure 2 represent descriptive statistics of the percentage of deltas and the comparison of measures between the 2 groups adjusted for age and % fat mass. The production of IL-6, a pro-inflammatory cytokine, in supplement group was the only one with significant decrease of 54,6% compared to that of the placebo group (p = 0.039), when adjusted for the age and fat mass.

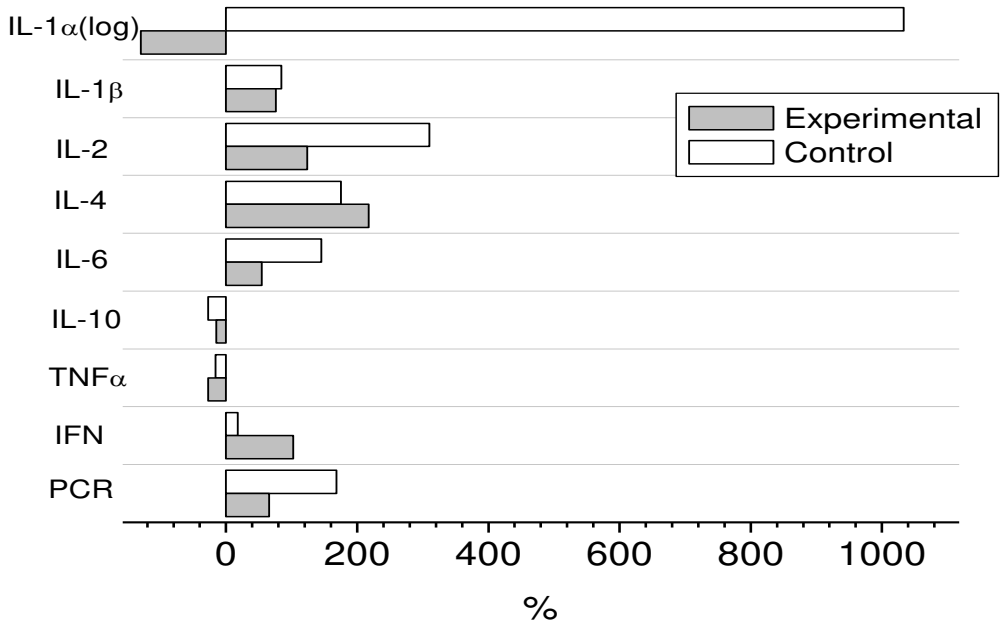

FIGURE 2: Mean percentage changes on immunological parameters between the placebo and supplemented groups. P < 0,05 ANCOVA. Analyses were adjusted for age and fat mass

4. DISCUSSION

In this study, the intake of 7.4 g FOS of FDY for 9 weeks by elderly people had measurable immunomodulatory effect. Prebiotics and similar complex carbohydrates could modulate the gut-associated lymphoid tissue (GALT), as well as the systemic immune system (14), promoting immunoestimulatory effects, especially in the elderly population (16,17),

Recent data from animal studies shown a positive effect of prebiotics intake on immune function (35,36). However, studies with human are variable, some reporting positive effects on the immune system (16,17) while others report no effects (26). Relating results observed in animal experiments is difficult and cannot always be reproduced in humans

The daily intake of 8 g of FOS for 3 weeks for 19 elderly showed a decrease in phagocyte activity of granulocytes and monocytes, as well as decrease on the expression of IL-6 m-RNA in PBMC. These results suggesting a decrease in the inflammatory process in elderly (17). The possibility that the findings arose by chance cannot be excluded because the study was not blinded. The consumption of 5.5 g of GOS was evaluated in a double-blind, placebo-controlled, crossover study among 44 elderly people for 10 weeks. The results showed an increase in phagocytosis, NK cell activity, production of IL-10 and a decrease in IL-6, IL-1β and TNF-α (16). Free-living elderly persons receiving a prebiotic mixture (70% raftilose and 30% raftiline) for 28 weeks were found to have no immunological effects between the patients (26). Positive effects in patients hospitalised for infectious diarrhea or ulcerative colitis and Crohn's disease have been observed after the consumption of prebiotics (18,37). Lindsay *et al.* (37) showed a decrease in disease activity and an increase on IL-10 following an intake of 15 g prebiotics in Chron´s disease patients. Schiffrin *et a.*. (18) concluded that the administration of a liquid supplement containing 1.3 g of prebiotics/250 mL to malnourished elderly subjects had a positive effect on the inflammation.

The effect of FOS from FDY observed in our study was a significant decrease in IL-1α and when adjusted for age and fat mass was demonstrated a significant decrease of IL-6 production compared to that of placebo group in the FDY-supplemented group, which suggest a positive effect of FOS on the immune system in elderly. Some factors could justify the absence of other immunological effects of FOS in our study. Our elderly subjects had an optimal conditions, i.e. they were free-living, were in good health, with normal range total cholesterol and HDL, LDL, VLDL-cholesterol and C-reactive protein, and nutrition state. Finally, it is possible that the duration of intervention was too short or the dose not enough to provide more beneficial effects in the patients.

Nutritional deficiencies induce decreased immune responses (10) and some studies have shown that prebiotics supplement because increased immune responses in elderly. However, the effects of nutritional supplementation on the immune function of health elderly subjects are much less clear, and most trials show only modest results (16,24). New trials with prebiotics in ill elderly are recommended.

5. CONCLUSIONS

Our results show that a daily supplementation of 7.4 g FOS in FDY for 9 weeks caused a significant decrease of IL-1α and a decrease IL-6 when adjusted for age and fat mass in not institutionalized elderly people, suggesting a possible immunomodulation in inflammatory response. This paper is the first to describe that consumption of freeze-dried powdered yacon rich in FOS has a positive effect in immune system of elderly people.

Elderly people with an adequate nutritional status have an appropriate immune function, which cannot be further stimulated by dietary supplements. Further trials with a supplement dose of 7.4 g FOS in FDY is could modulate the inflammatory response in hospitalised elderly.

Acknowledgements

The authors would like to thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - São Paulo Research Foundation - (Research Project 2010/50689-7) for financial support and Corn Products Brazil for the supply of maltodextrin

REFERENCES

[1] Aspinal, R, Andrew, D. Immunosenescence: potential causes and strategies for reversal. Biochem Soc Trans. 2000; 34:512-518

[2] Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J Dairy Sci. 1995;78: 491–497

[3] Meydani SN. Status of nutritional immunology studies: where we are and where we should be going. J Nutr Immunol. 1994; 2: 93–97

[4] Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartín S, Sanderson IR, Loo JV, Dias WV, Watzl B. Markers to measure immunomodulation in human nutrition intervention Studies Brit J Nutr, 2005; 94:452-81

[5] Abbas AK, Janeway CA, Jr. . Immunology: Improving on nature in the twenty first century, Cell 2000; 100: 129-138

[6] Delves PJ, Roitt IM. The immune system. First of two parts. N Engl J Med 2000; 343: 108-117.

[7] Zhou, Y. Immunosenescence: Mechanisms, clinical impacts and Therapeutic strategies. Clinical Infectious Diseases. 2012;Nov.21:.235

[8] Lesourd B. Undernutrition: a factor of accelerated ageing in healthy and diseased aged persons. In Handbook of Nutrition in the Aged Persons, [RR Watson, editor]. New York: CRC Press.2000.p.145-158

[9] Hodes R. Aging and the immune system. Immunol Rev 1997;160:5–35.

[10] Lesourd B, Mazari L. Nutrition and immunity in the elderly. Proc Nutr Soc 1999; 58:685–695.

[11] Guigoz Y, Doré J, Schiffrin EJ. The inflammatory status of old age can be nurtured from the intestinal environment. Curr opin nutr metab care. 2008; Jan;11(1):13-20

[12] Chandra RK. Nutrition and the immune system: an introduction. Am J C,in Nutr. 1997; 66: 460-3

[13] Scrimshaw NS, SanGiovanni JF. Synergiem of nutrition, infection, and immunity: an overview. Am J Clin Nutr, 1997: Aug;66(2): 646-477

[14] Seifert S, Watzl B. Inulin and oligofrutose: Review of experimental data on immune modulation. Journal of Nutrition. 2007;137: 2563-2567

[15] Biagi E, Candela M, Fairweather-Tait S, Franceschi C, & Brigidi P. (2012). Aging of the human metaorganism: the microbial counterpart. *Age (Dordr). 2012; 34:* 247-267.

[16] Vulevic J, Drakoularakou A, Yaqoob P, Tzortzis G, Gibson GR. Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. American Journal of Clinical Nutrition. 2008; 88:1438 – 1446

[17] Guigoz Y, Rochat F, Perruisseau-Carrier G, Rochat I, Schiffrin EJ. Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. Nutrition Research. 2002; 22: 13–25.

[18] Schiffrin EJ, Thomas DR, Kumar VB, Browm C, Hager C, Van´t Hof MA, Morely JE, Guigoz, Y. Systemic inflammatory markers in older persons: the effect of oral nutritional supplementation with prebiotics. Journal of Nutrition Health and Aging. 2007;11(6): 475 -479

[19] Hague A, Butt A J, & Paraskeva C. The role of butyrate in human colonic epithelial cells: An energy source or inducer of differentiation and apoptosis? *Proceedings of the Nutrition Society. 1996; 55*: 937-943.

[20] Donohoe DR, Garge N, Zhang X, Sun W, O´Connell TM, Bunger MK, Bultman SJ. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colo. Cell Metabolism. 2011;13:517-526

[21] Marcil V, Delvin E, Seidman E, Poitras L, Zoltowska M, Garofalo C, & Levy E. Modulation of lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly by butyrate. *Am J Physiol Gastrointest Liver Physiol. 2002; 283:* 340-346.

[22] Weber T E, & Kerr BJ. Butyrate differentially regulates cytokines and proliferation in porcine peripheral blood mononuclear cells. *Veterinary Immunology and Immunopathology. 2006; 113:* 139-147.

[23] Bosscher, D. Fructan Prebiotics derived from inulin. In: Charalampopoulos, d, Rastall, R.A. Prebiotics and probiotics Science and technology. 1, Springer, 2009. pp163-205

[24] Seminario J, Valderrama M & Manrique I. El yacón: fundamentos para el aprovechamiento de um recurso promissório. Centro Internacional de la Papa (CIP), Universidad Nacional de Cajamarca, Agencia Suiza para el Desarrollo y la Cooperación (COSUDE), 2003; Lima, Perú, 60p.

[25] Bunout D, Barrera G, Hircg S, Gattas V, de la Maza MP, Haschke F, Steenhout P, Klassen P, Hager . Effects of a nutritional supplement on the

immune response and cytokine production in free-living Chilean elderly. JPEN J Parenter Enteral Nutr. 2004; 28:348–54

[26] Bunout D, Hirsch S, Pia de la Maza M, Munoz C, Hascke F, Steenhout P, Klassen P, Barrera G *et al.*. Effects of prebiotics on the immune response to vaccination in the elderly. *Journal of Parenteral and Enteral Nutrition. 2002;* 26(6): 372–376

[27] Zenebon, Odair; Pascuet, Neus Sadocco (Coord.). Métodos físico-químicos para análise de alimentos. 4 ed. Brasília: Ministério da Saúde/ANVISA São Paulo: Instituto Adolfo Lutz, 2005a, cap. 4, met. 037, p. 124.

[28] Zenebon, Odair; Pascuet, Neus Sadocco (Coord.). Métodos físico-químicos para análise de alimentos. 4 ed. Brasília: Ministério da Saúde/ANVISA São Paulo: Instituto Adolfo Lutz, 2005b, cap. 4, met. 018, p. 105.

[29] Zenebon, Odair; Pascuet, Neus Sadocco (Coord.). Métodos físico-químicos para análise de alimentos. 4 ed. Brasília: Ministério da Saúde/ANVISA São Paulo: Instituto Adolfo Lutz, 2005c, cap. 4, met. 034 B, p. 119.

[30] World Health Organization. Physical Status: the use and interpretation of anthropometry. Report. Geneva; 1995. (WHO Technical Report Series, 854)

[31] Nutrition Screening Initiative (NSI). Nutrition interventions manual for professionals caring for older Americans. Washington DC: Nutrition Screening Initiative, 1992

[32] World Health Organization. Defining the problem of overweight and obesity. In: World Health Organization. Obesity: preventing and managing the global epidemic: report of a Who Consultation. Geneva; 2000. p. 241-3. (WHO Technical Report Series, 894).

[33] Durnin JVGA, Womersley, J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutrition 1974; 32: 77-97.

[34] Institute of Medicine (IOM). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington (DC): National Academy Press; 2005

[35] Choque-Delgado GT, Thomé R, Gabriel DL, Tamashiro MSC, Pastore GM. Yacon (Smallanthus sonchifolius) derived fructooligosaccharides improves immune parameters in the mouse Nutr Research 2012; 32:884-892

[36] Lemos ACG. Avaliação in vitro e in vivo das propriedades funcionais e efeitos prebióticos dos galactooligosaccharides (GOS) Campinas, 2012, 244p. Dissertação (doutorado em Ciência dos Alimentos), Faculdade de Engenharia dos Alimentos, Universidade Estadual de Campinas (UNICAMP)

[37] Lindsay JO, Whelan K, Stagg AJ, Gobin P, Al-Hassi HO, Rayment N, Kamm MA, Knight SC *et al*.. Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease. Gut. 2006; 55: 348–355.

CONCLUSÕES GERAIS

O presente trabalho foi desenvolvido com o objetivo de avaliar as alterações físicas e o teor de carboidratos do yacon fresco durante o armazenamento e analisar o efeito do consumo diário de yacon liofilizado contendo 7,4g FOS durante nove semanas no perfil lipídico, glicêmico, hábito intestinal, ingestão alimentar e sistema imunológico de idosos frequentadores da Universidade Aberta da Terceira Idade.

Os resultados obtidos no presente estudo mostraram que:

- O yacon fresco é uma fonte de FOS. Com o armazenamento ocorre redução no teor de FOS e aumento no teor de glicose e frutose, sendo que estas variações são menores quando as raízes foram armazenadas em geladeira (25º C) e até 17 dias. Durante o armazenamento as raízes tornam-se murchas e escuras, prejudicando a aceitação pelo consumidor.

- O yacon liofilizado é um produto bem aceito pelos idosos.

- A ingestão diária de yacon liofilizado com 7,4 FOS durante nove semanas melhora o perfil glicêmico e imunológico, mas não altera o perfil lipídico de idosos.

- O consumo diário de 18g yacon liofilizado contendo 7,4g FOS por 9 semanas não melhorou o hábito intestinal, e não produziu efeitos adversos, intolerância intestinal, flatulência, e foi bem aceito pelos idosos.

- Desta forma, consideramos que o yacon liofilizado é uma fonte de FOS, é de fácil consumo e seguro para armazenamento e apresenta efeitos positivos no perfil glicêmico e possível resposta imunomodulatória inflamatória em idosos.

PERSPECTIVAS FUTURAS

As pesquisas de consumo de yacon em humanos são escassas, portanto novos estudos são necessários para avaliar seu efeito lipídico e imunomodulador em idosos saudáveis e doentes.

É importante determinar a dosagem de FOS a ser administrada, o tempo de tratamento e a população idosa a ser estudada (se hígidos ou doentes), além de avaliar as alterações na microbiota intestinal de idosos.

ANEXO 1: Constipation Scoring (Agachan, 1996):

Constipation Scoring System (Minimum Score, 0; Maximum Score, 30)

Frequency of bowel movements	Score
1–2 times per 1–2 days	0
2 times per week	1
Once per week	2
Less than once per week	3
Less than once per month	4
Difficulty: painful evacuation effort	
Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4
Completeness: feeling incomplete evacuation	
Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4
Pain: abdominal pain	
Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4
Time: minutes in lavatory per attempt	
Less than 5	0
5–10	1
10–20	2
20–30	3
More than 30	4
Assistance: type of assistance	
Without assistance	0
Stimulative laxatives	1
Digital assistance or enema	2
Failure: unsuccessful attempts for evacuation per 24 hours	
Never	0
1–3	1
3–6	2
6–9	3
More than 9	4
History: duration of constipation (yr)	
0	0
1–5	1
5–10	2
10–20	3
More than 20	4

ANEXO 2 : **Aprovação do Comitê de Ética em Pesquisa**

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 27/10/09.
(Grupo III)

PARECER CEP: N° 949/2009 (Este n° deve ser citado nas correspondências referente a este projeto)
CAAE: 4272.0.000.146-09

I - IDENTIFICAÇÃO:

PROJETO: **"AVALIAÇÃO DO EFEITO DA INGESTÃO DE FRUTOOLIGOSSACARÍDEOS DE YACON EM IDOSOS".**
PESQUISADOR RESPONSÁVEL: Marlene Maria Amaral Scheid
INSTITUIÇÃO: Faculdade de Odontologia de São José dos Campos
APRESENTAÇÃO AO CEP: 09/10/2009
APRESENTAR RELATÓRIO EM: 27/10/10 (O formulário encontra-se no *site* acima)

II - OBJETIVOS

Produzir o extrato liofilizado de yacon (ELY) e avaliar o efeito de seu consumo sobre o hábito intestinal, sistema imunológico, perfil metabólico glicêmico e lipídico e estado nutricional de idosos.

III - SUMÁRIO

Trata-se de estudo prospectivo, duplo cego, randomizado, controlado por placebo com 80 idosos de ambos os sexos, que serão suplementados com FOS de yacon (10 mg/dia) ou maltodextrina durante 8 semanas. Os sujeitos de pesquisa serão selecionados entre os freqüentadores da Universidade da Terceira Idade (FTI) Unesp-faculdade de Odontologia da UNESP de São José dos Campos. Serão excluídos sujeitos com uso crônico de antibióticos, doença intestinal crônica, seguidores de dieta específica, portadores de câncer de TGI, antecedente de cirurgia bariátrica, uso crônico de laxante, fumante, uso regular de pré bióticos ou portadores de demência. Os sujeitos que preencham os critérios de inclusão e concordem em participar do estudo serão avaliados (avaliação clinica, imunológica, metabolismo lipídico e glicêmico, função intestinal, avaliação antropométrica e estado nutricional). Serão então randomizados em 2 grupos, e receberão o liofilizado por 8 semanas. Ao final do estudo as mesmas avaliações iniciais. Em dois momentos, antes e após 15 semanas de intervenção, serão avaliados o hábito intestinal, através de um questionário, o perfil lipídico e glicêmico, pela dosagem sangüínea de glicose, colesterol total e frações, a função imunológica através da dosagem de IgA secretória e citocinas e o estado nutricional dos idosos. Todos os idosos terão acompanhamento nutricional durante o estudo. Os exames laboratoriais serão coletados por profissionais do curso de Biomédicas da Faculdade de Ciências de Saúde da UNIVAP. Os marcadores imunológicos (TNF, IL1, IL2, IL6, IgA, IgG e proteina C reativa) serão dosados utilizando kits comerciais.Os dados serão analisados através de teste ANOVA de 2 vias, com teste de significância se $p<0.05$.

IV - COMENTÁRIOS DOS RELATORES

Projeto claro, com objetivos e metodologia bem definidos. Apesar de se tratar de projeto de doutorado da Faculdade de Engenharia de Alimentos da Unicamp, os sujeitos de pesquisa serão recrutados junto à UNESP de São José dos Campos. Assim o projeto deveria ser encaminhado para apreciação do CEP responsável por aquela instituição.

Comitê de Ética em Pesquisa - UNICAMP
Rua: Tessália Vieira de Camargo, 126
Caixa Postal 6111
13083-887 Campinas – SP

FONE (019) 3521-8936
FAX (019) 3521-7187
cep@fcm.unicamp.br

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, o Termo do Consentimento Livre e Esclarecido, bem como todos os anexos incluídos na pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e).

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII – DATA DA REUNIÃO

Homologado na X Reunião Ordinária do CEP/FCM, em 27 de outubro de 2009.

Profa. Dra. Carmen Silvia Bertuzzo
VICE-PRESIDENTE do COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

Comitê de Ética em Pesquisa - UNICAMP
Rua: Tessália Vieira de Camargo, 126
Caixa Postal 6111
13083-887 Campinas – SP

FONE (019) 3521-8936
FAX (019) 3521-7187
cep@fcm.unicamp.br