**RENORBIO**
**Programa de Pós-Graduação em Biotecnologia**
**Rosane Souza Cavalcante**

# Efeitos das tecnologias emergentes não térmicas empregadas no processamento de suco prebiótico de maçã

Tese submetida à Coordenação do Curso de Pós-graduação em Biotecnologia - Renorbio, da Universidade Federal do Ceará, como requisito parcial para obtenção do grau de Doutor em Biotecnologia.

Fortaleza - CE
2016

**ROSANE SOUZA CAVALCANTE**

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Orientadora: Profª Drª Sueli Rodrigues.

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## ATA DE SESSÃO DE DEFESA DE TESE DE DOUTORADO

Ata da Sessão de Defesa de Tese de Doutorado da Pós-Graduanda **ROSANE SOUZA CAVALCANTE** Realizado no dia 26/02/2016.

Às 13 horas e 30 minutos do dia 26/02/2016 realizou-se a sessão de Defesa de Tese de Doutorado de **ROSANE DE SOUZA CAVALCANTE**. O trabalho tinha como título "Efeitos das tecnologias emergentes não térmicas empregadas no processamento de suco prebiótico de maçã.". Compunha a banca examinadora os professores doutores SUELI RODRIGUES (presidente), EDY SOUSA DE BRITO, ELENILSON DE GODOY ALVES FILHO, ANA PAULA DIONÍSIO E MARIA CRISTIANE RABELO. (examinadores). A sessão foi aberta pela orientadora da tese, que apresentou a banca examinadora e passou a palavra para a candidata. Após a exposição do trabalho, seguiu-se o processo de arguição da candidata. Em seguida, a banca examinadora se reuniu reservadamente a fim de avaliar seu desempenho e trabalho. A banca examinadora considerou o trabalho APROVADA. Nada mais havendo a relatar a sessão foi encerrada às 16 horas e 15 minutos, e eu Profa. Dra. Sueli Rodrigues lavrei a ata que depois de lida e aprovada foi assinada por mim e pelos membros da banca examinadora.

Fortaleza, 26/02/2016.

Profa. Dra Sueli Rodrigues
Presidente

Prof. Dr. Edy Sousa de Brito
Examinador

Profa. Dra. Maria Cristiane Rabelo
Examinadora

Profa. Dra. Ana Paula Dionísio
Examinadora

Prof. Dr. Elenilson de Godoy Alves Filho
Examinador

Dedico este trabalho aos meus pais que sempre me proporcionaram boa educação, em especial à minha mãe Eliane, pelo apoio incondicional.

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**“**Há que ser mudada a perspectiva e a atitude discente quanto ao ato de aprender, uma vez que este é um elemento de construção do seu ser pessoal e profissional, e não mais um mero cumprimento para obtenção de certificados.**”**

(Paulo Freire)

## RESUMO

O aumento no consumo de suco de maçã aliado ao apelo por alimentos funcionais tem evidenciado a necessidade de mais pesquisas em relação a sucos prebióticos de maçã. O processo usual de conservação por calor vem mostrando perda de qualidade desses sucos. O objetivo deste trabalho foi verificar os efeitos de novas tecnologias que não fazem uso de aquecimento sobre a qualidade desses sucos contendo oligossacarídeos (prebióticos), principalmente no que diz respeito à sua propriedade funcional. Em um primeiro momento, foram aplicados plasma e alta pressão em sucos adicionados de fruto-oligossacarídeos (FOS) a 7%. No caso do plasma, foram testados dois tipos de exposição (direta e indireta) e diferentes tempos de aplicação do tratamento (15, 30, 45 e 60 segundos). O processamento por alta pressão foi a 450 MPa por 5 min. Foram avaliados dados de cor e de concentração dos oligossacarídeos e dos ácidos orgânicos. Em relação à cor, todas as amostras de suco tratadas por plasma e também por alta pressão mostraram-se com uma cor significativamente mais vívida, baseados nos dados de croma. Os resultados mostraram despolimerização dos FOS, aumentando a concentração de 1-kestose (oligossacarídeo com 3 monômeros). Ao final dos tratamentos com exposição direta e indireta de plasma, a concentração total de FOS mostrou-se dentro dos padrões exigidos pela legislação vigente, confirmando ser o plasma uma tecnologia aplicável. As amostras tratadas com alta pressão tiveram comportamento parecido. No caso da concentração de ácidos orgânicos, o ácido málico se mostrou predominante e teve decaimento conforme o aumento da aplicação do plasma em ambas as exposições. O mesmo ocorreu para o ácido cítrico. No caso dos sucos a alta pressão, houve um aumento significativo na concentração de ambos os ácidos quando comparado ao controle, à amostra tratatada a alta pressão e à amostra a alta pressão contendo os FOS, nesta ordem. Na segunda etapa do trabalho, iniciou-se o processo biotecnológico. Por meio de uma fermentação submersa utilizando a bactéria *Leuconostoc mesenteroides*, foi produzida a enzima dextrana-sacarase, sendo então purificada e utilizada para fazer a conversão dos açúcares simples presentes no suco de maçã em gluco-oligossacarídeos, que têm propriedade prebiótica. Após a obtenção do suco prebiótico, foram aplicados plasma nas mesmas condições da primeira etapa e ozônio utilizando diferentes cargas: 0,057; 0,128; 0,230; 0,386 e 0,671 mg $O_3$/ml. Após os tratamentos, foram medidas as concentrações dos açúcares simples (frutose, glicose e sacarose), dos oligossacarídeos e dos ácidos orgânicos por HPLC além do conteúdo total de fenólicos pelo método de Folin-Ciocalteau e da atividade antioxidante por ABTS. Para os dois tratamentos houve despolimerização dos oligossacarídeos, porém a concentração total dos oligossacarídeos permitiu que os sucos permanecessem com atividade prebiótica, sendo evidente que o ozônio preservou bem melhor essa concentração, seguida de plasma com exposição direta. A concentração de ácido cítrico só teve diferença significativa em relação ao controle em sucos após o ozônio ser aplicado, enquanto o ácido málico teve um decaimento significativo. No caso do plasma, houve variações de ambos os ácidos. O conteúdo de fenólicos mostrou-se inalterado após exposição direta de plasma e também após tratamento por ozônio, mostrando uma pequena diminuição após exposição indireta (de $0.73 \pm 0.10$ a $0.53 \pm 0.02$). A atividade antioxidante teve pequenas alterações após a aplicação de ozônio, enquanto para as amostras tratadas por plasma foi obtido aumento significativo em ambas as exposições. As tecnologias não térmicas aqui estudadas podem ser utilizadas em sucos prebióticos, preservando sua funcionalidade e sua qualidade. Considerando serem tecnologias novas, estudos para avaliar outros parâmetros também importantes devem ser realizados.



**Palavras-chave:** Tecnologias não térmicas; alta pressão; plasma; ozônio; prebióticos; gluco-oligossacarídeos; fruto-oligossacarídeos; suco de maçã

## ABSTRACT

The increase in apple juice consumption of the demand for functional foods has shown the need for more research regarding prebiotics juices apple. The traditional heat conservation usually imparts quality loss of fruit juices. The objective of this study was to verify the effects of new non-thermal technologies on the quality of fruit juices containing prebiotic oligosaccharides with regard to its functional property. At first, it was applied plasma and high pressure processing in apple juice with added fructo-oligosaccharides (FOS, 7 % w/v). In the case of plasma it was tested two types of exposure (direct and indirect) and different processing times (15, 30, 45 and 60 seconds). The high pressure processing was carried out at 450 MPa for 5 min. Colour, oligosaccharides concentration and organic acids were evaluated. Regarding colour, all juice samples treated by plasma and by high pressure demonstrated a significantly more vivid colour according to on the Chroma value. The results showed some depolymerisation of FOS, increasing the concentration of 1-kestose (oligosaccharides with 3 monomers). However, after the plasma treatment, the total concentration of FOS still meets the standards required by law, confirming that plasma is a suitable non-thermal technology for apple juice containing FOS. The high pressure treated samples presented similar behaviour. Regarding the organic acids concentration, malic acid was the predominant one and presented a decay after both plasma exposure modes (direct and indirect). The same happened for citric acid. For the juices treated by high pressure, there was a significant increase in the concentration of both acids when compared to the control. In the second stage of the research, the biotechnological process started. Through a fermentation using the bacterium *Leuconostoc mesenteroides* it was produced the dextransucrase enzyme. The enzyme was purified and used to convert the simple sugars present in the apple juice in prebiotic gluco-oligosaccharide. After synthesizing the prebiotic juice plasma treatment was applied under the same conditions of the first stage. Ozone was also applied using different loadings: 0.057; 0.128; 0.230; 0.386 and 0.671 mg $O_3$ / mL. After the treatments the concentrations of simple sugars (fructose, glucose and sucrose), oligosaccharides and organic acids by HPLC were measured .The total phenolic content was determined by Folin-Ciocalteu method and the antioxidant activity by ABTS. For both treatments oligosaccharides depolymerisation was observed, but the total concentration of oligosaccharides attested that the juices remained with prebiotic activity. The oligosaccharides were better preserved after ozone treatment, followed by plasma with direct exposure. The citric acid concentration presented significant difference only for juices treated by ozone, while malic acid had a significant decay for both treatments. In the case of plasma there were changes in both acids concentration. The phenolic content unchanged after plasma direct exposure and also after ozone treatment, showing a decrease after indirect exposure ($0.73 \pm 0.10$ to $0.53 \pm 0.02$). The antioxidant activity presented slight differences after ozone treatment. After plasma, the antioxidant activity presented significant increase for both exposures kind. Then non-thermal technologies studied herein can be applied in prebiotic juices, preserving their functionality and quality.



**Key words:** Non-thermal technologies; high pressure; atmospheric cold plasma; ozone; prebiotics; gluco-oligosaccharides; fruto-oligosaccharides; apple juice

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## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| ABTS | 2,2 – azinobis (ácido 3-etilbenzotiazolina - 6 – sulfônico) |
| ACP | Atmospheric cold plasma |
| ANVISA | Agência Nacional de Vigilância Sanitária |
| DP | Degree of polymerization ou Grau de polimerização |
| FOS | Fructooligosaccharides ou Frutoligossacarídeos |
| GOS | Galactooligosaccharides ou Galactoligossacarídeos |
| IMO | Isomaltooligosaccharides ou Isomaltoligossacarídeos |
| NTP | Non-thermal plasma ou plasma não térmico |
| DBD | Dieletric barrier discharge ou Descarga de barreira dielétrica |
| SOS | Soybean oligosaccharides ou Oligossacarídeos de soja |
| XOS | Xylooligosaccharides ou Xiloligossacarídeos |
| SCFA | Short chain fatty acids |
| AGCC | Ácidos graxos de cadeia curta |
| IUPAC | International Union of Pure and Applied Chemistry |

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# 1 INTRODUÇÃO

Os consumidores estão cada vez mais preocupados com a saúde e buscam na alimentação não apenas uma fonte de nutrientes, mas também propriedades benéficas ao organismo (MENRAD, 2003). Alguns compostos que têm sido associados aos benefícios de bem-estar, controle de peso, saúde digestiva e resistência são os prebióticos (SLOAN, 2014), que são definidos como carboidratos não digeridos pelo organismo que promovem o crescimento e/ou atividade das bactérias do cólon (CHARALAMPOPOULOS; RASTALL, 2012; GIBSON; ROBERFROID, 1995a; HUEBNER; WEHLING; HUTKINS, 2007; RASTALL, 2010). Alimentos prebióticos têm ganhado importância no mercado por proporcionar bem-estar físico e mental em quem os consome (BIGLIARDI; GALATI, 2013; HUEBNER; WEHLING; HUTKINS, 2007), beneficiar a microbiota intestinal (HUEBNER; WEHLING; HUTKINS, 2007; RASTALL, 2010), promover a formação de ácidos graxos de cadeia curta (AGCC) (HUEBNER; WEHLING; HUTKINS, 2007) e auxílio na absorção de cálcio (BIGLIARDI; GALATI, 2013b). Oligossacarídeos desenvolvem uma importante função no organismo como, por exemplo, o controle da obesidade por promover saciedade (SIRÓ et al., 2008).

O mercado de alimentos funcionais vem crescendo nos últimos anos, com destaque para o Japão, berço desse tipo de alimento e que, junto com Estados Unidos (EUA) e Europa (principalmente Reino Unido, Alemanha, França e Itália), totaliza 90% desse mercado (BIGLIARDI; GALATI, 2013b). Apesar da preocupação por alimentos funcionais, o mercado de prebióticos ainda é pequeno e fragmentado e, tomando como exemplo a Europa, só a Alemanha que de fato está com um mercado em ascensão, graças ao sucesso da bebida tipo ACE (bebida não alcóolica fortificada com vitaminas A, C e E ou outros ingredientes funcionais) (BIGLIARDI; GALATI, 2013b; SIRÓ et al., 2008).

A indústria mundial voltada para produtos para saúde e bem-estar movimentou o equivalente a US$52 bilhões de dólares em 2013 segundo a Euromonitor (2014), o que corresponde a um crescimento de 7% comparado com o ano anterior. A pesquisa aponta ainda que produtos com propriedades funcionais como leite formulado funcional/fortificado (FF), bebidas energéticas FF e iogurtes pre/probióticos estão entre os mais aceitos, estando o suco de fruta FF na quinta posição na categoria dos que mais vêm aumentando o consumo. Dentre os países que lideram o *ranking* estão a China em primeiro lugar, os EUA em segundo, seguido pelo Brasil, Rússia e México, respectivamente.

Com o rápido e crescente aumento da classe média, com orçamento mais disponível e um grande número de mulheres trabalhando e dispondo de mais informação no mercado ascendente, o mercado de alimentos e bebidas funcionais é considerado um potencial para vendas desse tipo de produto (SLOAN, 2014).

O presente estudo foi realizado em parceria com o Dublin Institute of Technology (DIT), no qual foram realizados os tratamentos de plasma e ozônio, com o Teagasc (Dublin, Ireland) com a empresa HPP Tolling Ltd (Dublin, Ireland), na qual foram ralizados os tratamentos de  alta pressão. Foram então realizadas análises de cor, concentração de açúcares simples e oligossacarídeos, ácidos orgânicos, compostos fenólicos e antioxidantes.

O objetivo geral deste trabalho foi avaliar os efeitos dos tratamentos não térmicos em suco prebiótico de maçã.

# *Revisão Bibliográfica*

# 2 REVISÃO BIBLIOGRÁFICA

## 2.1 Alimentos funcionais com enfoque em prebióticos

Consumidores vêm em busca de alimentos que não só proporcionem nutrientes e saciem a fome, mas também alimentos que previnam doenças relacionadas com a alimentação e proporcionem bem estar. Assim, alimentos com propriedades funcionais têm ganhado força do mercado, e a indústria de alimentos está se adequando a essas novas demandas (BIGLIARDI; GALATI, 2013b).

O conceito de alimento funcional (prebióticos e probióticos) surgiu no Japão em 1984, com divulgação dos efeitos benéficos de alimentos enriquecidos com componentes especiais pelos cientistas japoneses. Há uma série de definições para prebióticos, entretanto, ainda não há um consenso para uma definição universalmente aceita para este grupo de alimentos e, na maioria dos países, não há uma legislação específica para os mesmos (SIRÓ et al., 2008). Porém muitos autores defendem a definição de que são alimentos não digeridos pelo organismo humano e que chegam ao intestino intactos, estimulando o crescimento e/ou a atividade das bactérias do cólon (BIGLIARDI; GALATI, 2013a; CHARALAMPOPOULOS; RASTALL, 2012; GIBSON; ROBERFROID, 1995b; HUEBNER; WEHLING; HUTKINS, 2007; LICHT; EBERSBACH; FRØKIÆR, 2012; SAAD et al., 2013).

Para classificar um alimento como prebiótico, o composto qualificado como tal deve resistir à acidez gástrica, à hidrólise por enzimas de mamíferos e à absorção gastrointestinal; ser fermentado pela microbiota intestinal, além de estimular seletivamente o crescimento e/ ou atividade de bactérias intestinais associadas à saúde e ao bem-estar (DA SILVA; RABELO; RODRIGUES, 2012; GIBSON; ROBERFROID, 1995b; GIBSON et al., 2004).

## 2.2 Benefícios associados ao consumo de prebióticos

Todos os prebióticos são misturas de oligossacarídeos indigeríveis, sendo carboidratos de cadeia curta, com baixo grau de polimerização (DP), em geral contendo de 3 a 10 monômeros de carboidratos (segundo a nomenclatura da IUPAC), tendo como monômeros mais comuns glicose, frutose, galactose e xilose. A inulina é uma exceção a essa definição, haja vista ser uma mistura de fruto- e polissacarídeos (SAAD et al., 2013). Outros autores classificam qualquer sacarídeo que contenha de 3 a 19 unidades de monossacarídeos como oligossacarídeos. O que os diferem quimicamente são o comprimento da cadeia, a composição dos monômeros, o grau de ramificação e a pureza (MUSSATTO; MANCILHA, 2007).

Atualmente já estão comprovados os benefícios dos prebióticos sobre a microbiota do cólon. Além disso, estão envolvidos também com a diminuição dos índices de colesterol, especialmente o LDL (SIRÓ et al., 2008). Apesar dos produtos prebióticos terem seu preço mais elevado, tais benefícios têm levado ao aumento da procura e da compra por tais produtos (SCOTT, 2010).

Vários estudos comprovam a eficácia em reduzir o colesterol devido ao consumo de aveia, que apresenta elevadas concentrações de fibras solúveis (BURRIS, 1990; CHARLTON et al., 2012; YOKOYAMA et al., 1998). Um desses estudos avaliou o efeito do consumo de produtos de aveia (que contém $\beta$-glucana como prebiótico) a uma dose de 3,0 g/dia e demonstrou a redução nos níveis de colesterol após seis semanas em homens e mulheres adultos com colesterol alto e sobrepeso (CHARLTON et al., 2012).

Algumas pesquisas vêm confirmando os benefícios no consumo de prebióticos. Um estudo que avaliou a dextrana como um novo gluco-oligossacarídeo demonstrou seu potencial prebiótico, aumentando as concentrações das bactérias do cólon *Bifidobacterium spp.* e as de ácidos graxos de cadeia curta, mesmo em indivíduos obesos (Sarbini et al., 2014). Em outra

pesquisa o oligossacarídeo arabinoxilano foi um prebiótico bem tolerado a uma dose diária de 10 g. Sua ingestão aumentou a quantidade de bifidobactérias e reduziu a excreção urinária de *p*-cresol (indicando menor degradação das proteínas do cólon) (CLOETENS et al., 2010). Já Bouchaud et al. (2014) reportaram que dietas suplementadas com os prebióticos galactoligossacarídeo e inulina durante período perinatal em ratas protegem contra alergia alimentar. Pesquisas indicam o efeito benéfico prebiótico do consumo de oligossacarídeos obtidos pela enzima dextranasacarase, estimulando o crescimento *in vitro* de bifidobactérias (Machida et al., 1986) e de lactobacilos (VERGARA et al., 2010).

Fruto-oligossacarídeos atuam em efeitos fisiológicos específicos como a diminuição dos níveis de colesterol total e de lipídios séricos, o alívio da constipação, a melhora em geral da saúde humana e a estimulação do crescimento de bactérias benéficas ao trato digestório como as bifidobactérias (RENUKA et al., 2009). Estas bactérias seletivamente consomem os prebióticos e não são consumidos por bactérias patogênicas como enterobactérias e clostrídio (SARBINI et al., 2014). As bifidobactérias são conhecidas por serem eficientes micro-organismos capazes de manter a saúde humana e prevenir o crescimento de micro-organismos patogênicos (MANDERSON et al., 2005; RABELO; FONTES; RODRIGUES, 2009).

A Agência Nacional de Vigilância Sanitária Brasileira (ANVISA) menciona que, assim como fibras alimentares, os fruto-oligossacarídeos (FOS) têm alegações funcionais, desde que a recomendação de consumo diário do produto pronto para consumo forneça no mínimo 5,0 g de FOS/fibras (BRASIL, 2008) e a porção, no mínimo 2,5 g, não devendo ultrapassar o consumo de 30 g diários.

## 2.3 Oligossacarídeos prebióticos

Oligossacarídeos são açúcares não digeríveis e que chegam ao intestino para serem consumidos por bactérias do cólon (COELHO et al., 2014; MADHUKUMAR;

MURALIKRISHNA, 2012). Assim, fazem parte de um dos tipos de compostos tidos com propriedades funcionais.

Os oligossacarídeos prebióticos incluem fruto-oligossacarídeos (FOS), glico-oligossacarídeos, isomalto-oligossacarídeos (IMO), oligossacarídeos de soja (SOS), galacto-oligossacarídeos (GOS), gentilo-oligossacarídeos, isomaltulose, lactosacarose, malto-oligossacarídeos e xilo-oligossacarídeos (XOS) (LOMAX; CALDER, 2009; PATEL; GOYAL, 2010). Muitos deles são sintetizados ou isolados de plantas e algas através de despolimerização de polissacarídeos. Oligossacarídeos primários como SOS, GOS e XOS são encontrados para comercialização no Japão (SAAD et al., 2013).

Devido à sua estrutura química, os oligossacarídeos prebióticos apenas podem ser metabolizados por um grupo limitado de bactérias, estimulando seu crescimento. Neste grupo, estão incluídos os gêneros *Bifidobacterium* e *Lactobacillus* (MUSSATTO; MANCILHA, 2007).

Concomitante ao crescimento de bifidobactérias e lactobacilos no cólon, a fermentação dos oligossacarídeos prebióticos resulta na formação de ácidos graxos de cadeia curta (AGCC), principalmente, acetato, butirato e propionato, que promovem a acidificação na região do intestino, desencadeando diversos benefícios à saúde (MADHUKUMAR; MURALIKRISHNA, 2012); afetam o transporte e o metabolismo do epitélio celular, bem como seu crescimento e diferenciação, além de proporcionar controle hepático de lipídios e carboidratos, enquanto proveem energia para órgãos como, rins, coração e cérebro e para os músculos (MACFARLANE; MACFARLANE, 2012). O decréscimo do pH no cólon suprime o crescimento de bactérias patogênicas enquanto estimula o crescimento de bifidobactérias e outras espécies láticas (MUSSATTO; MANCILHA, 2007).

Muitos oligossacarídeos prebióticos são naturalmente encontrados em plantas como, por exemplo, a chicória (inulina), a alcachofra e o alho (CHARALAMPOPOULOS;

RASTALL, 2012). Oligossacarídeos funcionais são encontrados em diferentes concentrações em leite, mel, caldo de cana-de-açúcar, soja, lentilha, mostarda, frutas e vegetais como cebola, aspargo, beterraba, alcachofra, chicória, alho-poró, banana, centeio, cevada, yacon, trigo, tomate e brotos de bambu (MUSSATTO; MANCILHA, 2007; PATEL; GOYAL, 2010). Os oligossacarídeos podem ser obtidos por extração, por hidrólise da inulina obtendo oligofrutose, ou por síntese química por reações de transglicosilação de monossacarídeos e de dissacarídeos como a sacarose (fruto-oligossacarídeos), a lactose (galacto-oligossacarídeos), bem como através de síntese enzimática (utilizando enzimas como glicosiltransferases, inulinases, pectinases e outras) de polissacarídeos de plantas (xilo-oligossacarídeos) (CHARALAMPOPOULOS; RASTALL, 2012; RASTALL, 2010; SAAD et al., 2013). As glicosiltransferases catalisam a transferência de resíduos de glicosil de uma molécula doadora para um aceptor particular (RABELO et al., 2006; RODRIGUES; LONA; FRANCO, 2005). A rafinose pode ser extraída diretamente de materiais de plantas com o uso de água ou de soluções alcóolicas (MUSSATTO; MANCILHA, 2007).

Alguns carboidratos prebióticos disponíveis no mercado são a inulina, os FOS, a lactulose, os GOS (RASTALL, 2010), assim como os mais recentes: IMO, XOS e SOS (CHARALAMPOPOULOS; RASTALL, 2012).

O poder de doçura dos oligossacarídeos depende da estrutura química, do grau de polimerização e da proporção dos monômeros na cadeia. Porém, a doçura diminui conforme a extensão da cadeia. Essa característica é importante em alimentos com restrição de sacarose. Podem atuar como agentes de corpo junto com edulcorantes em produtos doces, além de mascarar o sabor residual peculiar dos edulcorantes. Outra vantagem é que tem como propriedade físico-química o aumento da viscosidade devido ao seu alto peso molecular (MUSSATTO; MANCILHA, 2007).

O pH de sucos de maçã é 3,13 de acordo com Patil et al. (2010). Em outro estudo, encontrou-se que, com pH < 4,0 e tratamentos a temperaturas elevadas ou armazenamento prolongado a condição ambiente, os oligossacarídeos presentes nos alimentos podem ser hidrolisados resultando em perda nutricional e em propriedades físico-químicas (MUSSATTO; MANCILHA, 2007).

Os IMO são oligossacarídeos compostos por unidades de maltose e até oito unidades de glicose unidas por ligações α-1,6-glicosídicas (RABELO et al., 2009), sendo a maltose reconhecidamente o melhor aceptor para sua produção por via enzimática (DÍEZ-MUNICIO et al., 2012). Os IMO são também conhecidos pelo seu potencial para ativar o sistema imunológico e para melhorar o metabolismo lipídico, bem como as funções do fígado e dos rins (RABELO et al., 2012).

Por sua vez, os FOS ajudam a regular o colesterol, a suprimir a produção de toxinas, melhorando as taxas de potássio, cálcio e magnésio, regulando-as. Além disso, os FOS previnem diarreia, ajudando a manter o intestino saudável, ajudam na síntese de vitaminas e previnem ou até mesmo curam constipação, diarreia e/ou infecções por *Candida albicans* (SCHEER, 1997), estimulam o crescimento de bifidobactérias e diminuem os níveis de triacilglicerois no sangue (MATUSEK et al., 2008).

Yacon, uma planta andina, está atraindo a atenção mundial por suas características prebióticas e benefícios devido ao seu elevado teor de oligossacarídeos não digeríveis, tais como FOS e inulina. Seu efeito benéfico se deve à diminuição de infecções por patógenos intracelulares, diminuindo as alergias do trato digestório (DELGADO et al., 2013).

Os oligossacarídeos podem ser extraídos ou obtidos por hidrólise enzimática através de uma variedade de fontes de biomassa ou sintetizados a partir de açúcares simples por reação enzimática de transferência (RASTALL, 2010), além de poderem ser obtidos por métodos físicos e químicos (GIESE et al., 2011).

Muitas pesquisas vêm ocorrendo visando a produção por via enzimática desses açúcares na qual a enzima dextrana-sacarase (E.C. 2.4.1.5.), uma enzima extracelular, é produzida por via fermentativa utilizando a bactéria industrial ácido lática *Leuconostoc mesenteroides* NRRL B-512 F (COELHO et al., 2014; RABELO et al., 2006; YAMANER; SEZEN; TANRISEVEN, 2010). A síntese por via enzimática tem o intuito de agregar valor aos sucos de frutas como a síntese enzimática de oligossacarídeos em suco de caju (ALMEIDA et al., 2015; RABELO; FONTES; RODRIGUES, 2009), em suco de limão (COELHO et al., 2014) e em suco de acerola para produção de suco em pó utilizando leito de jorro (ARAÚJO et al., 2014). A sacarose é o único substrato capaz de induzir a produção dessa enzima pela espécie nativa (SANTOS; TEIXEIRA; RODRIGUES, 2000).

O mecanismo de reação do aceptor da enzima dextrana-sacarase ocorre de forma que parte dos grupos glicosil são desviados da cadeia de dextrana e transferidos por transglicosilação para a molécula receptora. Dependendo do tipo de aceptor e das condições de reação, pode-se formar pouca dextrana, havendo formação dos oligossacarídeos desejados, além de frutose (HONORATO et al., 2007; RABELO et al., 2006; RODRIGUES; LONA; FRANCO, 2005; VERGARA et al., 2010).

A dextrana-sacarase catalisa a síntese de dextrana a partir da sacarose e também transfere uma glicose vinda da sacarose para outros carboidratos (aceptores) principlamente por ligação α-1,6 – glicosil (reação de transglicosilação) (DÍEZ-MUNICIO et al., 2012). A panose, um IMO, pode ser sintetizada por via enzimática, utilizando a enzima dextrana-sacarase, tendo a maltose como aceptora (RABELO et al., 2006).

## 2.4 Suco de maçã

A maçã e o seu suco são amplamente conhecidos por serem uma importante fonte de polifenois, que têm grande potencial preventivo devido à sua habilidade de capturar espécies

reativas de oxigênio, responsáveis por causar estresse oxidativo no organismo com consequentes danos. As principais classes de polifenois encontradas na maçã são flavonoides, ésteres, procianidinas e dihidrochalconas (BELLION et al., 2008). Assim, são reconhecidas pela promoção de saúde devido às suas propriedades antioxidantes (PATIL et al., 2010a).

O consumo de maçã e/ou seu suco é benéfico à saúde humana pelo conteúdo de seus constituintes como açúcares, álcoois derivados de açúcares, aminoácidos e compostos fenólicos. São esses metabólitos primários e secundários que determinam sua qualidade. Além disso, o balanço entre os açúcares e os ácidos orgânicos é o responsável pelo sabor da fruta (ZHANG; LI; CHENG, 2010). Um estudo sobre a composição química de diferentes cultivares de maçã teve como resultado que a frutose é o açúcar mais abundante, seguido de glicose e sacarose, o ácido málico é o principal ácido orgânico, o perfil de ácidos graxos mais encontrados são C16:0, C18:0, C18:1, C18:2 e C18:3, os aminoácidos mais comuns sendo asparagina e serina, e principais compostos fenólicos o ácido protocatecóico e clorogênico. Maçãs têm compostos biologicamente ativos, como ácido ascórbico, compostos fenólicos, particularmente flavonoides (incluindo catequinas e proantocianidinas). Os ácidos graxos e aminoácidos têm papel importante sobre o atributo de aroma (WU et al., 2007). O consumo de suco de maçã por pessoas de todas as idades se deve aos seus atrativos sensoriais e nutritivos, sendo rico em antioxidantes, porém pobre em sódio e gordura (PATIL et al., 2010b).

## 2.5 Processos térmicos

Os sucos de frutas são normalmente tratados por pasteurização na indústria de alimentos. Apesar dos tratamentos térmicos convencionais serem amplamente adotados por aumentar a vida útil do produto, preservando o suco, muitos consumidores têm preferido alimentos mais nutritivos, naturais ou minimamente processados (TORRES et al., 2011).

Os processos térmicos são usados para inativar micro-organismos patógenos e enzimas indesejadas em sucos de frutas, no qual a temperatura chega a alcançar 80 °C e causando perdas sensoriais e nutricionais (SUROWSKY et al., 2013), como os compostos voláteis, responsáveis pelo aroma e sabor, e algumas vitaminas termo sensíveis (POLYDERA; STOFOROS; TAOUKIS, 2003). Por isso, o desenvolvimento de tecnologias não térmicas vem crescendo como alternativa de substituição dos processos térmicos (SUROWSKY et al., 2013).

Tradicionalmente, sucos de frutas são pasteurizados a temperaturas abaixo de 100 °C por segundos ou minutos (em torno de 73 °C por 15 segundos ou de 63 °C por 30 minutos). Geralmente o método que mais se aplica para sucos de maçã é a pasteurização curta (HTST) de 77 a 88 °C por 25-30 s (AGUILAR-ROSAS et al., 2007; DE PAEPE et al., 2014). Apesar do tempo curto de aquecimento, há perdas nutricionais, químicas e sensoriais, que são associadas ao processo térmico convencional de pasteurização (AGUILAR-ROSAS et al., 2007; PATIL et al., 2010b). Sucos prebióticos contendo oligossacarídeos sofrem perdas devido às altas temperaturas quando expostas por longos períodos. Uma pesquisa avaliou a aplicação de diferentes temperaturas (60, 70, 80, 90 e 100 °C) e pH baixos (2,7; 3,0 e 3,3) por até duas horas em soluções contendo 100g/L de fruto-oligossacarídeos (considerados prebióticos) e encontrou que, nessas condições, houve despolimerização desses açúcares. Isso provoca a perda dos benefícios funcionais, inclusive, a ponto do produto não poder ser vendido com alegações funcionais (MATUSEK et al., 2008).

Os efeitos negativos dos tratamentos térmicos incluem o escurecimento não-enzimático, a perda de nutrientes e a formação de produtos tóxicos indesejáveis como o 5-hidroximetilfurfural (5-HMF) (DAMASCENO et al., 2008).

## 2.6 Tecnologias não térmicas emergentes

Além das técnicas usuais de processamento já existentes que não fazem uso do calor, há também processamentos emergentes por meio de campo elétrico pulsante, alta pressão hidrostática, luz pulsante, ultrassom, irradiação, ozônio e plasma, sendo que a última ainda está em fase de testes para o uso em alimentos (MISRA et al., 2014; NIEMIRA, 2012; SUÁREZ-JACOBO et al., 2011).

O plasma como uma tecnologia não térmica (NTP) tem se tornado uma das mais emergentes e, recentemente, tem despertado interesse no uso para descontaminação de alimentos e superfícies de áreas de processamento de alimentos (PANKAJ et al., 2013) (PANKAJ; MISRA; CULLEN, 2013). É gerado pela aplicação de energia na forma de calor, voltagem ou campo eletromagnético de um gás, levando a reações como ionização, excitação e dissociação (SUROWSKY et al., 2013). A geração de NTP (temperatura de 30-60°C) a pressão atmosférica é de interesse para a indústria de alimentos porque não requer condições extremas. NTP pode ser gerado por descarga elétrica em um gás a baixa pressão ou usando micro-ondas. No primeiro caso, à pressão atmosférica, o plasma pode ser gerado por descarga de corona, descarga de barreira dielétrica (DBD), plasma de rádiofrequência e plasma de arco deslizante (Misra et al., 2011).

DBD é um dos métodos que oferece versatilidade no seu modo de operação e configuração de sistema. Pelo DBD, o plasma é gerado entre dois eletrodos, separados por uma ou mais barreiras dielétricas. Essa camada dielétrica tem um importante papel, limitando a carga aplicada, proporcionando um tratamento homogêneo (PANKAJ et al., 2013). DBD é usado por ser um dos métodos mais eficientes para produzir ozônio (MISRA et al., 2015).

O plasma é um gás ionizado que tem partículas reativas como elétrons, íons, radicais livres e átomos que estão no estado excitado, as quais emitem fótons, incluindo fótons de UV (TAPPI et al., 2014). Espécies reativas de oxigênio (como átomos de oxigênio e radicais

hidroxila) formadas pela aplicação de plasma têm um papel importante, pois atacam partes da membrana celular e começam a causar reações oxidativas, como é o caso dos lipídios insaturados transformados em peróxidos (SUROWSKY et al., 2013). Há três mecanismos primários da inativação microbiana por plasma. O primeiro é a interação química entre radicais, espécies reativas ou partículas carregadas e a membrana celular. O segundo é pelo dano à membrana celular e seus componentes internos pela radiação UV. E o terceiro é quando atingem as fitas de DNA. Essas sofrem clivagem pelo UV gerado durante a recombinação de espécies de plasma (NIEMIRA, 2012).

O uso de plasma como tecnologia não térmica já é aplicado em indústrias para tratamentos de superfícies (BONANDINI et al., 2010; HUR et al., 2014), incluindo biomassa e também para despoluição do ar (DURME et al., 2008). Pesquisas têm sido feitas para avaliar seu uso em outras aplicações, como no uso de plasma para a despolimerização de celulose em substituição à tecnologia usual, sendo a última menos vantajosa segundo o estudo (BENOIT et al., 2011). Uma erva chamada alface-de-cordeiro sofreu tratamento por plasma e, após 120 segundos de tratamento, mostrou-se um aumento no conteúdo de compostos fenólicos, independente da voltagem aplicada (GRZEGORZEWSKI et al., 2010).

Um estudo mostrou a eficiência do plasma por DBD no qual o plasma promoveu uma redução de 7 log UFC/ml de *Escherichia coli* patogênica após 20 segundos de exposição direta e 45 segundos de indireta (ZIUZINA et al., 2013), mostrando ser um método eficiente de esterilização. Para ser considerado um método esterilizante a redução necessária para micro-organismos patógenos (em geral se usa a *E. coli*) é de 5 log UFC/mL (CULLEN et al., 2010). As espécies formadas pelo NTP contribuem para a ação letal pela interação com material biológico. O oxigênio do plasma pode ser um eficiente bactericida. Os micro-organismos são expostos a um intenso bombardeamento de radicais, provocando lesões na superfície das células, de forma que elas não conseguem se recuperar a um tempo

suficientemente rápido. Outro parâmetro importante é o tipo de exposição: direta ou indireta. Por exposição indireta, o calor transmitido para a amostra é reduzido e as partículas carregadas não desempenham seu papel desde que se recombinem antes de atingir a amostra, além de muitas espécies neutras reativas de meia-vida curta também não atingem a amostra (MISRA et al., 2011).

Um estudo sobre os custos operacionais demonstrou um consumo de 0,017 – 0,051 kWh para um sistema de processamento por plasma em escala de laboratório suficiente para diminuir a população de patógenos. O consumo de energia desses sistemas experimentais em escala de laboratório varia de acordo com o tamanho do emissor, variando de 15 W a 900W (NIEMEIRA; SITES, 2008). Porém, a tecnologia vai em direção a uma escala comercial e, evidentemente, os custos aumentarão. Os custos também dependerão da finalidade do uso do plasma e a forma como ele será obtido. Os custos fixos serão fontes de alimentação, câmaras de tratamento, controles eletrônicos, tipo de gás utilizado, etc. Os custos recorrentes vão depender de qual tecnologia utilizada para a obtenção do plasma e irão resultar do uso e desgaste dos selos, transportadores, eletrodos, monitoramento de sondas, termopares, etc (NIEMIRA, 2012).

Já o ozônio é reconhecidamente seguro (*Generally Recognized as Safe* – GRAS) e seu efeito bactericida é devido ao seu alto poder de oxidação e à sua rápida difusão pelas membranas biológicas (STEENSTRUP; FLOROS, 2004). O ozônio é um poderoso agente, de largo espectro antimicrobiano, ativo contra fungos, vírus, protozoários, bactérias e esporos fúngicos, devido ao seu alto potencial de oxidação (CULLEN et al., 2009; PATIL et al., 2010a, 2010b), apesar das bactérias serem mais sensíveis (CULLEN et al., 2010). O ozônio como oxidante é usado em tratamento de água natural, lavagem e desinfecção de frutas e legumes, e processamento de suco para inativar microrganismos patogênicos e deteriorantes (CULLEN et al., 2009; PATIL et al., 2010a). É um efetivo sanitizante com amplo espectro de

ação. Além disso, decompõe-se rapidamente, não deixando resíduos tóxicos. Juntamente com seu alto poder de oxidação torna-se atrativo para uso industrial (TIWARI et al., 2009).

Em 2001 o *Food and Drug Administration* (FDA), órgão governamental dos Estados Unidos da América (EUA) responsável pelo controle dos alimentos, aprovou o uso de ozônio como aditivo direto para alimentos, desencadeando grande interesse na sua aplicação em processos na indústria de alimentos. Alguns processadores de sucos de frutas comerciais nos EUA e na Europa começaram então a empregar ozônio em substituição à pasteurização, resultando em um guia industrial emitido pelo FDA (PATIL et al., 2010a; TIWARI et al., 2009). Reduções da ordem de 5 log UFC/ml de patógenos resistentes pela aplicação de ozônio foram confirmadas (PATIL et al., 2010b).

A inativação bacteriana foi efetivamente comprovada para suco de maçã, no qual foi aplicado um tratamento de 5 minutos a um taxa de fluxo de 0,12 L/min e uma concentração de ozônio de 0,048 mg de ozônio/mL (PATIL et al., 2010a). Além disso, o ozônio é considerado uma tecnologia “limpa”, devido a sua rápida decomposição em oxigênio molecular, não deixando resíduo para o meio ambiente (PATIL et al., 2009, 2011), além de ser um agente antimicrobiano seguro. Sua decomposição varia de acordo com temperatura, pH e matéria orgânica, enquanto suas propriedades orgânicas são atribuídas à oxidação progressiva de componentes vitais da célula (PATIL et al., 2011).

O processamento por alta pressão (HPP) é um outro método de conservação não convencional que vem ganhando espaço em substituição aos métodos tradicionais. HPP é um método eficiente para inativação enzimática e redução da carga microbiana em alimentos. O processo utiliza água como uma forma de elevar a pressão de 300 a 700 MPa (KEENAN et al., 2010; POLYDERA; STOFOROS; TAOUKIS, 2003). Uma das vantagens é o aumento do tempo de prateleira dos produtos sem a necessidade de submeter o alimento a temperaturas elevadas, e, consequentemente, preservando as suas características nutricionais,

principalmente em produtos de frutas, que têm alta quantidade de antioxidantes sensíveis ao calor (KEENAN et al., 2010). Um estudo que avaliou parâmetros sensoriais e de qualidade como compostos voláteis, pigmentos e vitaminas mostrou que o processamento não causou alterações (PATRAS et al., 2009).

Tendo em vista a crescente pesquisa no uso de tecnologias não térmicas para o processamento de alimentos, neste trabalho é estudado o efeito do plasma dielétrico, do ozônio e do tratamento de alta pressão em suco de maçã.

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# *Capítulo 1*

# Comparison between plasma and high pressure as non-thermal technologies for apple juice containing fructo-oligosaccharides

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**Abstract**

Prebiotic oligosaccharides have been included in the human diet as an alternative to improve health and well-being. Atmospheric Cold Plasma (ACP) and High-Pressure Processing (HPP) have been successfully applied for food preservation. This work aimed to study the effects of ACP and HPP on the quality and oligosaccharides stability of a prebiotic apple juice. The ACP treatment was applied with direct and indirect plasma exposure at different times (15, 30, 45 and 60 s). Also, the organic acids (citric and malic acids) were evaluated. The characteristic colour was kept with an increase in chroma values indicating that the juice became more vivid after the treatments. Oligosaccharides suffered depolymerization due to the both treatments (ACP and HPP), and the organic acids were preserved in the samples. Thus the final product remained a prebiotic juice due to the high FOS content (about 7 % w/v). The kind of exposure had slight differences in the parameters analysed after ACP. Better results were found for HPP.

Keywords: Prebiotic apple juice , Atmospheric Cold Plasma, High-Pressure Processing.

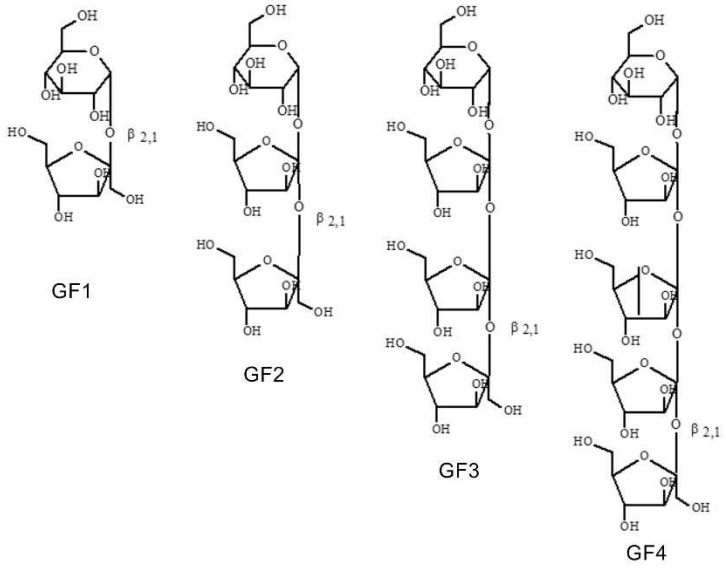
## 1. Introduction

Alternatives for partial or total substitution of milk and dairy products by other products are of industrial interest (Ranadheera et al., 2012). One of the alternatives is the consumption of innovative non-dairy products that provide health benefits such as carbohydrates like fructo-oligosaccharides (FOS) (Saad et al., 2013) or juices containing FOS (Koh et al., 2010). The fortification of fruit juices with prebiotic FOS confers functional characteristics to the product (Renuka et al., 2009).

Prebiotics are non-digestible carbohydrates that provide additional healthy benefits improving gut functionality and stimulating the growth and bioactivity of beneficial bacteria (Rabelo et al., 2009; Vergara et al., 2010). Oligosaccharides with a degree of polymerization from 2 to 10 are usually classified as functional carbohydrates (Rabelo et al., 2009). Among the known prebiotic oligosaccharides, the fructo-oligosaccharides are the most widely used and studied prebiotic carbohydrate. Consumption of FOS is associated with some positive health effects such as the enhanced growth of probiotic bacteria in the colon, which is associated with short-fatty acid production (Hess et al., 2011).

FOS are composed of a glucose unit linked to fructose moieties by a β-2,1 glycosidic bond. Kestose (GF2) is the oligosaccharide with the lower degree of polymerization (DP3) followed by nystose (GF3) and frutofuranosylnystose (GF4) and FOS with a higher degree of polymerization (> DP4). The FOS structure is shown in Figure 1.

Fig 1 – Disaccharide and fructo-oligosaccharides chemical structures. GF1 – Sucrose, GF2 – kestose, GF3 – nystose, GF4 – frutofuranosyl-nystose.




Although thermal processing is widely employed for juice preservation, there are concerns over quality losses. Lately, the interest in non-thermal technologies such as ionizing radiation, pulsed light, pulsed electric fields, supercritical gas pasteurization, high pressure processing (HPP), pulsed electric field (PEF), UV radiations, ozone and cold plasma has increased (Caminiti et al., 2011; Misra et al., 2014b; Patil et al., 2010; Suárez-Jacobo et al., 2011; Torres et al., 2011b).

Among these technologies, cold plasma has been successfully applied for pathogen inactivation in foods (Ziuzina et al., 2014). According to Niemira, (2012), microbial inactivation by cold plasma is due to the UV radiation generated during plasma recombination and the chemical interactions of ions, photons, atoms and free electrons formed during the plasma application (a neutral charge is obtained in the end). One novel approach to treating food with plasma is to generate it within in a sealed package or container (Misra *et al*., 2011). The non-thermal atmospheric plasma is generated by a dielectric barrier discharge (DBD) ensuring a homogeneous process (Pankaj et al., 2013).

High-pressure processing (HPP), also described as high hydrostatic pressure (HHP), or ultra high pressure (UHP) processing, subjects liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa resulting in a reduction in microbial numbers and enzyme activity (Torres et al., 2011a). The HPP offers many advantages over conventional techniques due to the instantaneous transmission of isostatic pressure to the product, independent of size, shape and food composition (Patterson et al., 1996).

Apple juice (AJ) is widely consumed throughout the world due to its associated nutritional properties and protective effects against some diseases. In this study, the effect of plasma and high pressure on apple juice containing FOS was evaluated. The addition of FOS to this juice enhances its functionality due to the prebiotic characteristics of these oligosaccharides. The efficacy of plasma for microbial inactivation in foods has been previously demonstrated (Baier et al., 2013; Misra et al., 2011; Ziuzina et al., 2014, 2013). However, when an emerging technology is applied to a functional product such as apple juice containing FOS, it is necessary to ensure that the final product will retain the functional characteristics post treatment (Matusek et al., 2008).

Benoit et al. (2011) reported the depolymerisation of cellulose, amide and inulin by non-thermal atmospheric plasma. Inulin is a polymer with terminal glucose unit and fructose units linked by β-1,2 bonds. Inulin is, in fact, the raw material for FOS production by enzyme or chemical hydrolysis. To propose an alternative route for the synthesis of FOS, inulin (from chicory) was subjected to atmospheric plasma treatment (11 kV, 2 kHz). Under these conditions, the authors reported that inulin (DP = 46) was selectively converted into FOS within 7 min of plasma treatment along with the production of fructose in 16 wt % yield. These results indicate that the FOS linkage can be broken by plasma reducing the degree of polymerization. Ma et al., (2012) studied the chitosan plasma depolymerisation. Chitosan is a glucosamine polymer linked by β - 1,4 glycosidic bond. The chitosan depolymerisation was

found to be a function of the processing time and the molecular weight was reduced by 50% after 30 min of treatment. Although the plasma depolymerization mechanism was not elucidated, it is clear that plasma can break the beta glycosidic linkage in carbohydrates promoting their depolymerization.

The main interest in the present study was to assure the preservation of the prebiotic characteristic of the apple juice, which means that after the treatment, FOS must be still present in the juice. The aim of this research was to study the effects of atmospheric cold plasma (ACP) treatment and high-pressure processing on the colour, sugars and organic acids concentration of prebiotic apple juice.

## 2. Material and Methods

### *2.1 Juice samples*

Apple juice (Squeez©, Fruit Juices Ltd, Ireland) was purchased from a local supermarket (Dunnes, Dublin-Ireland) and maintained at 4 °C before use. Fructo-oligosaccharides (FOS) (ORAFTI© P95, Beneo GmbH, Mann, Germany) composed of kestose, nystose and fructofuranosyl-nystose was added to the juice at a concentration of 70 g/L according to Gobinath et al. (2010).

### *2.2 Non-thermal treatment*

The prebiotic juices were treated by two non-thermal processing: atmospheric cold plasma (ACP) and high pressure (HPP). Atmospheric Cold Plasma was generated within a polypropylene container sealed container containing the juice samples (20 mL) with processing times of 15, 30, 45 and 60 s. These treatment times were selected based on previously determined times that would achieve a useful microbial inactivation (Ziuzina et al., 2013). The applied voltage was 70 kV and treatment was carried out at ambient air and

atmospheric pressure conditions. The plasma was generated by a Dielectric Barrier Discharge (DBD) system with the experimental setup following Ziuzina et al., (2013), comprising of two circular aluminum plate electrodes (outer diameter = 158 mm) over polypropylene (PP) dielectric layers (2 mm thickness). This experimental set up facilitates two modes of plasma exposure; direct exposure where the samples were placed inside the plasma field between the electrodes and indirect exposure, where the samples were placed outside the plasma field and only exposed to the plasma afterglow. Samples were treated in sealed PP packages. The PP dielectric boxes were sealed within a polymeric film (Cryovac BB3050, 50 µm of thickness, Sealed Air Corporation, USA). The samples were stored for 24h post-treatment at room temperature (20 °C). The researchers achieved the enough time of exposition: 20 s and 45 s for direct and indirect exposure, respectively.

For the HPP treatment, high pressure industrial equipment (Hiperbaric, model 300) was used to process the juice. The juice samples containing FOS were packed in 250 mL polyethylene bottles, which were placed in polyethylene plastic bags, and vacuum sealed. A control sample, containing the same concentration of FOS dissolved in water was prepared and packed in the same way the samples. The process was subjected to a pressure of 450 MPa for 5 min at HPP Tolling Ltd (Dublin, Ireland) followed by analyses.

### *2.3 Analyses*

#### *2.3.1 Colour*

Juice colour was measured using an L*–a*–b* colorimeter (ColourQuest XE Hunter Lab, Northants, UK). The calibration was performed using white (L* = 93.97, a* = 0.88 and b* = 1.21) and black standard tiles. The L* parameter (lightness index scale) ranges from 0 (black) to 100 (white). The parameter a* measures the degree of red (+a) or green (-a*) colour and the b* parameter measures the degree of yellow (+b) or blue (-b*) colour. Thereby it was

possible calculate chroma (Eq. 1), total colour difference (TCD) (Eq. 2) that expresses the magnitude of colour change after the treatment and Hue angle (0 – 360°) (Eq. 3) that indicates the dominant colour were calculated by the given equation.

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$\text{TCD} = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (2)$$

$$\text{Hue} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

Where $L_0$, $a_0$ and $b_0$ are the colour values of untreated juice.

*2.3.2 Sugar quantification*

Thin Layer Chromatography (TLC) analysis was used to quantify the FOS. The prebiotic apple juice samples were diluted (1:2) cleaned in a C-18 SPE cartridge and filtered in a 0.45 µm cellulose acetated membrane. The samples were analysed by TLC, using a silica gel on TLC plates SIGMA-ALDRICH (20x20cm, 60 Å medium pore diameter; product number: 99570-25EA). Samples of 1 µL were applied on the plate at 1.0 cm from the edge with 1.0 cm of separation from each other. The plates were disposed into the TLC chamber pre-conditioned at room temperature. The solvent system used to separate the carbohydrate mixture was a n-butanol/2-propanol/$H_2O$ (10:5:4 [vol/vol/vol]) mixture (Shiomi et al., 1997). The TLC plate was irrigated by the solvent system three times. To visualize the separated carbohydrates in the plates, a fine spray of butanol/water (80% w/w) as solvent, phosphoric acid (6.78 mL), urea (3 g) and ethanol (8 mL) in 100 mL was used. After that, the plates were oven heated at 120 °C for 10 min. For the quantification of the oligosaccharides a TLC scanner CAMAG 4 20x20 cm densitometer was used, using the Planar winCATS Chromatografy Manager software. The wavelength used was 450 nm. Analyses of the assays were performed in triplicate.

*2.3.3 Organic acids quantification*

Organic acids were quantified by High Performance Liquid Chromatographic (HPLC). For quantification of citric acid (retention time at 8.0 min) and malic acid (retention time at 9.6 min) in prebiotic apple juice after ACP and HPP treatments, a calibration curve with a mixture of these acids (0.1 – 1.0 g/L and 0.2 – 2.0 g/L, respectively) was used.

The samples were diluted in water (1:4), filtered, using glass fiber prefilters AP25 13 mm diameter (Merck Millipore Ltd.) and cleaned using a C-18 SPE cartridge, followed by filtration, using a syringe (2.0 mL) and a support comprising a membrane (HA membrane cellulose ester 0,45 uM, 13 mm in diameter). The system (Agilent Technologies 1260 Infinity) was equipped with a pump system and a UV-DAD detector monitored at 210 nm. Organic acids were separated in an Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad) termostated at 50 °C. The mobile phase was sulphuric acid (0.01 M) in deionized water at 0.6 mL/min for 30 minutes. The assays were performed in triplicate.

*2.3.4 Statistical analysis*

Analysis of ANOVA and Tukey test at a 95% of confidence level was applied using the statistical software Statistica 13.0 (Statsoft).

## 3. Results and discussion

*3.1 Colour parameters*

According to the results presented in Table 1, there was a statistically significant effect ($p<0.05$) on the L* parameters (luminosity) after both ACP and HPP treatment when compared with the control. Comparing the plasma treated samples with the control, the L* value decreased slightly up to 30 s of treatment and increased further with plasma treatments of 45 and 60 s for both direct and indirect modes of exposure (Tables 1 and 2).

Table 1 - Colour parameters including total colour difference (TCD) for prebiotic apple juices after atmospheric cold plasma in different times of direct exposure.

| Time (s) | L* | Chroma | h° | TCD |
|---|---|---|---|---|
| 0 (control) | 70.83 ± 0,01$^{c}$ | 32.82 ± 0.02$^{e}$ | 91.91 ± 0.02$^{a}$ | 0 |
| 15 | 70.32 ± 0,00$^{e}$ | 39.50 ± 0.01$^{b}$ | 91.14 ± 0.00$^{b}$ | 6.72 ± 0,01$^{b}$ |
| 30 | 70.50 ± 0,00$^{d}$ | 39.92 ± 0.01$^{a}$ | 90.75 ± 0.01$^{c}$ | 7.15 ± 0,01$^{a}$ |
| 45 | 71.05 ± 0,01$^{b}$ | 39.25 ± 0.01$^{c}$ | 90.12 ± 0.01$^{d}$ | 6.52 ± 0,01$^{c}$ |
| 60 | 71.25 ± 0,01$^{a}$ | 39.02 ± 0.02$^{d}$ | 90.01 ± 0.00$^{e}$ | 6.33 ± 0,02$^{d}$ |

Means with the same letters in the same column are not statistically different according to the Tukey's test ($p<0.05$).

Table 2 - Colour parameters including total colour difference (TCD) for prebiotic apple juices after atmospheric cold plasma in different times of indirect exposure.

| Time (s) | L* | Chroma | h° | TCD |
|---|---|---|---|---|
| 0 (control) | 70.83 ± 0,01$^{c}$ | 32.82 ± 0.02$^{d}$ | 91.91 ± 0.02$^{a}$ | 0 |
| 15 | 70.22 ± 0,00$^{e}$ | 40.00 ± 0.01$^{a}$ | 91.00 ± 0.01$^{b}$ | 7.21 ± 0.01$^{a}$ |
| 30 | 70.44 ± 0,01$^{d}$ | 39.99 ± 0.01$^{a}$ | 90.48 ± 0.00$^{c}$ | 7.23 ± 0.01$^{a}$ |
| 45 | 70.92 ± 0,00$^{b}$ | 38.62 ± 0.02$^{b}$ | 89.98 ± 0.01$^{e}$ | 5.93 ± 0.02$^{b}$ |
| 60 | 71.10 ± 0,00$^{a}$ | 38.46 ± 0.01$^{c}$ | 90.13 ± 0.01$^{d}$ | 5.81 ± 0.01$^{c}$ |

Means with the same letters in the same column are not statistically different according to the Tukey's test ($p<0.05$).

Table 3 shows a slight statistic difference between the sample treated by HPP and the control.

Table 3 - Colour parameters for high pressure processing (HPP) on apple juice and its prebiotic.

| Samples | L* | Chroma | h° | TCD |
|---|---|---|---|---|
| Apple juice (control) | $70.49 \pm 0.01^{a}$ | $14.97 \pm 0.01^{a}$ | $92.68 \pm 0.07^{a}$ | 0.0 |
| Prebiotic apple juice | $71.18 \pm 0.38^{b}$ | $16.74 \pm 0.19^{b}$ | $92.57 \pm 0.14^{a}$ | $1.25 \pm 0.16$ |

Means with the same letters in the same column are not statistically different according to the Tukey's test ($p<0.05$).

Almost all samples were statistically different at 95% of significance compared to the non-treated samples (control), except for hue angle. In fact, according to the L* parameter the changes in L* were irrelevant taking into account these parameter scale. L* changes were less than 2% with L* values ranging from 0 to 100. Strawberries treated plasma also presented differences in L colour values (Misra et al., 2014a). The chroma value indicates the colour intensity or saturation while the Hue angle (h°) is the characteristic colour of the sample represented by its characteristic angle in the colour wheel. The angle 0° represents pure red, 90° pure yellow, 180° pure green and pure blue 270°. The results showed an increase in the chroma values when compared with the control in both direct and indirect exposure of plasma with statistical difference ($p<0.05$). The Hue angle of the prebiotic apple juice samples treated by plasma is located in the yellow range (from 89.98 ° to 91.91 °). Thus, the HPP treated samples also remained in the yellow range. An increase in chroma means that the colour is more vivid while a decrease indicates that the colour is more pale. The chroma value increased along the plasma treatment time from 32.82 to 40.00.

Despite the statistically significant differences obtained by the instrumental measures, it is important to note that the characteristic colour of the product was not lost due to the plasma neither high pressure treatment with juices more vivid and great luminosity.

Table 3 showed no statistical difference in hue angle indicating that the characteristic colour was preserved. A small significant difference ($p<0.05$) in L* parameter indicates that

the juice became more luminous. The increase in the chroma value indicates more intense colour. Despite the statistical significant difference, the both ACP and HPP had no deleterious effect on the juice colour. Aside preserving the characteristic colour, the treated juice became more vivid.

For plasma, according to the total colour difference (TCD) obtained (Tables 1 and 2), it was observed that the lowest colour difference was obtained using 60 s of both plasma exposure. Specifically, the lowest TCD obtained was 5.81 ± 0.01 for the sample treated for 60 s using indirect exposure. This value is higher than the threshold considered for the colour difference percieved by the human eye (TCD > 3). However, a perceptive colour difference does not mean that the product would be rejected and/or the colour after the treatment is worst than the non treated sample (Tiwari et al., 2009).

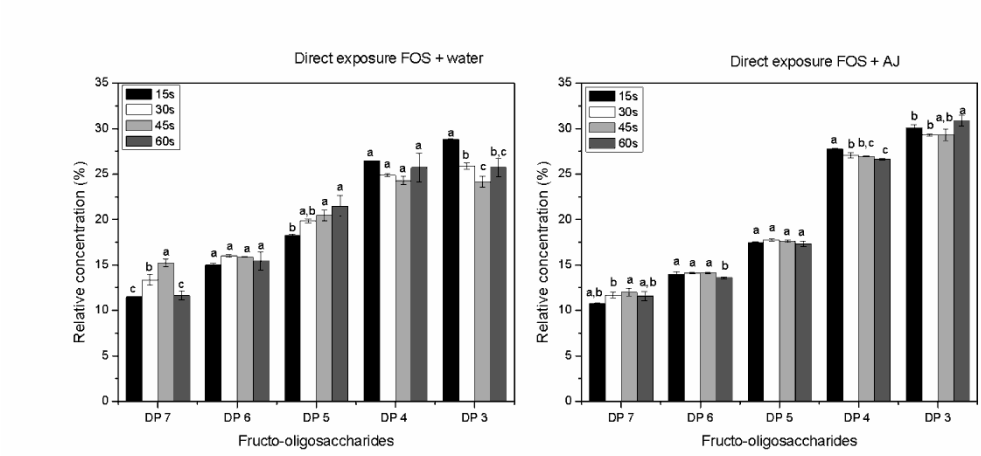
Interestingly, the process was found to have a much lower effect on the overall colour parameters than found with similar treatment processes such as ozone wherein TCD value was 30.84 at the highest processing conditions employed i.e. at ozone concentration of 4.8% w/w and processing time of 10 min (Torres et al., 2011b).

About TCD of samples treated by HPP, there was no difference when compared with the control, taking into account that differences in perceivable colour can be analytically classified as small differences when TCD < 1.5.

### *3.2 ACP treatment effect on apple juice carbohydrates*

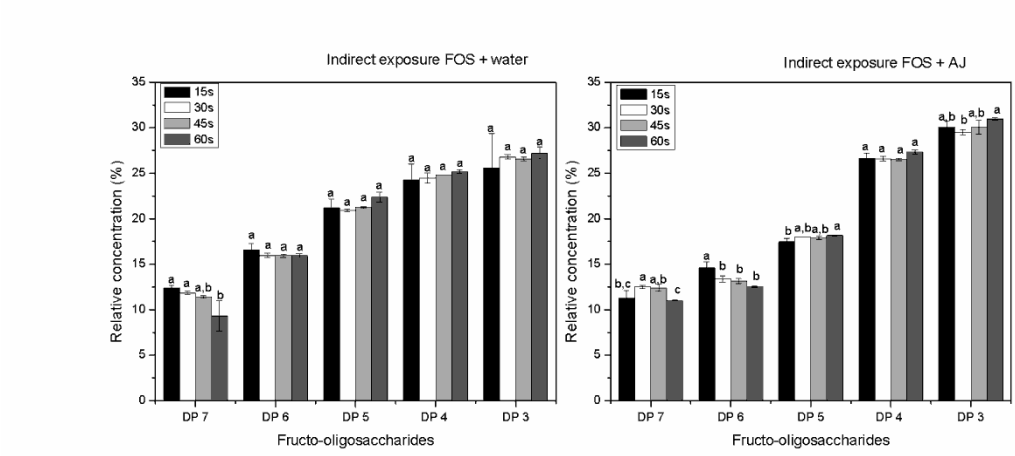
After plasma treatment, all carbohydrates according theirs degree of polymerization (DP 3 up to DP 7) were visualized on the TLC plate (data not shown). The oligosaccharides were then quantified by densitometer. The results were expressed as the relative percentages of the concentration obtained on densitometer and are shown in Figures 2 (direct exposure) and 3 (indirect exposure).

Fig 2 – Comparison of relative concentration (%) of samples containing fructo-oligosaccharides (FOS) solubilized in water or apple juice (AJ) and their different degrees of polymerization (DP) after plasma direct exposure.




Means with the same letters in the same DP are not statistically different according to the Tukey's test ($p<0.05$).

Fig 3 - Comparison of relative concentration (%) of samples containing fructo-oligosaccharides (FOS) solubilized in water or apple juice (AJ) and their different degrees of polymerization (DP) after plasma indirect exposure.




Means with the same letters in the same DP are not statistically different according to the Tukey's test ($p<0.05$).

For direct exposure it could be noticed a difference in the relative concentration comparing FOS in water and in apple juice. The higher amount of DP 3 oligosaccharides in apple juice could be due to depolymerisation of these sugars, which explains the decrease in

DP 7, DP 6 and DP 5. In the Fig. 2 and 3 it can be observed a similar behaviour for the two kinds of exposure along the treatment time, except for the samples of FOS and water, with not differences, in general. However statistically differences were smaller for indirect exposure. This kind of exposure can be less aggressive promoting a production of less reactive species on the sample as it is not directly under the plasma field. Small losses in oligosaccharides were found in other similar study (Almeida et al., 2015). In general, it can be noticed small differences in samples treated for 15 until 45 s. Plasma application for 60 s is the most aggressive condition. However, a previously study demonstrated that a complete bacterial inactivation was achieved after 20 s of direct exposure and 45 s of indirect plasma treatment (Ziuzina et al., 2013). Thus, for the pathogens inactivation, 45 s under indirect exposure is enough. The mode of plasma exposure influenced in small proportion the prebiotic relative concentration.

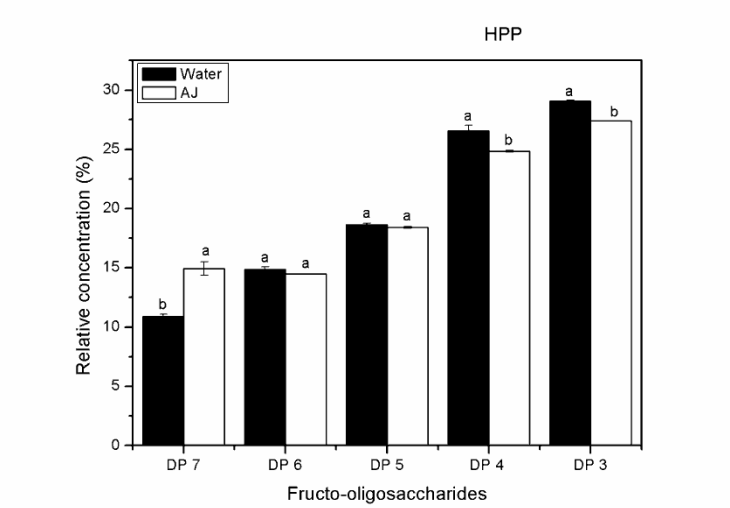
Following some researches, in the present study the FOS was degraded by the treatment, however in a small quantity due to the short time applied, different of the other studies. Therefore, the short time of plasma treatment did not cause enough losses and ensure a prebiotic juice. The short treatment times selected for this study based in the time needed for microbial inactivation resulted in the preservation of the oligosaccharide in the apple juice. After 60 s of plasma treatment, the FOS content did not present great differences, i.e., the juice maintained its prebiotic properties since non substantial FOS degradation was observed.

Another research reported that indirect ACP exposure had higher loss of oligossacharides (Almeida et al., 2015). In the present study, this kind of exposure was more tenuous. It is difficult compare our results on plasma treatment with other groups because only one paper, published by our group is available.

*3.3 HPP treatment effect on apple juice carbohydrates*

After HPP, the FOS content was the same in apple juice, compared to the water for DP 6 e 5 (Figure 4). High levels of FOS remained in the juices after this non-thermal treatment, i.e., it is possible use HPP as technology and to maintain prebiotic functionality in apple juice.

Fig 4 – Comparison of relative concentration (%) of samples containing fructo-oligosaccharides (FOS) solubilized in water or apple juice (AJ) and their different degrees of polymerization (DP) after high pressure processing (HPP).



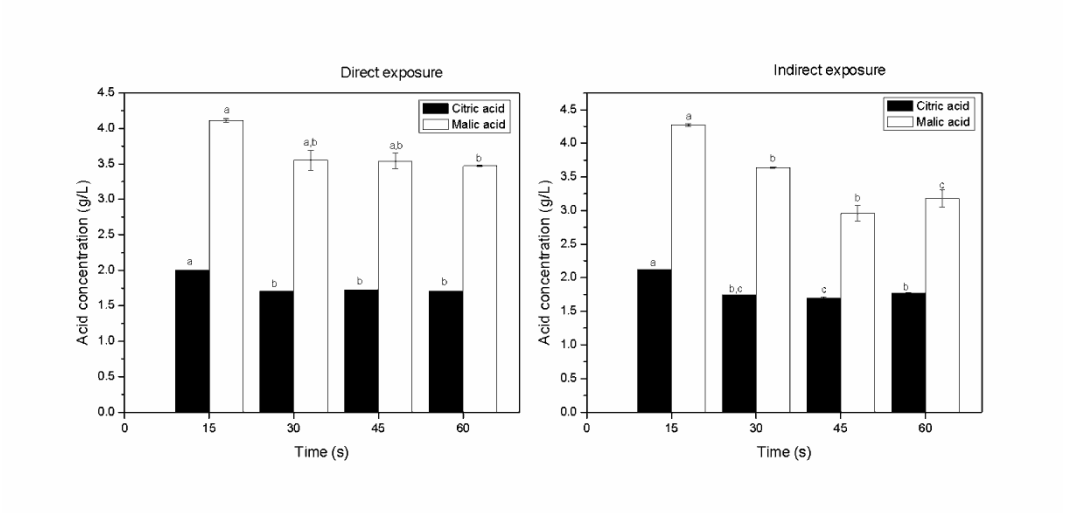

Means with the same letters in the same DP are not statistically different according to the Tukey's test ($p<0.05$).

A study evaluated apple purees enriched with prebiotic inclusions treated by HPP and found similar results about the oligosaccharides quantity where inulin and FOS were stable throughout 30 days and were present in sufficient quantities to deliver a prebiotic effect (Keenan et al., 2011).

*3.4 Organic acids*

Figure 5 shows decrease in the concentration of malic acid (from 4.11 ± 0.03 g/L to 3.47 ± 0.01 g/L) and citric acid (from 2.00 ± 0.03 g/L to 1.71 ± 0.02 g/L). For indirect exposure is noticed similar behaviour. Significant decreases occurred in the analyses of these acids at different times and forms of plasma exposure.

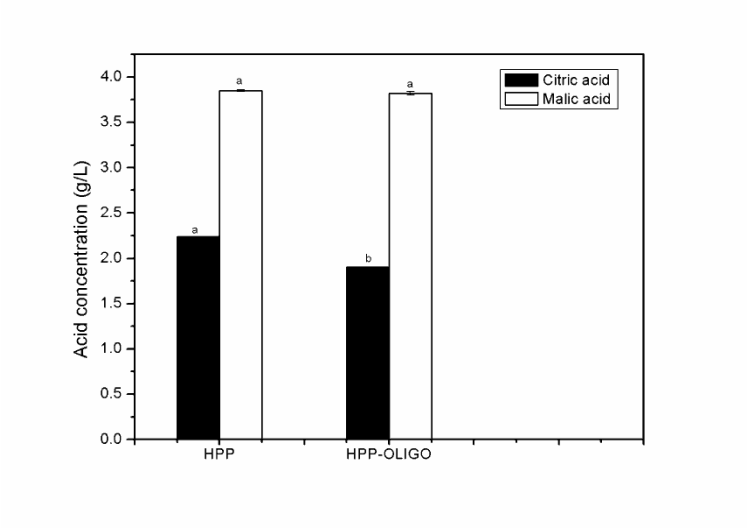
Fig 5 – Concentration of organic acids on prebiotic apple juices after plasma direct and indirect exposure in different treatment times.




Means with the same letters for the same acid are not statistically different according to the Tukey's test ($p<0.05$).

After HPP application no significant differences were found for malic acid. It was possible to observe a slight decrease in the concentration of citric acid (Fig. 6).

Fig 6 – Concentrations of organic acids on prebiotic apple juices after high pressure processing (HPP) in different treatments (HPP: apple juice treated by HPP; HPP-OLIGO: apple juice containing FOS treated by HPP).




Means with the same letters for the same acid are not statistically different according to the Tukey's test ($p<0.05$).

Analyses conducted after HPP show malic acid concentration remained statistically the same. For citric acid occurred a decrease of their concentration. A study made by Igual, García-Martínez, Camacho, & Martínez-Navarrete, (2010) have shown that thermal techniques, such as conventional pasteurisation, degraded organic acids on grapefruit juice.

## 4. Conclusion

Prebiotic apple juice submitted to two non-thermal treatments suffered losses in relation to acid and oligosaccharides concentrations. However, the prebiotic proprieties, were kept because the overall losses were less than 5%. About the colour, the treatments also improved their parameters making the juices more vivid. The results showed that taking into account the FOS integrity, HPP and ACP can be applied to prebiotic apple juice. Moreover, the TCD was below the threshold for the human perception (TCD < 3).

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# *Capítulo 2*

# Effects of Ozone and Atmospheric Cold Plasma on prebiotic apple juice

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**Abstract**

Due to the increase in functional foods consumption and the increase of research in non-thermal technology for food processing, this work evaluated the effects of atmospheric cold plasma (ACP) and ozone on prebiotic apple juice. The functional juice was obtained through enzyme synthesis using the juice sugars ~~were,~~ partially polymerized into gluco-oligosaccharides by the dextransucrase acceptor reaction. The samples were assayed regarding total oligosaccharides, simple sugar concentration and organic acids by HPLC and total phenolic content (TPC) by Folin-Ciocalteau. The antioxidant activity was determined by the ABTS method. ACP and ozone treatment were able to preserve the functional prebiotic characteristics maintaining the amount of the total oligosaccharides in a level enough to attest the juice functionality, despite some depolymerisation was observed. Ozone preserved better the oligosaccharides compared to plasma. Besides the TPC showed slight changes after plasma indirect exposure (from 0.73 ± 0.10 up to 0.53 ± 0.02). Antioxidants content increased after plasma treatment.

**Keywords:** Non-thermal treatment, prebiotic juices, dextransucrase acceptor reaction, gluco-oligosaccharides.

## 1 Introduction

Healthy food and juice consumption has increased around the world due to the consumers concern on the use of food as a way to improve the well-being. Regarding the consumers seek for practical and healthy foods, ready to drink fruit juices with prebiotics oligosaccharides might be a good option to ingest health and beneficial foods. The consumption of prebiotics has raised in high amounts affirming an increase on the demand for this kind of product (Sloan, 2014). A way to make fruit juices more attractive is to add functional properties to them. Fructo-oligosaccharides, inulin, galacto-oligosaccharides and other similar carbohydrates are classified as functional carbohydrates named prebiotics. Prebiotic oligosaccharides are non-digestible carbohydrates that benefit the host health by stimulation of growth and/or activity of probiotic bacteria in the colon (Charalampopoulos & Rastall, 2012; Huebner, Wehling, & Hutkins, 2007). Thereby, the prebiotic juices can be obtained when oligosaccharides are added or synthesized (da Silva, Rabelo, & Rodrigues, 2012; Rabelo, Fontes, & Rodrigues, 2009; Rabelo, Honorato, Gonçalves, Pinto, & Rodrigues, 2009).

Many researches have demonstrated functional benefits besides nutritionals when prebiotics are ingested. Oligosaccharides develop important role in the organism such as obesity control, besides promoting satiety and reducing hunger (Siró, Kápolna, Kápolna, & Lugasi, 2008).

On the other hand, the heat may also accelerate undesirable biochemical and nutritious changes, which may affect the overall quality of the juice. Alternative non-thermal technologies have been investigated in order to obtain a safe product for consumption (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007).

Apple juice has good acceptability due to its flavour and the high antioxidant capacity, which is attributed to its biologically active compounds, such as phenolics (Patil, Valdramidis, Cullen, Frias, & Bourke, 2010a; Wu et al., 2007).

Brazilian Health Surveillance Agency establishes that liquid foods (ANVISA, 1999) must contain 1.5 g of oligosaccharide per portion for functional properties allegations. Thus an intake of 200 mL of a juice containing 7.5 g/L of prebiotic oligosaccharides reaches this regulation.

Usually prebiotic foods are obtained adding the oligosaccharides into the food matrix. However, our group has studied options to obtain a prebiotic juice through enzymatic synthesis. Dextransucrase, from *Leuconostoc mesenteiroides* B512F, have been applied to produce oligosaccharide in fruit juices. The oligosaccharides are produced when, besides sucrose, there is other sugar (glucose, fructose or maltose) present in the reaction medium, which acts as acceptor for glucose and fructose (Coelho et al., 2014; Rabelo, Fontes, et al., 2009; Rabelo, Honorato, Gonçalves, Pinto, & Rodrigues, 2006) The oligosaccharides produced by *L. mesenteroides* B512F dextransucrase present α-1,6- glucosidic bonds (Rabelo et al., 2009, 2006), which are non-digested by humans reaching the colon where they can be metabolized by the probiotic bacteria and then acting as functional carbohydrates. Despite several juices showed to be suitable for oligosaccharides synthesis, apple juice has not been studied yet.

Atmospheric cold plasma (ACP) is one of the most promising technologies for food preservation. ACP are produced by the excitation of atmospheric air within a dielectric barrier discharge (DBD) at room temperature and atmospheric pressure (Bárdos & Baránková, 2010; Fernández & Thompson, 2012).

To minimizing the losses caused by thermal process ACP has being evaluated as an emerging non-thermal technology for several applications in foods. ACP refers to a partially

or wholly ionized gas composed basically of free electrons, photons, atoms and ions, which results in a neutral charge at the end of the processing (Misra, Tiwari, Raghavarao, & Cullen, 2011). Previous studies proved to be secure and suitable for pathogens inactivation with only seconds of plasma application. Ziuzina et al. (2013) demonstrated that 20 s of plasma direct exposure and 45 s of plasma indirect exposure were enough to promote a 7 log CFU/mL of *Escherichia coli*, which attends the minimum need (5 log CFU/mL) to be considered a sterilization protocol. Despite several studies on microbial inactivation by ACP have been published, few ones take into account the treatment effect on the product quality and none takes into account apple juice containing gluco-oligosaccharides synthesised directly into the juice.

As ozone was also already studied as non-thermal alternative technology for pathogens and spoilage microorganisms inactivation (Achen & Yousef, 2001; Cullen et al., 2010; Patil, Valdramidis, et al., 2010a; Patil, Valdramidis, Cullen, Frias, & Bourke, 2010b), the effect of ozone was also evaluated in the present study. Ozone is a potential oxidant gas with high reactive power (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009). The aim of this study was to evaluate ACP and ozone as a non-thermal technology suitable to maintain apple juice quality and its prebiotic characteristics.

## 2 Material and Methods

*Prebiotic apple juice*

Clarified apple juice (Squeez©, Fruit Juices Ltd, Ireland) purchased from a local supermarket (Dunnes, Dublin-Ireland) was used as raw material. The carbohydrates were synthesized into the juice using *L. mesenteroides* B-512 F dextransucrase produced as previously reported (Fernandes & Rodrigues, 2007; Rodrigues, Lona & Franco, 2003; Rabelo, Honorato, & Rodrigues, 2007; Rodrigues, Lona, & Franco, 2005).

*Strain activation*

Stock culture of *Leuconostoc mesenteroides* B512F obtained from ARS Culture Bacterial Collection (NRRL Culture collection, United States Department of Agriculture, Peoria, Illinois) was prepared in MRS broth. The strain was maintained in protective beads (Technical Services Consultants Ltd, Lancashire, UK) at - 80°C. One protective bead was used to inoculate the bacteria in MRS medium on Petri dish.

*Enzyme preparation and activity*

The strain was activated in MRS broth until a cell concentration of approximately 9.0 log CFU $mL^{-1}$, equivalent to an absorbance reading of 0.600 at 590 nm in the McFarland scale (Fonteles et al., 2012). An aliquot of 1% (v/v) of this culture was used as inoculum to produce the dextransucrase enzyme (1,6-α-D-glucan-6-α-glucanosil transferase, EC. 2.4.1.5). Fermentation was carried out using the following culture medium: sucrose, 50 g/L (food grade); yeast extract, 20 g/L; $MgSO_4.7H_2O$, 0.20 g/L; $MnSO_4.H_2O$, 0.01 g/L; $FeSO_4.7H_2O$, 0.01 g/L; $CaCl_2.2H_2O$, 0.02 g/L; NaCl, 0.01 g/L; and $K_2HPO_4$ (anhydrous), 20 g/L (Rodrigues et al., 2003). The operating conditions were 30 °C with initial pH at 6.5 (±0.1) and mechanical agitation of 150 rpm. The process was carried out in a rotatory shaker for 6 hours. After that the pH was allowed to drop to 5.2.

The cells were harvested by centrifugation at 4,600 rpm for 20 min at 4 °C. The enzyme was recovery by precipitation with polyethylene glycol (PEG 1500 50 % v/v) at 4 $^oC$. The partially purified enzyme was then diluted in a sodium acetate buffer (20 mM) containing 0.05 g/L of $CaCl_2$ with pH adjusted to 5.2. The enzyme was stored frozen at −80 °C prior to use. Enzyme activity was determined by quantifying the released fructose by the DNS (3,5-dinitrosalicylic acid) method (Miller, 1959). The substrate was a sucrose solution in sodium

acetate buffer (20 mM containing 0.05 g/L of $CaCl_2$) pH 5.2. Results were expressed in international unit. One international unit (IU) is defined as the amount of enzyme that releases 1 μmol of fructose per minute under ideal reaction conditions (30 °C and pH 5.2) (Rabelo, Fontes, et al., 2009; Rabelo et al., 2006; Rodrigues et al., 2003).

*Synthesis of oligosaccharides*

Dextransucrase was added to apple juice with sugars concentration adjusted to 75 g/L of sucrose and 75 g/L of reducer sugar (fructose and glucose at equimolar proportions). The juice pH was adjusted to 5.2 (optimum for the enzyme activity) at 30 °C to obtain a juice with oligosaccharides (Araújo et al., 2014; da Silva et al., 2012). Synthesis was carried out in 1000 mL Erlenmeyer flask at 30 °C for 24 h (Rabelo et al., 2006). The enzyme activity for oligosaccharide synthesis was 0.05 IU/mL. The oligosaccharides synthesized by *L.mesenteriodes* B-512F dextransucrase present α-1,6 glycosidic bonds, which are not digested by intestinal bacteria (Araújo et al., 2014; da Silva et al., 2012; Rabelo, Fontes, et al., 2009).

*Non-thermal treatments*

Ozone gas was generated by an ozone generator (Model OL80, Ozone services, Canada) in a 100 mL glass bubble column. Ozone was produced by a corona discharge generator. Pure oxygen was supplied via an oxygen cylinder (Air Products Ltd., Dublin, Ireland). Ozone concentration was recorded using an ozone analyser (built in ozone module OL80A/DLS, Ozone services, Burton, Canada). The ozone concentration in the feed flow rate was 0.048 mg/min/mL and was fed at 0.12 L/min. To prevent excess foaming, 5 drops of anti-foaming agent (Antifoam B emulsion, Sigma Aldrich, Ireland Ltd.) were added before the

ozone treatment. An active carbon cartridge was used to destroy the ozone excess at the system exit.

Ozone loads from 0.057 up to 0.671 mg $O_3$/mL were applied in the prebiotic apple juice. These loads were within the minimum applied by Patil et al. (2010a) to inactivate 5 log UFC/mL of *Escherichia coli* (0.057 mg $O_3$/mL). Higher loads (up to 0.671 mg $O_3$/mL) were also evaluated to check the effect of ozone if more drastic conditions were necessary.

Plasma treatment was applied in 20 mL of juice samples in an open Petri dish. The Petri dish was put in polypropylene dielectric boxes, which were sealed with a polymeric film (Cryovac BB3050, 50 µm of thickness, Sealed Air Corporation, USA). The system was comprised of two circular aluminium plate electrodes (outer diameter = 158 mm) over polypropylene (PP) dielectric layers (2 mm thickness). The treatment was done at 70 kV with processing times of 15, 30, 45 and 60 seconds in two kinds of exposure: direct exposure where the samples were placed inside the plasma field between the electrodes; and indirect exposure where the samples were placed outside the plasma field and only exposed to the plasma afterglow. The samples were stored for 24h post-treatment at room temperature (20°C).

The operating conditions used herein were based on the evidence of pathogens inactivation by plasma reported in previously published studied (Rød, Hansen, Leipold, & Knøchel, 2012; Ziuzina, Patil, Cullen, Keener, & Bourke, 2013, 2014). Rød et al. (2012) have shown that indirect cold atmospheric pressure plasma treatment can reduce *Listeria innocua* on the surface of a ready-to-eat meat product (bresaola). Inoculated samples were treated at 15.5, 31, and 62 W for 2 - 60 s and resulted in a reduction of *L. innocua* ranging from 0.8 ± 0.4 to 1.6 ± 0.5 log CFU/g. Ziuzina et al. (2013) demonstrated that 20s of plasma direct exposure and 45s of plasma indirect exposure were enough to 7 log CFU/mL reduction of *E. coli*.

The treated samples were stored in fridge at 10 °C, approximately.

*High Performance Liquid Chromatography (HPLC) analyses*

All samples were previously treated precipitating the dextran formed during the synthesis of the prebiotic juice by adding pure ethanol to avoid HPLC column obstruction (da Silva et al., 2012; Rodrigues et al., 2003; Honorato & Rodrigues, 2008). Then the supernatant was cleaned through C-18 cartridges (Alltech) and pre-conditioned once with methanol and twice with ultrapure water. The samples were filtered through nylon membranes (0.45µm) and 20 µL of samples were injected into the HPLC system.

Sucrose, fructose and glucose were measured in an Agilent Technologies HPLC series 1260 Infinity (Wilmington, Delaware, EUA) equipped with a quaternary pump (model G1311B), an automatic sampler (model G1329B), an oven column (model G1316A) at 80 °C and a refractive index detector (model G1362A) at 35 °C. Supelcogel $Ca^{++}$ column (product nº. 5930-U), 30 cm x 7.8 mm I.D. with ultrapure water (MilliQ) at 0.5 mL/min flow rate was used to separate the simple sugars.

The synthesized oligosaccharides were analysed by a Varian Pro Star HPLC system (Varian Inc., Palo Alto, CA) equipped with two high-pressure pumps model 210, refractive index detector model 350 and a column oven Eldex CH model 150 at 80 $^{o}$C. The software Pro Star WS 5.5 was used to acquire and process the data. Ultrapure water (MilliQ System, Millipore, Bilberica, MA, USA) at 0.3 mL/min was used as eluent and the detector temperature was 35 °C. Oligosaccharide concentration was calculated from peak areas using malto-oligosaccharides (Sigma-Aldrich, USA) as standard.

*Organic acid quantification*

Organic acids were measured by High Performance Liquid Chromatographic. For quantification of citric acid (retention time at 8.0 min) and malic acid (retention time at 9.6 min) was used a calibration curve with a mixture of these acids (0.1 - 1.0 g/L and 0.2 - 2.0 g/L, respectively) in prebiotic apple juice after ACP and ozone treatments. The samples were diluted in water (1:4), filtered, using glass fiber prefilters AP25 13 mm diameter (Merck Millipore Ltd.) and cleaned using a C-18 SPE cartridge, then was filtrated with a syringe and a support comprising a membrane (HA membrane cellulose ester 0,45uM, 13 mm in diameter). The mobile phase was sulphuric acid in deionized water (0.01 M) at 0.6 mL/min for 30 minutes. The system (Agilent Technologies 1260 Infinity) was equipped with a pump system and a UV-DAD detector monitored at 210 nm. The organic acids were separated in Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad) termostated at 50 °C. The analyses were made in triplicate.

*Total phenolic content (TPC)*

The total phenolics were quantified through Folin-Ciocalteau method. Analyses were done in triplicate. The sample (10 µL) was mixed with 1:10 diluted Folin-Ciocalteau reagent in a microtiter 96-well plate, and then homogenized. After 3 minutes at room temperature, 100 µL of calcium carbonate 20% (w/v) was added and the sample was homogenized again. The absorbance was measured at 765 nm in a spectrophotometer (Model Epoch, Biotek, Biosystems – Brazil). Results were made in triplicate and expressed as g of gallic acid equivalents (GAE)/L. A standard curve was made with gallic acid.

*Antioxidant activity*

*ABTS method*

The method is based on the reaction of single electron transfer and used to measure the specific oxidant-reducing power of fruit and vegetables (Suárez-Jacobo et al., 2011). Total antioxidant activity was measured by improved Azinobis (ethylbenzothiazoline-6-sulphonic acid) radical scavenging (ABTS) method with modifications. Briefly 10 mL of an 7.0 mM ABTS solutions in ethanol and 176 µL of a 140mM potassium persulphate were mixed to produce the radical ABTS cation (ABTS·+) and kept in dark for 16 h at 20°C. The ABTS radical was diluted with ethanol (mixture) till an absorbance of 0.700 ± 0.05 at 734 nm. For the assays it was mixed 1.5 µL of samples (diluted 1:10 with ethanol) with 1500 µL of the mixture and the absorbance at 754 nm was measured during 6 min for the reaction against an ethanol blank in a spectrophotometer (model Evolution 201, Thermo Scientific - USA). Results were compared with a standard curve of Trolox in different concentrations (from 100 up to 2000 µM) (Re et al., 1999) and expressed as relative concentration between the sample and the control.

*Statistical Analysis*

Results were analysed by Tukey test using the software Statistica 13.0 (Statsoft).

## 3 Results and discussion

*Sugar concentration*

As atmospheric cold plasma (ACP) generates reactive compounds like ozone and free radicals and some depolymerisation of oligosaccharides were observed after the treatment. Regarding simple sugars, glucose was less affected by the treatment, while fructose decreased and sucrose increased for both kinds of plasma exposure as showed in the Tables 1 and 2.

Table 1 – Simple sugars concentration over the time (s) in plasma direct exposure.

| Direct Exposure | | | | | |
|---|---|---|---|---|---|
| Time (s) | 0 | 15 | 30 | 45 | 60 |
| Fructose (g/L) | $65.77 \pm 0.36^{a}$ | $60.34 \pm 0.01^{b}$ | $52.10 \pm 0.05^{c}$ | $48.94 \pm 0.21^{d}$ | $48.38 \pm 0.09^{d}$ |
| Glucose (g/L) | $29.76 \pm 0.21^{a}$ | $28.94 \pm 0.17^{b}$ | $28.33 \pm 0.17^{c}$ | $28.18 \pm 0.06^{c}$ | $28.45 \pm 0.01^{c}$ |
| Sucrose (g/L) | $24.47 \pm 0.15^{d}$ | $30.72 \pm 0.18^{c}$ | $39.57 \pm 0.22^{b}$ | $42.88 \pm 0.27^{a}$ | $43.17 \pm 0.10^{a}$ |

Means with the same letters in the same line are not statistically different according to the Tukey's test ($p<0.05$).

Table 2 – Simple sugars concentration over the time (s) in plasma indirect exposure.

| Indirect Exposure | | | | | |
|---|---|---|---|---|---|
| Time (s) | 0 | 15 | 30 | 45 | 60 |
| Fructose (g/L) | $65.77 \pm 0.36^{a}$ | $58.68 \pm 0.15^{b}$ | $49.14 \pm 0.21^{c}$ | $50.75 \pm 0.45^{d}$ | $47.89 \pm 0.31^{e}$ |
| Glucose (g/L) | $29.76 \pm 0.21^{a}$ | $29.14 \pm 0.04^{b}$ | $28.19 \pm 0.00^{c}$ | $28.26 \pm 0.12^{c}$ | $29.02 \pm 0.07^{b}$ |
| Sucrose (g/L) | $24.47 \pm 0.15^{d}$ | $32.18 \pm 0.18^{c}$ | $42.67 \pm 0.21^{a}$ | $40.99 \pm 0.58^{b}$ | $43.08 \pm 0.24^{a}$ |

Means with the same letters in the same line are not statistically different according to the Tukey's test ($p<0.05$).

Ozone treatment also releases reactive compounds imparting changes in the simple sugar concentration according to ozone load increase (Table 3). Glucose concentration did not present statistical differences all over the loads whereas fructose and sucrose presented some variations. Regarding the simple sugars, a slight fructose decrease is observed along a sucrose increase with the raise of ozone load (Table 3). This behaviour was similar in other study evaluating orange juice treated for ACP in the same conditions (Almeida et al., 2015) Glucose did not present any significant change. In all ozone loads, the total simple sugar concentration was kept at 120g/L. The sucrose increase may be due to some moieties released from the oligosaccharides or some reactions promoted by the radical species that can be formed due to ozone application. This fact can be observed mainly when the ozone load reached a concentration of 0.230 mg $O_3$/mL with fructose concentration of $53.85 \pm 0.06$ and sucrose, $36.34 \pm 0.16$.

Table 3 – Simple sugars concentration over the ozone loads (mg $O_3$/mL).

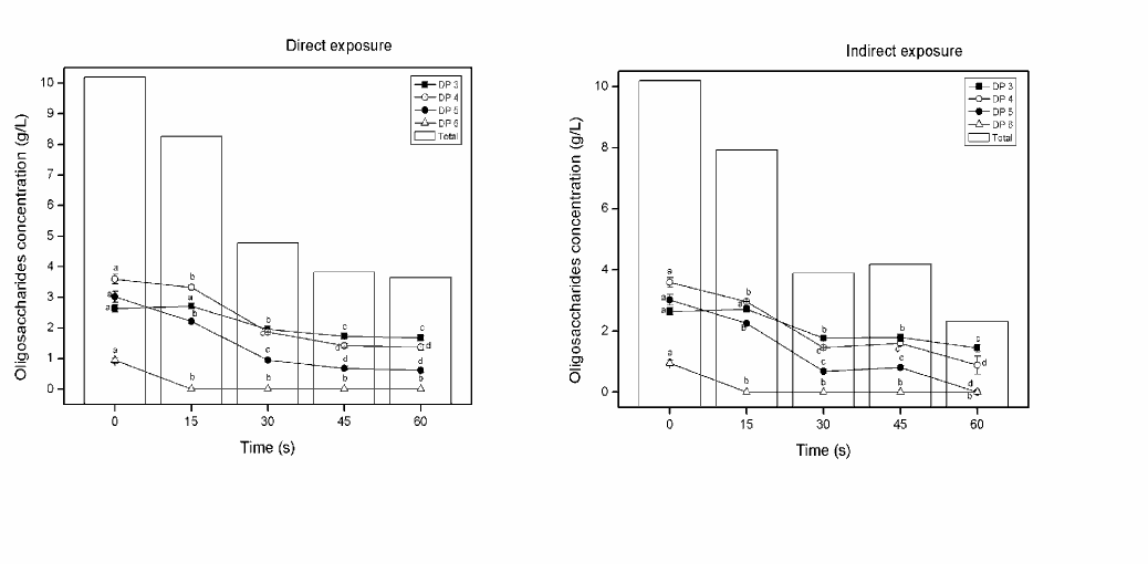
| Ozone | | | | | | |
|---|---|---|---|---|---|---|
| Loads (mg $O_3$/mL) | 0 | 0.057 | 0.128 | 0.230 | 0.386 | 0.671 |
| Fructose (g/L) | $65.77 \pm 0.36^{a}$ | $63.36 \pm 0.47^{b}$ | $60.63 \pm 0.07^{d}$ | $53.85 \pm 0.06^{e}$ | $62.03 \pm 0.12^{c}$ | $60.80 \pm 0.11^{d}$ |
| Glucose (g/L) | $29.76 \pm 0.21^{a}$ | $29.81 \pm 0.14^{a}$ | $29.78 \pm 0.08^{a}$ | $29.81 \pm 0.22^{a}$ | $29.90 \pm 0.02^{a}$ | $29.82 \pm 0.04^{a}$ |
| Sucrose (g/L) | $24.47 \pm 0.15^{e}$ | $26.83 \pm 0.33^{d}$ | $29.60 \pm 0.16^{b}$ | $36.34 \pm 0.16^{a}$ | $28.07 \pm 0.14^{c}$ | $29.38 \pm 0.15^{b}$ |

Means with the same letters in the same line are not statistically different according to the Tukey's test ($p<0.05$).

According to Almeida et al. (2015), the increase in sucrose concentration might have been due to the combination of fructose and the glucose moiety released from the oligosaccharides degradation.

Ben'ko, Manisova, & Lunin, 2013 claimed that the main route of carbohydrate ozonolysis induces the cleavage of glycoside bonds, oxidising functional groups to form carbonyl and carboxyl compound such as: lactones, and hydroperoxides. After thermal pasteurisation, they found greater losses for tetrasaccharides and higher saccharides. Part of that sugars were turned into trisaccharides, supplementing in part the amount of trimers that had been hydrolysed to di- and monosaccharides (Klewicki, 2007). Oligosaccharide degree of polymerization and total oligosaccharides concentration after ACP and ozone can be observed through the Figures 1 and 2, respectively.

Figure 1 – Oligosaccharides concentration in different DP (degree of polymerization) and the




total concentration of all oligosaccharides over the Atmospheric Cold Plasma time.

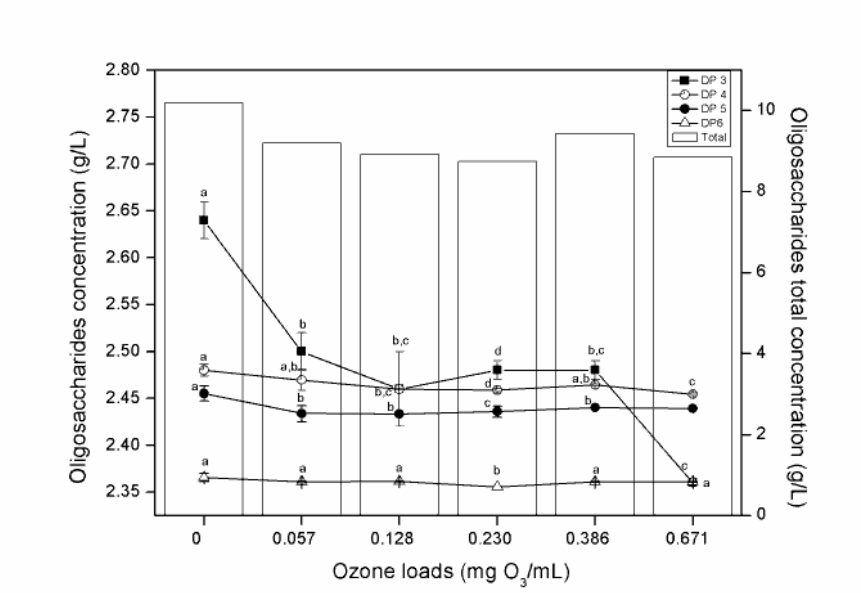
Means with the same letters for the same DP are not statistically different according to the Tukey's test ($p<0.05$).

Despite total oligosaccharide decay after plasma direct exposure from 10.19 to 3.64 g/L and indirect exposure from 10.19 to 2.32 g/L, the total oligosaccharide concentration in the juice after 60s of ACP treatment not remained within the Brazilian Health Surveillance Agency (ANVISA, 2014). The ANVISA recommended intake, for prebiotic liquid foodsis 1.5 g of oligosaccharides per portion ( > 7.5 g/L for a 200 mL portion). However, the ANVISA recommended contet is not achieved after 15 s of both ACP exposure, which it is not enough to microbiologic safety according a previous research (Ziuzina et al., 2013). It is recommended one teaspoon a day of the FOS powder that it can be mixed in water or juices or sprinkled on cereals (Scheer, 1997). A consumption of 16 g/day of short-chain inulin-type fructan prebiotics has favorably increased satiety peptides, reduces hunger, food intakes and significantly lowered energy intake by 10% (Cani et al., 2009).

In previous studies 30s and 45s of direct and indirect exposure, respectively, were enough for microbial stability. Thus, to reach the recommended intake determined by ANVISA, the consumption should be two portions (200 mL) per day, which means 2 regular

glasses of juice. Oligosaccharides total concentration decreased from 10.19 to 8.85 g/L along the ozone load increase (Figure 2).

Figure 2 – Oligosaccharides concentration in different DP (degree of polymerization) and the total concentration of all oligosaccharides over the ozone loads.




Means with the same letters for the same DP are not statistically different according to the Tukey's test ($p<0.05$).

Despite ANVISA determines a final concentration of 1.50 g per portion of prebiotic oligosaccharide: fructo-oligosaccharides (FOS); there is not studies about other kind of oligosaccharides. The juice produced through enzymatic process in this work can be classified as prebiotic because contains gluco-oligosaccharides (GOS). It was considered diary intake to FOS. Even at high ozone loads (a portion of 200 mL would supply at least 1.77 g of oligosaccharides). The regular consumption of prebiotics improves the mineral absorption (calcium). The supplementation of oligofructose enriched inulin (8 g/d) in diet of girls with a high habitual calcium intake showed the increase absorption of this mineral (Bosscher, Van Loo, & Franck, 2006).

The decrease of oligosaccharide can be due its conversion in some other species because of ozone cause oxidative disintegration of the ozonide and formation of carbonyl

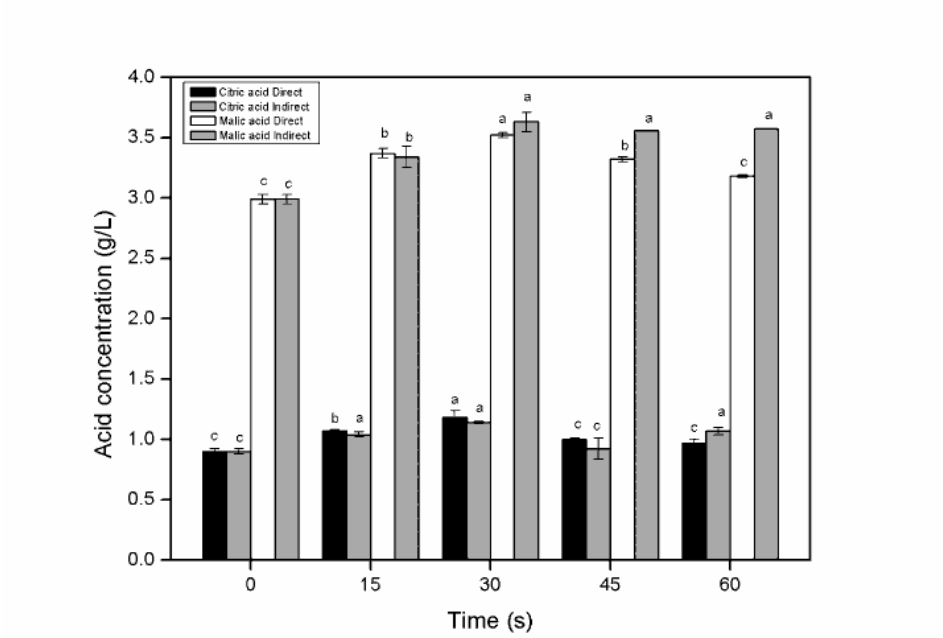
compounds, while oxidative work-up leads to carboxylic acids or ketones (Tiwari et al., 2009). ACP treatment releases ozone gas that can cause carbohydrate depolymerisation, including polysaccharides with 1,6-linked α-D-glucose bonds, and producing sugars with shorter chains as in this research (Wang, Hollingsworth, & Kasper, 1999).

A research on prebiotic orange juice non-thermal treatment showed similar results (Almeida et al., 2015). The researchers applied plasma and ozone at similar conditions on the juice. Even though the oligosaccharides degree of polymerization (DP), the non-thermal treatments did not affect the DP of the oligosaccharides in the juice since the DP of the treated samples was the same as the untreated sample in both cases.

*Organic acids quantification*

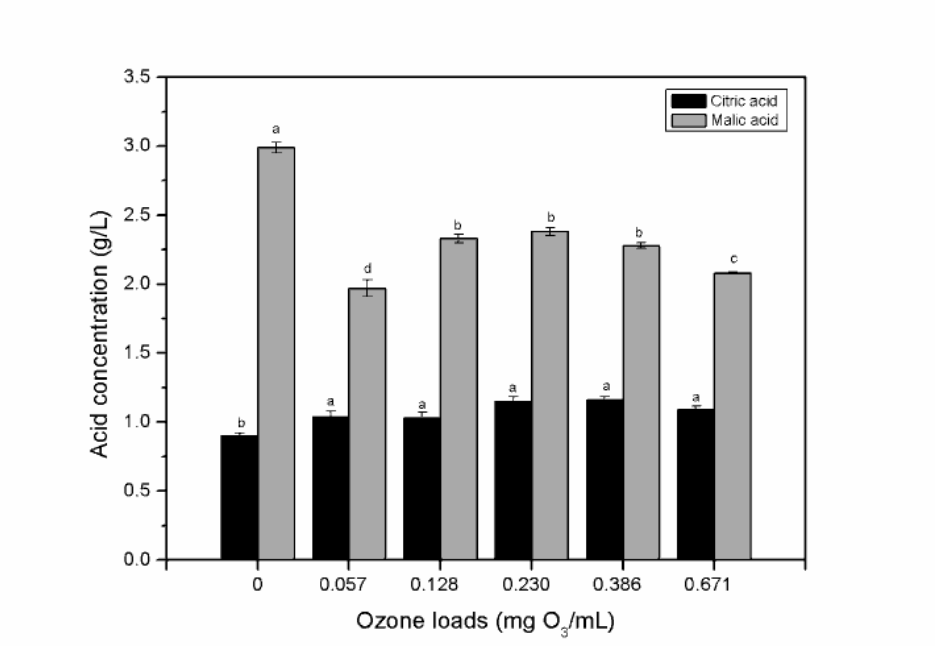
Figures 3 and 4 show the results of organic acids in apple juice after ACP and ozone treatment, respectively. In the samples treated by ACP it was observed similar behaviour compared to ozone treatment, with relation to citric acid concentration, because significant changes were observed. About malic acid, there was a small variation for both sort of exposure in the case of ACP treatment. The malic acid concentration increased ($2.99 \pm 0.04$ to $3.18 \pm 0.01 - 3.57 \pm 0.00$). Significant differences occurred in the analyses of these acids at different times and plasma exposure.

Figure 3 – Organic acids concentration of the prebiotic apple juice after atmospheric cold plasma treatment and direct and indirect exposure.




Means with the same letters for the same organic acid and exposure are not statistically different according to the Tukey's test ($p<0.05$).

Figure 4 – Organic acids concentration of the prebiotic apple juice after ozone treatment.




*Means with the same letters for the same organic acid are not statistically different according to the Tukey's test ($p<0.05$).

In ozone treatment, it was observed a significant change in the concentration of citric acid compared to the control (0.90 ± 0.02). Furthermore, it was observed a variation about 30% in malic acid concentration (from 2.99 ± 0.04 of the control to 2.08 ± 0.01 – 2.38 ± 0.03 g/L) after the treatment showing that the treatment affected more this kind of organic acid.

Some studies have shown that thermal treatments, such as conventional pasteurization, degraded organic acids (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010). Other studies showed that after pulsed electric field treatment, unchanged acid contents were obtained. In this case the difference between the mean values of untreated and treated samples was less than 1%. Malic acid concentration in untreated sample (2.56 ± 0.09 g/L) was similar to the one treated (2.46 ± 0.02 g/L) in lemon juice (Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006).

Regarding to other studies, it is not possible to compare our organic acids results with other groups because so far, these treatments in juices containing oligosaccharides were never performed before.

*Total Phenolic content (TPC)*

TPC did not present change compared the control sample with the samples in direct and indirect exposure to the plasma field except for 60s of indirect exposure demonstrating that plasma causes little or no negative effect on apple juice phenolic content (Table 4).

Table 4 – Total phenolic concentration (TPC) for apple juice for plasma direct and indirect exposure in different times.

| Treatment time (s) | TPC (g of GAE/L) | |
|---|---|---|
| | Direct Exposure | Indirect Exposure |
| 0 | $0.73 \pm 0.10^{a}$ | $0.73 \pm 0.10^{a}$ |
| 15 | $0.72 \pm 0.02^{a}$ | $0.69 \pm 0.04^{a}$ |
| 30 | $0.64 \pm 0.02^{a}$ | $0.59 \pm 0.01^{a,b}$ |
| 45 | $0.66 \pm 0.02^{a}$ | $0.64 \pm 0.04^{a,b}$ |
| 60 | $0.61 \pm 0.02^{a}$ | $0.53 \pm 0.02^{b}$ |

Same letters in the same column indicate no significant difference at the 0.05 level between values.

Similar results (Bellion et al., 2008) were reported for fermented apple juice extracts, where phenol content range between 0.15 and 0.97 g/L, dependent on the apple variety. A study on thermal degradation of TPC in cloudy apple juice (De Paepe et al., 2014) showed 42 compounds susceptible in high-temperature short time (HTST) processing (the main method applied in food industry for fruit juices). Aguilar-Rosas et al. (2007) reported a greater loss of phenolic compound in HTST (32.2%) compared with non-thermal treatment (pulsed electric field), which only caused 14.49% of reduction. Therefore, their lost or decrease in concentration will prejudice seriously apple juice sensory attributes. After ozone, TPC was preserved comparing to the control (no treated prebiotic juice) (Table 5).

Table 5 – Total phenolic concentration (TPC) of clarified apple juice for ozone in different loads.

| Ozone loads (mg $O_3$/mL) | TPC (g of GAE/L) |
|---|---|
| 0 | $0.73 \pm 0.10^{a}$ |
| 0.057 | $0.65 \pm 0.10^{a}$ |
| 0.128 | $0.70 \pm 0.10^{a}$ |
| 0.230 | $0.62 \pm 0.01^{a}$ |
| 0.386 | $0.72 \pm 0.03^{a}$ |
| 0.671 | $0.80 \pm 0.02^{a}$ |

Same letters in the same column indicate no statistical difference at $p<0.05$.

A previous research that evaluated the TPC in fresh apple juice submitted to ozone in different times observed a decrease of TPC according to ozone load increase. However, the quantity continued similar of other studies: from 0.63g/L for fresh apple juice, 0.32 g/L for 4 min and 0.27 g/L for 10 min (ozone concentration of 0.048 $O_3$ mg/min/mL) (Patil, Torres, et al., 2010). Another research reported values of 6.38 ± 1.23 g GAE/L for apple juice treated by ozone (Torres et al., 2011). In other study about the influence of ultra-high pressure homogenization (UHPH) on polyphenol content of clear (filtered) apple juice showed that the pasteurization process affected more the raw apple juice (0.120 ± 0.01 g GAE/L) with no significant difference for UHPH non-thermal treatment (from 0.013 ± 0.00 up to 0.0143 ± 0.00 g GAE/L) and with significant differences for pasteurisation (0.0145 ± 0.00 g GAE/L) (Suárez-Jacobo et al., 2011).

*Antioxidants*

*ABTS radical scavenging*

After plasma treatment (Table 6) it was noted an increase in the ABTS relative value along with the treatment time for juices treated with direct exposure. For assays conducted through indirect plasma exposure it was noticed the same phenomenon. Plasma treatment enhanced the antioxidant capacity the product probably due to the formation of free radicals with antioxidant activity.

Table 6 – ABTS relative concentration in relation with the control on prebiotic apple juice after plasma treatment.

| | ABTS relative concentration (%) | |
|---|---|---|
| Time (s) | Direct exposure | Indirect exposure |
| 15 | $83.92 \pm 1.0^{a}$ | $121.06 \pm 19.0^{a}$ |
| 30 | $87.19 \pm 3.0^{b}$ | $117.46 \pm 18.0^{b}$ |
| 45 | $125.47 \pm 19.0^{a,b}$ | $145.78 \pm 10.0^{a,b}$ |
| 60 | $154.40 \pm 1.0^{a,b}$ | $138.81 \pm 25.0^{a,b}$ |

Means with the same letter in the same column are not significantly different by the Tukey test ($p < 0.05$).

The study of antioxidant activity of pasteurised pomegranate juice showed losses in ABTS scavenging (Mena et al., 2013) demonstrating that thermal treatment reduced the

antioxidant potential. Our group evaluated plasma and ozone effects on prebiotc orange juice with different results for this food matrix – a slight reduction for direct exposure of ACP and no alterations for indirect (Almeida et al., 2015).

Apple juice has a great scavenging capacity due its high phenolic content. The results indicated a great ABTS scavenging for the antioxidants from the extracts taking into account the ozone treatment (Table 7) and confirming that ABTS and TPC values were strongly correlated.

Table 7 – ABTS relative concentration in relation with the control on prebiotic apple juice for different ozone loads applied.

| Ozone loads (mg $O_3$/mL) | ABTS relative concentration (%) |
|---|---|
| 0.057 | $104 \pm 28^{a,b}$ |
| 0.128 | $145 \pm 19^{a}$ |
| 0.230 | $73 \pm 15^{b}$ |
| 0.386 | $91 \pm 12^{a,b}$ |
| 0.671 | $100 \pm 15^{a,b}$ |

Same letters indicate no statistical difference at $p < 0.05$.

After ozone treatment for orange juice with ozone loads from 0.057 to 0.230 mg $O_3$/mL, our group found no changes up to 0.128 mg $O_3$/mL. However, the stronger load modified ABTS capacity causing its decreased (Almeida et al., 2015).

## 4 Conclusion

It is possible to use atmospheric cold plasma by 30 s and 45s of direct and indirect exposure time, respectively, to ensure a safe and prebiotic product, taking in account the consumption twice a day, considering diary intake to FOS. It is important to remember that no studies on the amount of gluco-oligossacharides daily intake is available up to now. Thus, the desired prebiotic effect might be achieved with a lower intake. Moreover, there were high

rates of phenolic compounds and antioxidant activity in apple juice after ozone and ACP treatments.

Ozone treatment is a better alternative than ACP to substitute thermal treatment in order to preserve apple juice quality and prebiotic properties, because the ANVISA recommended daily intake is reached with a singleportion per day. Best results were found to TPC when the juices were treated by ozone instead of pasteurisation claiming that non-thermal process migh produce prebiotic apple juice maintaining their antioxidant characteristics.

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# CONSIDERAÇÕES FINAIS

O plasma, a alta pressão e o ozônio como tecnologias emergentes em substituição às tecnologias usuais, principalmente a pasteurização, têm grande potencial de serem usados para preservar as características funcionais do suco, mantendo uma concentração aceitável de oligossacarídeos segundo a Agência Nacional de Vigilância Sanitária. Mais estudos podem ser feitos em continuidade aos presentes, principalmente para plasma, que é uma tecnologia ainda não usada comercialmente.