UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE ENGENHARIA

DEPARTAMENTO DE ENGENHARIA QUÍMICA

Concentração de suco de uva por osmose direta e estudo tecnológico para o aproveitamento do bagaço

Tese de Doutorado

Voltaire Sant'Anna

Porto Alegre

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PROGRMA DE PÓS-GRADUAÇÃO EM ENGENHARIA QUÍMICA

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Voltaire Sant'Anna

Tese de Doutorado apresentada como requisito parcial para obtenção do título de Doutor em Engenharia. Área de concentração: Fenômenos de Transporte e Operações Unitárias

Orientadora: Dra. Isabel Cristina Tessaro
Co-orientadora: Dra. Ligia Damasceno Ferreira Marczak

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A banca examinadora, abaixo assinada, aprova a defesa de Tese de Doutorado, cujo projeto é intitulado "*Concentração de suco de uva por osmose direta e estudo tecnológico para o aproveitamento do bagaço*", elaborado por Voltaire Sant'Anna, como pré-requisito para obtenção do grau de Doutor em Engenharia Química.

_____

Dra. Florencia Cladera-Olivera (ICTA/UFRGS)

_____

Dra. Lourdes Maria Correa Cabral (EMBRAPA/RJ)

_____

Dr. José Carlos Cunha Petrus (EAQ/UFSC)

“Quanto mais eu treino, mais sorte eu tenho”
Arnald Palmer

“Quem quer dá um jeito, quem não quer arruma uma desculpa”
Caio Fernando de Abreu

“Mão Santa? Mal sabem eles quanto treino e quantas repetições faço todos os dias para acertar a cesta”
Oscar, Mão Santa

“O nosso sonho é o próximo objetivo”
Steve Jobs

“I can’t get no satisfaction”
Rolling Stones

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RESUMO

A uva é uma fruta amplamente consumida em todo o mundo e importante fonte de compostos com atividade antioxidantes, principalmente compostos fenólicos. A operação mais tradicional de conservação de alimentos é pelo uso de calor, porém ele acarreta em alterações sensoriais e nutricionais do produto acabado. A utilização do bagaço de uva, resultante do processo de extração de suco, também tem demonstrado grande potencial para elaboração de produtos ricos em compostos com atividade antioxidante e fibras. Logo, estudos tecnológicos para o apropriado processamento industrial do suco de uva e de seus resíduos são necessários para incrementar o seu uso como componente funcional na indústria de alimentos. Os resultados sobre a concentração do suco de uva por osmose direta mostraram que o aumento da diferença de pressão osmótica, da vazão de alimentação e da temperatura acarretam em aumento do fluxo de água e sódio através da membrana. Também, foi observado que os fenômenos de polarização por concentração são fatores determinantes no desempenho do processo e podem ser minimizados pelo controle dos parâmetros de processo de osmose direta. Esse estudo mostrou que o suco concentrado por osmose direta não perde suas propriedades antioxidantes e não há redução da concentração de compostos fenólicos na bebida. Os resultados mostraram que há o aumento do teor de sódio de 0,9 mg L^{-1} para 1,75 mg L^{-1}, quando o suco foi concentrado por osmose direta, porém esse aumento não acarreta em níveis altos para o consumo humano. Os resultados de secagem do bagaço de suco de uva mostram que maiores temperaturas e velocidades de ar de secagem implicaram em um processo de desidratação mais rápido. A retenção de compostos bioativos se mostrou maior em menores temperaturas. Em relação à velocidade do ar de secagem, a retenção da concentração dos compostos fenólicos totais foi maior quando foram utilizadas maiores fluxos de ar. Os flavonóis e flan-3-óis foram sensíveis ao aumento da velocidade de ar de secagem devido a degradações oxidativas. A atividade sequestrante de radicais ABTS aumentou no resíduo tratado a altas temperaturas, possivelmente devido à formação de produtos da reação de Maillard que também apresentam tal característica. A extração de compostos fenólicos do bagaço de suco de uva mostrou que grande parte de compostos fenólicos, principalmente taninos condensados, estão fortemente ligados na matriz do resíduo e não são facilmente extraíveis com solventes orgânicos. Além disso, esses compostos apresentam grande capacidade antioxidante, principalmente poder quelante de ferro. O estudo cinético de extração de compostos fenólicos e de antocianinas de bagaço de uva mostrou que o modelo de pseudo-primeira ordem foi o modelo que melhor representou a extração dos polifenóis. Em relação à estabilidade da farinha de bagaço de uva, os resultados mostraram que os compostos fenólicos e compostos com atividade sequestrante de radiais DPPH e de ferro se mostraram estáveis durante o armazenamento do produto a 25ºC por um período de 6 meses. Antocianinas e compostos com a atividade de sequestrar radiais ABTS se mostraram

sensíveis à degradação durante o armazenamento. Foi observado que a farinha se mostrou livre de *Salmonella* sp., *Bacillus cereus* e coliformes fecais, havendo a necessidade de cuidados com o crescimento de bolores e leveduras durante a armazenagem. Finalmente, foram elaboradas massas tipo fettuccini, substituindo farinha de trigo por farinha de bagaço de uva, em que foi verificado que a adição do subproduto do suco de uva não interferiu na absorção de água e na perda de sólidos da massa durante o seu cozimento. Houve grande incremento de compostos fenólicos, antocianinas e de compostos com atividade antioxidante nos produtos adicionados de farinha de bagaço de uva, sendo a massa tipo fettuccini com melhor aceitação aquela em que se adicionou 2,5% do subproduto na sua preparação. Assim, farinha de bagaço de uva se mostra como um potencial componente funcional para ser utilizado tanto na indústria de alimentos quanto na agricultura familiar.

ABSTRACT

Grapes are widely consumed fruits throughout the world and important source of compounds with antioxidant activity, particularly phenolic compounds. The most traditional operation of food preservation is the use of heat, but it leads to changes on sensory and nutritional properties of the industrialized product. The use of grape pomace, resulting from the extraction of juice, has also shown great potential for development of products rich in compounds with antioxidant activity and fiber. So, technology studies to the propper industrial processing of grape juice and their waste ar essential to enhance the use of them as functional components in food and pharmaceutical industries. The results of the concentration of grape juice by forward osmosis showed that the concentration polarization phenomena are determining factors on process performance. Also, the study showed that the juice concentrated by FO does not degrade the compounds with antioxidant activity and does not change the nutritional properties of the beverage, despite of the small sodium transport for juice. The drying procedures of the grape marc show that higher temperatures and air velocity implied on a faster process of dehydration. The retention of bioactive compounds was greater at lower temperatures. In relation to the air velocity, the retention of the phenolic compounds was higher when higher values of air flows were used. Flavonols and flan-3-ols were sensitive to the increasing of the air velocity due to oxidative degradation. The ABTS-radical scavenging activity was increased when high temperatures were applied, possibly due to the formation of Maillard reaction products which also have this characteristic. The extraction of phenolic compounds from grape juice marc showed that most of the phenolic compounds, mainly tannins, are strongly linked in the matrix of residue and are not easily extractable with organic solvents. Furthermore, these compounds still have great antioxidant capacity, mainly iron chelating power, which shows the great potential of grape pomace be used in food applications, both in the form of phenolic extracts, as in the form of flour. The kinetics of extraction of phenolic compounds and anthocyanins in grape pomace showed that the model of pseudo-first order was the equation that best represented the extraction of polyphenols. Much of polyphenols is strongly linked to bagasse and not extracting solvent, as well as much of bioactive with antioxidant activity, reducing power and chelating activity is linked. In relation to the stability of the marc powder, the results showed that the phenolic compounds and compounds with DPPH radial and iron scavenging activity were stable during storage of the product at 25 ° C for a period of 6 months. Anthocyanins compounds with the capability of scavenging ABTS radicals were sensitive to degradation during storage. It was observed that the dried residue was free of *Salmonella* sp., *Bacillus cereus* and faecal coliforms, although it may be of great concern the growth of yeasts and molds during storage. The sorption isotherm curve of grape marc at 25 ° C had sigmoidal behavior, and the GAB model was the best equation to describe the experimental data. Finally, fettuccini

type pastas were prepared, substituting wheat flour by grape marc powder. It was observed that the addition of the dried by-product did not interfere in water absorption capability and the solid loss of the past during cooking procedures. High enhance of the concentration of phenolic compounds, anthocyanins and compounds with antioxidant activity was observed in the blend added of grape marc powder, being the fettuccini pasta with better acceptance that in which it was added 2.5% of the residue to the preparation. These results so far show that grape pomace and the its phenolic extracts have great potential to be use as functional components in the food industry.

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CAPÍTULO 1

INTRODUÇÃO

A crescente busca dos consumidores por alimentos prontos para o consumo, mas com as mesmas características do alimento *in natura*, tem despertado o interesse das indústrias de alimentos na manutenção da qualidade desses produtos após sua industrialização. Além disso, o crescimento industrial que se observa nos últimos anos tem gerado grandes quantidades de resíduos, que, na sua grande maioria, devido às suas características, podem ser reaproveitados quando processados de modo adequado gerando produtos de elevado valor agregado.

A uva é uma fruta amplamente consumida em todo o mundo, tanto *in natura* quanto processada como doces, sucos ou vinho. As uvas são importantes fontes de compostos com atividade antioxidante, principalmente compostos fenólicos, e seu consumo tem sido motivo de amplo estudo devido sua contribuição à saúde humana.

Os processos tradicionais de conservação de alimentos envolvem o uso de calor, destacando-se, entre estes, os processos de pasteurização, esterilização e evaporação. Porém, o uso de altas temperaturas pode acarretar perdas significativas de componentes sensoriais e nutricionais como vitaminas, carotenóides, aromas, cor e sabor, considerados importantes para a qualidade final do produto. Devido a isto, muitos esforços na área de ciência, tecnologia e engenharia de alimentos têm atentado a novas tecnologias que visam maior retenção de compostos bioativos, maior rendimento, menores custos de processamento e de consumo de energia.

CAPÍTULO 1 - INTRODUÇÃO

Processos de separação com membranas, tais como osmose inversa e nanofiltração, têm sido amplamente empregados na concentração de sucos de frutas devido às inúmeras vantagens que esta tecnologia apresenta: operação à temperatura ambiente ou inferiores, seletividade, equipamentos modulares, simplicidade de operação, economia de energia. A Osmose Direta (OD), também chamada de concentração osmótica, é um processo com membranas, no qual se utiliza uma solução como agente osmótico para criar um gradiente de pressão osmótica através de uma membrana semi-permeável e, desta forma, remover água da solução de alimentação para a solução osmótica. Essa técnica já vem sendo utilizada na purificação de água do mar, tratamento de efluentes e concentração de alimentos líquidos. Porém, ainda há muito a ser explorado nesse campo, principalmente em relação ao tratamento de alimentos.

Além disso, no processo de extração de suco de uva e na produção de vinho, há a geração de grandes quantidades de bagaço, o qual apresenta potencial para elaboração de produtos ricos em compostos com atividade antioxidante e fibras. Assim, estudos tecnológicos para o apropriado processamento industrial do bagaço de uva são necessários para alavancar o uso deste subproduto como componente funcional na indústria de alimentos e farmacêutica. Estudos na área de engenharia são essenciais para a análise da viabilidade de novas tecnologias e do impacto de processamentos sobre a qualidade do produto final e das tecnologias utilizadas.

A escassa informação sobre o uso de OD para a concentração de suco de uva, aliado ao grande potencial industrial que a utilização do bagaço de uva apresenta, são temas atuais e de grande relevância a serem abordados, visando aperfeiçoar processos industriais para produção de alimentos com maior qualidade sensorial e nutricional.

CAPÍTULO 2

OBJETIVOS

Este trabalho tem como objetivo concentrar o suco de uva por osmose direta, estudando o impacto dessa tecnologia sobre características nutricionais da bebida. Este projeto também visa o estudo tecnológico do aproveitamento de bagaço de uva para utilização na indústria de alimentos.

Os objetivos específicos do projeto são:

- validação do processo de osmose direta quanto à influência dos principais parâmetros de processo sobre o fluxo de água transmembrana;
- concentração de suco de uva por osmose direta, avaliando a influência de variáveis de operação (concentração da solução osmótica, temperatura de operação, velocidade de alimentação de suco e da solução osmótica) sobre a transferência de água e de sal transmembrana;
- avaliação do impacto da concentração por OD do suco de uva sobre propriedades antioxidantes da bebida;
- avaliação de parâmetros de secagem (temperatura e velocidade de secagem) para obtenção da farinha de bagaço de uva;
- extração de componentes bioativos do bagaço de uva;
- estudo de estabilidade da farinha de bagaço de uva;
- preparação de produto adicionado de bagaço de uva.

CAPÍTULO 3

REVISÃO BIBLIOGRÁFICA

Neste capítulo, será apresentada uma revisão bibliográfica que, inicialmente, inclui a uva e seu suco com enfoque em suas características nutricionais. Após, é discutida a osmose direta como processo de separação por membranas, enfatizando aspectos relacionados à concentração de alimentos líquidos e à influência de parâmetros de processo sobre o desempenho da técnica. Em seguida, é discutida a potencialidade de resíduos de vegetais como ingrendientes na indústria de alimentos. Por fim, são apresentados aspectos teóricos de operações unitárias utilizadas para viabilizar o uso de bagaço de uva como um insumo para a indústria de alimentos.

3.1 Suco de uva

A uva é o fruto da videira, ou vinha, pertencendo, segundo classificação botânica, à ordem das *Ramnidea*, família das *Vitaceas*, ao gênero *Vitis* e espécies das mais variadas entre elas: *Vitis vinífera*, *Vitis labrusca*, *Vitis riparia*, *Vitis cinérea*, entre outras (GUERRA *et al*., 2009).

A uva é uma das frutas mais cultivadas e consumidas em todo o mundo. Nos cinco continentes, o mundo da uva e do vinho abrange mais de 40 países. A partir da introdução do cultivo da videira no Brasil, ocorrida em 1535, muitas regiões brasileiras experimentaram e desenvolveram o cultivo da uva e a produção de vinhos. Contudo, a vitivinicultura somente ganhou impulso e se tornou uma atividade de importância sócio-

econômica a partir do final do século XIX, com a chegada dos imigrantes italianos, sobretudo no Estado do Rio Grande do Sul (GUERRA *et al.*, 2009).

O Estado do Rio Grande do Sul foi o maior produtor de uvas do Brasil no ano de 2010, chegando a produzir cerca de 53% da produção nacional. A Figura 3.1 apresenta o gráfico da distribuição percentual da produção de uvas no Brasil para o ano de 2010.

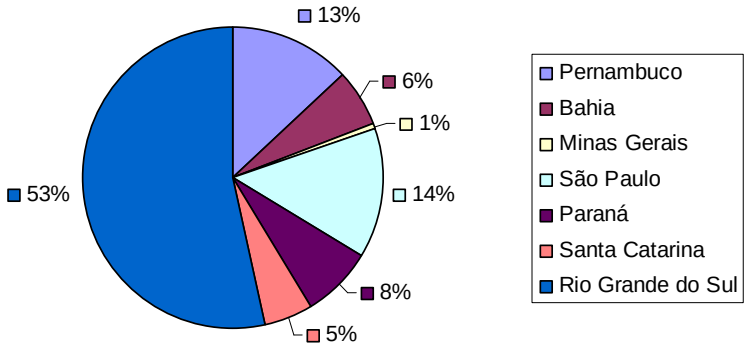


Figura 3.1 Distribuição percentual da produção de uvas no Brasil no ano de 2010 (Fonte: IBRAVIN, 2011).

Em 2010, foi estimado que, das 1.295.442 toneladas de uva foram produzidas, 557.888 toneladas foram destinadas ao processamento industrial para a produção de suco, vinho, geleia, entre outros produtos (IBRAVIN, 2011). Isto mostra o grande potencial que o setor vitífera apresenta no Brasil, principalmente visando o impacto dos processos industriais na qualidade do produto final.

Efeitos fisiológicos benéficos à saúde humana, relacionados ao consumo de vinho, têm sido amplamente investigados. O suco de uva, desprovido de teor alcoólico, apresenta efeitos similares. Estes efeitos estão relacionados principalmente à presença de compostos fenólicos tanto na fruta *in natura* quanto no alimento processado (ISHIMOTO, 2008).

Em estudos conduzidos por Arts e colaboradores (2001a,b), foi observado que o consumo de vinho reduziu a mortalidade por doenças crônicas de população com idade

entre 65 a 84 anos, além de mulheres na menopausa mostrarem menor tendência a apresentarem problemas coronarianos. Keevil e colaboradores (2000) verificaram que o consumo de cerca de 400 mL de suco de uva por dia diminuiu a agregação plaquetária em homens saudáveis. Já Freedman e colaboradores (2001) observaram que o consumo da mesma quantidade de suco de uva diminuiu a agregação plaquetária, assim como a produção de superóxido (radial livre relacionado a doenças ligadas ao estresse oxidativo). Contribuindo neste sentido, Chou e colaboradores (2001) verificaram que o consumo de 500 mL de suco de uva por dia esteve associado ao aumento da dilatação da artéria branquial em adultos com doença arterial coronariana. Ainda, os autores verificaram a redução da susceptibilidade do LDL à oxidação, sugerindo efeito antioxidante do suco *ex vivo*. Park e colaboradores (2004a) observaram um aumento da atividade antioxidante de plasma sanguíneo e prevenção a danos oxidativos ao DNA em homens e mulheres adultas que adicionaram 480 mL de suco de uva à sua dieta habitual. O mesmo grupo de pesquisa (PARK *et al*., 2004b) observou que o consumo de 400 mL de suco de uva por dia reduziu a pressão arterial de pacientes com hipertensão na Coréia do Sul. Castilla e colaboradores (2006) administraram 100 mL de suco de uva por dia e verificaram efeitos antioxidantes, hipolipodêmico e anti-inflamatório em pacientes submetidos ao tratamento de hemodiálise.

Todos os efeitos relatados pelos trabalhos acima citados são relacionados à presença de compostos fenólicos no produto ingerido. Com isso, há evidências científicas que o consumo de contínuo e moderado de compostos fenólicos pode ter grande potencial de prevenir doenças e melhorar a saúde de seres humanos.

3.2 Compostos fenólicos em uvas

Os compostos fenólicos, também chamados de polifenóis, encontram-se largamente em plantas e são um grupo muito diversificado de fitoquímicos derivados de fenilalanina e tirosina. Os fenólicos, em plantas, são essenciais ao crescimento e à reprodução dos vegetais, além de atuarem como agente antipatogênico e contribuírem na pigmentação (SHAHIDI e NACZK, 1995). Em alimentos, são responsáveis pela cor, adstringência, aroma (PELEG *et al.*, 1998) e estabilidade oxidativa (SHAHIDI *et al.*, 1992).

De acordo com a via biossintética, os compostos fenólicos podem ser reunidos em metabólitos primários e secundários, sendo o último grupo o mais importante, tanto em aspectos sensoriais, quanto fisiológicos (SHAHIDI *et al.*, , 1992). Esses compostos são encontrados em vacúolos e paredes de células vegetais, sendo essenciais para o seu crescimento e reprodução. Além disso, se formam em condições de estresse, como infecções, ferimentos, radiações UV, dentre outros (NACZK e SHAHIDI, 2004).

As principais fontes de compostos fenólicos são frutas vermelhas, como uva, mirtilo, ameixa, amora, além de outras frutas a exemplo da cereja, manga, limão, pêra, maçã e mamão. Pimenta verde, brócolis, repolho roxo, cebola, alho e tomate também são excelentes fontes destes compostos (PIMENTEL *et al.*, 2005).

Quimicamente, os fenólicos são definidos como substâncias que possuem anel aromático com um ou mais substituintes hidroxílicos, incluindo seus grupos funcionais. Mais de 8000 variantes estruturais têm sido identificadas, sendo classificadas de acordo com o número de anéis aromáticos e outros elementos ligados à sua estrutura. Desta forma são distribuídos em 4 grupos: ácidos fenólicos, flavonóides, proantocianinas e estilbenos (NACZK e SHAHIDI, 2004).

3.2.1 Ácidos fenólicos

Ácidos fenólicos são geralmente divididos em dois grupos principais: ácidos hidroxibenzóicos, contendo sete átomos de carbono, e ácidos hidroxicinâmicos, constituídos por nove átomos de carbono.

3.2.1.1 Ácido Hidroxibenzóico

Várias espécies de ácidos hidroxibenzóico foram identificadas em uvas. Os mais abundantes são os *para*-hidrobenzóicos, protocatecuico, vanílico, gálico e siníngico (BADERSCHNEIDER e WINTERHALTER, 2001), cujas estruturas químicas estão mostradas na Figura 3.2. Ácido gálico é descrito como o mais importante ácido

hidroxibenzóico, uma vez que é precursor de taninos hidrolisáveis e estão presentes na síntese de taninos condensados (GARRIDO e BORGES, 2011).

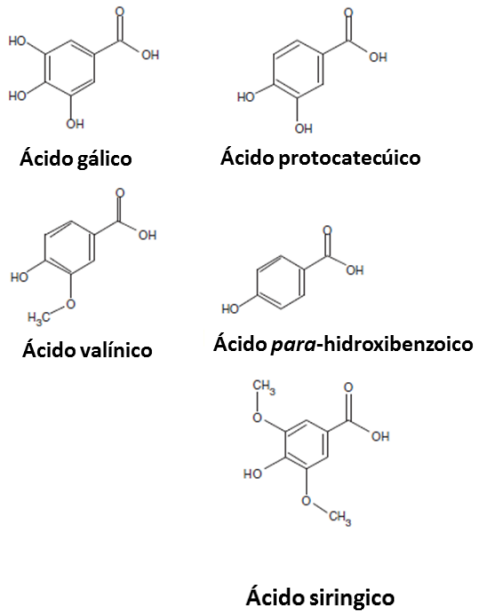


Figura 3.2 Estrutura química dos principais ácidos hidroxibenzóicos presentes em uvas (Fonte: MARTINS *et al.*, 2011).

3.2.1.2 Ácidos Hidroxicinâmicos

Ácidos hidroxicinâmicos são uma das principais classes encontradas em uvas (BADERSCHNEIDER e WINTERHALTER, 2001). Ácidos *para*-cumárico, caféico, felúrico e sinápico são alguns dos compostos mais referenciados na fruta. Eles estão associados ao processo de escurecimento de vinhos e são precursores de compostos fenólicos voláteis (KALLITHRAKA *et al.*, 2009). Os ácidos sinápticos, ferúlico e *p*-cumárico são antioxidantes mais ativos do que os derivados do ácido benzóico, tais como ácido protocatecuico, siríngico e vanílico, cujas estruturas químicas estão mostradas na Figura 3.3 (BALASUNDRAM *et al.*, 2006). Isso se deve à dupla ligação presente na molécula dos derivados do ácido cinâmico, que participa da estabilidade do radical por ressonância de deslocamento do elétron desemparelhado, enquanto que os derivados do ácido benzóico não apresentam essa característica (WANASUNDARA e SHAHIDI, 1994).

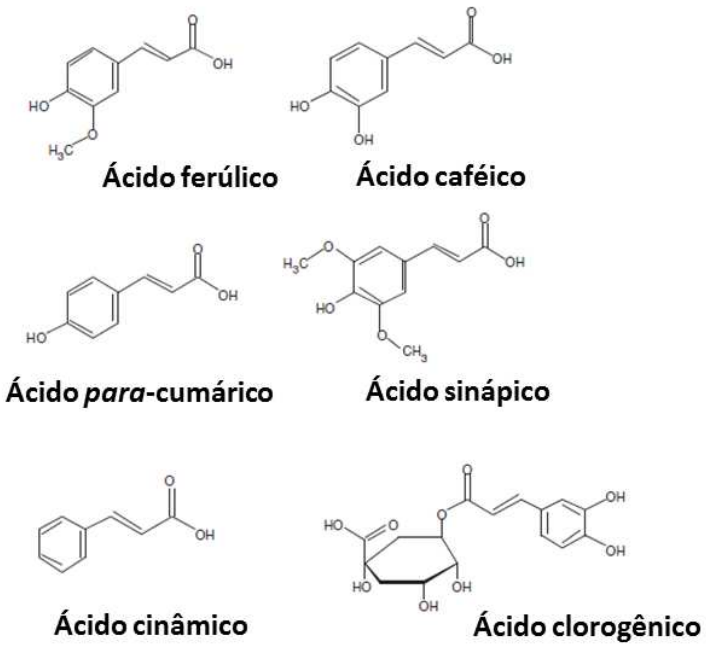


Figura 3.3 Estrutura química de ácidos hidroxicinâmicos mais comumente em uvas (Fonte: Martins *et al.*, 2011).

3.2.2 Flavonóides

Os flavonóides são compostos de baixa massa molar, consistindo em 15 átomos de carbono, que compreende dois anéis aromáticos, denominados anéis A e B, ligados através de uma cadeia de até três carbonos que formam um anel heterocíclico, denominado anel C, como mostrado na Figura 3.4. O anel aromático A é derivado do ciclo acetato/malonato, enquanto o anel B é derivado da fenilalanina (MERKEN e BEECHER, 2000). A maior concentração de flavonóides é encontrada na fase de floração das plantas, decrescendo com o seu crescimento (BADERSCHNEIDER e WINTERHALTER, 2001). Variações na substituição do anel C padrão resultam em importantes classes de flavonóides, como flavonóis, flavonas, flavanonas, flavanóis (ou catequinas), isoflavonas e antocianidinas (MERKEN e BEECHER, 2000).

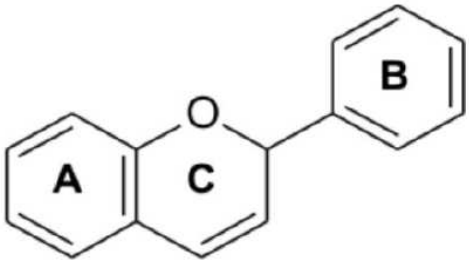


Figura 3.4 Estrutura química básica de flavonoides (Fonte: ANGELO e JORGE, 2007).

3.2.2.1 Flavonas

Flavonas, como mostrado na Figura 3.5, são caracterizadas pela presença de uma dupla ligação entre os carbonos C_2 e C_3 e pela ausência de um grupo hidroxila na posição C_3. Estes compostos são conhecidos por terem importante atividade farmacológica (MAXCHEIX *et al.*, 1990). Embora amplamente distribuídas em plantas, não estão presentes em grande quantidade em uvas, exceto para luteolina, eriodicitol e genisteína (ZOECKLEIN *et al.*, 1997).

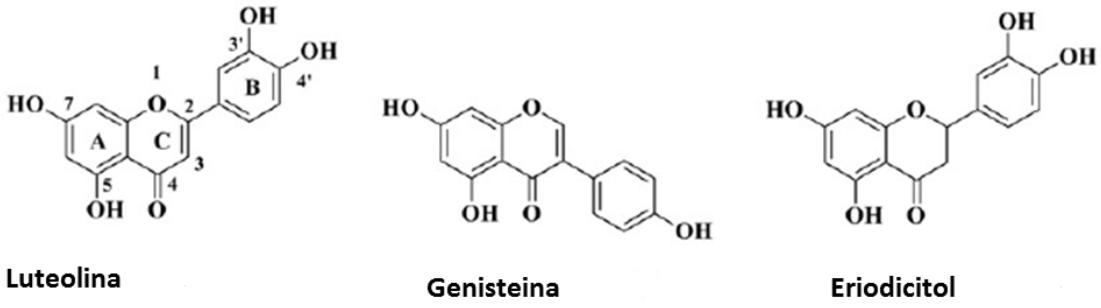
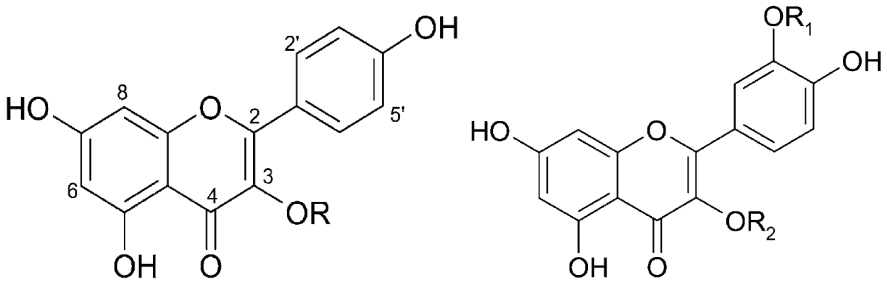


Figura 3.5 Estrutura química de flavonas (Fonte: ANGELO e JORGE, 2007).

3.2.2.2 Flavonóis

Os flavonóis são caracterizados pela presença de uma ligação dupla entre os átomos C_2 e C_3, além de um grupo hidroxila na posição C_3. Os principais flavonóis encontrados em uvas são a quercitina, a rutina e o kaempferol, cujas estruturas químicas estão mostradas na Figura 3.6 (SAGDIC *et al*., 2011). Eles encontram-se hidroxilados nos átomos C_3, C_5 e C_7 por moléculas de glucosídeos, glucoronídeos, galactosídeos e diglicosídeos. Amico e colaboradores (2004) identificaram quercitina como sendo o composto fenólico mais abundante em uvas "Nerelo Mascalese". Kaempferol foi identificado em pequenas quantidades nessa variedade de uva (AMICO *et al*., 2004).



R=H: Kaempferol; R_1=R_2=H: quercitina;

Figura 3.6. Estrutura química básica de kaempfenol e quercitina. (Fonte: AMICO *et al*., 2004).

3.2.2.3 Flavanóis

Flavanóis são benzopiranos que têm uma cadeia de carbono saturado entre C_2 e C_3, um grupo hidroxila no C_3 e nenhum grupo carbonila em C_4. Ambos, flavan-3-ols e flavan-3,4-dióis, podem ser encontrados na natureza, sendo este último grupo presente principalmente na madeira e casca de árvores, mas raramente encontrado em frutas. Flavan-3,4-dióis são também muitas vezes referidos como leucoantocianidinas. Os flavan-3-óis mais abundantes na natureza são as catequinas e as epicatequinas, cujas estruturas químicas estão mostradas na Figura 3.7. Estes compostos estão presentes na casca e na semente de uvas, sendo os principais responsáveis pelo sabor de vinhos brancos (LUNTE *et al*., 1988).

Em uvas da variedade “Narince”, “Gamay” e “Okuzgozu”, a catequina foi o flavanol encontrado em maior quantidade (SAGDIC *et al*., 2011).

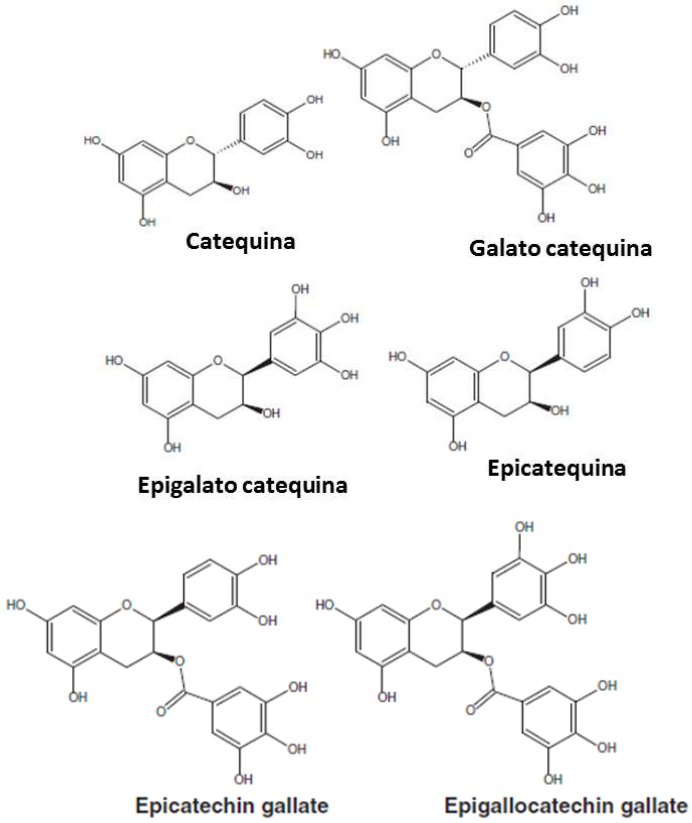


Figura 3.7 Estrutura química dos principais flavan-3-ols presentes em uvas (Fonte: SAGDIC *et al*., 2011).

3.2.2.4 Antocianinas

Antocianinas são pigmentos naturais presentes em frutas e verduras responsáveis pela cor rosa-violeta (LEE e WROLSTAD, 2005). Quimicamente, antocianinas são agliconas glicosiladas ou metoxiladas, sendo o aglicona chamado antocianidina. Elas apresentam estrutura baseada no cátion flavylium (2-fenilbenzopirilium) que consiste de dois anéis aromáticos unidos por uma unidade de três carbonos e condensados por um oxigênio (LEE e WROLSTAD, 2005). Na Figura 3.8 está apresentada a estrutura química

da molécula de antocianina que é constituída por dois ou três grupos funcionais: uma glicona (antocianidina), um grupo de açúcares e frequentemente um grupo de ácidos orgânicos (LEE e WROLSTAD, 2005.)

Em uvas vermelhas, cinco antocianidinas foram identificadas: cianidina (responsável pela cor vermelho-laranja), peonidina (vermelho), delfinidina (vermelho-azul), pelargonidina (laranja), petunidina e malvinidina (vermelho-azul) (Figura 3.8). Esta última é considerada a mais abundante antocianidina em *V. vinifera* (CASTILLO-MUÑOZ *et al*., 2010). Antocinidinas são encontradas na natureza geralmente na sua forma glucosilada, por exemplo, 3-monoglucosídeos, 3,5- e 3,7-diglucosídeos (KOPONEN *et al*., 2007). Monoglucosídeos, galactose, glicose, ramanose entre outros têm sido descritos como os açúcares mais comuns.

Nas videiras, as antocianinas acumulam-se nas folhas durante a senescência e são responsáveis pela coloração da casca da uva em cultivares tintos. A composição de antocianinas em frutas é considerada influenciada por vários fatores como a origem e o tipo de videira, o grau de maturidade, o tempo e as condições de cultivo da videira (SAGDIC *et al*., 2011).

Em bagaço de frutas vermelhas, as antocianinas tem demonstrado ser a classe de compostos fenólicos com maior poder antioxidante.

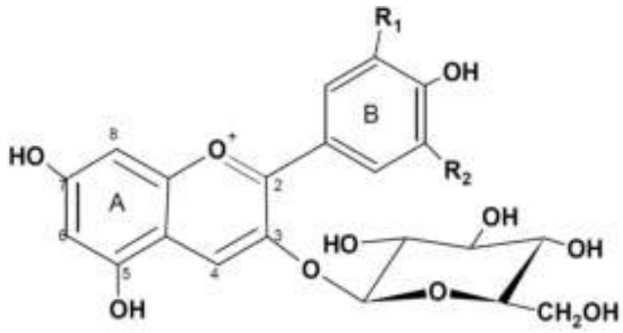


Figura 3.8 Estrutura química da molécula de antocianidina. Agliconas (estrutura do anel B): pelargonidina (R_1=R_2=H); cianidina (R_1=OH e R_2=H); delfinidina (R_1=R_2=OH); peonidina (R_1=OCH_3 e R_2=H); petunidina (R_1=OCH_3 e R_2=OH); malvinidina (R_1=R_2=OCH_3) (Fonte: GIUSTI e WROLSTAD, 2001).

Na Figura 3.9, as formas estruturais predominantes das antocianinas em pH 1,0, 4,5 e 7,0 estão apresentadas. Observa-se que a forma oxônio (que vai do laranja ao roxo) predomina em pH 1,0 e a forma hemiacetal (incolor) em pH 4,5. O método do pH diferencial, amplamente utilizado para quantificar antocianinas monoméricas (com correlação com a quantificação por cromatografia líquida de alta eficiência de 92%), baseia-se nesta reação, permitindo a quantificação mesmo na presença de pigmentos polimerizados degradados e de outros interferentes (GIUSTI e WROLSTAD, 2001).

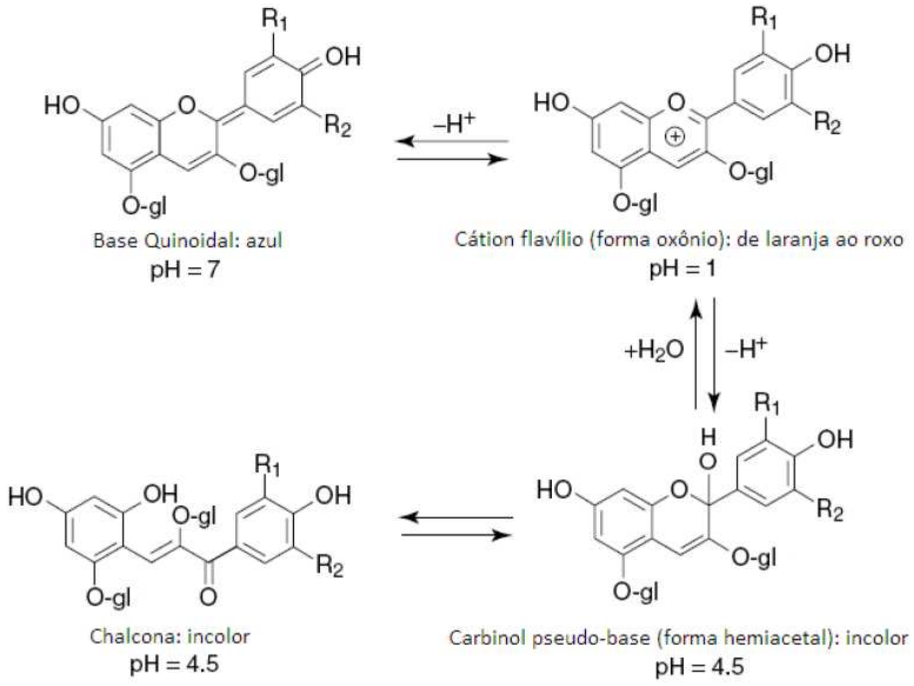


Figura 3.9 Formas estruturais de antocianinas em diferentes formas de pH (CASTAÑEDA-OVANDO *et al.*, 2009).

3.2.3 Taninos

Os taninos possuem massa molar relativamente alta e constituem uma classe de polifenóis que, segundo a estrutura química, são classificados em taninos hidrolisáveis e taninos condensáveis (LINSKENS e JACKSON, 1988; SCALBERT, 1993).

Os taninos condensáveis, também denominados proantocianidinas, são oligômeros e polímeros de flavan-3-ol (catequina) e/ou flavan-3,4-diol (leucocianidina), presentes principalmente, em uvas, derivados de catequinas e epicatequinas. Estes são a classe de taninos em maior quantidade em uvas (HE *et al.*, 2010; HE *et al*., 2008; ZHAO *et al*., 2010). As proantocianidinas, assim denominadas, provavelmente pelo fato de apresentarem pigmentos avermelhados da classe das antocianidinas, como cianidina e delfinidina, apresentam rica diversidade estrutural, resultante de padrões de substituições entre unidades flavânicas, diversidade de posições entre suas ligações e estereoquímica de seus compostos (MONTEIRO *et al*., 2005). São encontrados tanto na casca quanto nas sementes da uva. Procianidinas e prodelfinidinas, taninos condensados que, quando hidrolisados liberam cianidinas e delfinidinas, são os mais abundantes em *V. vinífera* (HE *et al*., 2010; HELLSTROM *et al*., 2009; ZHAO *et al*., 2010). A identificação e o estudo destes compostos são de grande relevância para a indústria de alimentos, uma vez que estes são responsáveis por características sensoriais (cor, sabor, adstringência e amargor) de alimentos derivados de uva. Além disso, eles têm papel de destaque no processo de envelhecimento de vinhos, devido à sua capacidade de oxidação, condensação e polimerização (GARRIDO e BORGES, 2011).

Taninos hidrolisáveis são polifenóis complexos que podem ser degradados pela mudança de pH, assim como por hidrólise enzimática ou não-enzimática, em fragmentos menores, principalmente açúcares e ácidos fenólicos. A unidade básica de taninos hidrolisáveis do tipo poliéster é o ácido gálico ou seus derivados. Eles estão geralmente esterificados com glicose, rendendo mais de 500 espécies possíveis de taninos hidrolisáveis (GARRIDO e BORGES, 2011). Taninos hidrolisáveis estão presentes principalmente nas sementes da fruta.

3.2.4 Estilbenos

Estilbenos são compostos fenólicos compostos de dois anéis aromáticos ligados por uma ponte de eteno. Resveratrol, cuja estrutura química está mostrada na Figura 3.10, é o estilbeno mais conhecido em frutas e vinhos. Ele está presente em folhas e na casca de uvas, e sua concentração decresce significativamente com o amadurecimento da fruta. Resveratrol também é considerado como uma toxina para a fruta se proteger contra ataques de fungos e sua biossíntese é fortemente influenciada por condições de estresse da fruta (LIMA *et al*., 1999; MORENO-LABANDA *et al*., 2004; PEZET *et al*., 2003; PÜSSA *et al*., 2006).

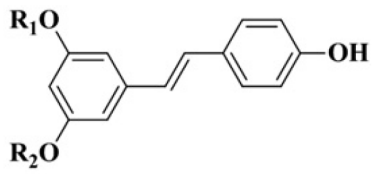


Figura 3.10. Estrutura básica de estilbenos. R_1=R_2=H: *trans*-resveratrol.

3.3 Osmose direta

A concentração é uma etapa crucial para indústrias processadoras de alimentos líquidos. Os principais objetivos de tal operação são (DOVA *et al*., 2007a):

1. redução de volume e massa, resultando em menores custos de armazenamento, embalagem e transporte;
2. redução da atividade de água, aumentando a estabilidade microbiológica e bioquímica do produto;
3. pré-tratamento para uma etapa de secagem do produto, caso seja necessário.

O processamento térmico permanece como o método mais empregado para a conservação e concentração de alimentos. No entanto, os tratamentos térmicos industriais

podem ter impactos negativos sobre os fatores nutritivos, como as antocianinas, carotenóides, vitaminas, proteínas bioativas (VAN DEN HOUT *et al.*, 1999; KECHINSKI *et al*., 2010; PROVESI *et al.*, 2011; BARROS *et al*., 2011), sobre parâmetros sensoriais, como cor, aroma, sabor (NISHA *et al*., 2009) e propriedades tecnológicas (ZAVAREZE e DIAS, 2011).

Para atingir a demanda atual de mercado, pesquisas recentes têm focado em tecnologias não térmicas. Processos de separação por membranas (PSM) são alternativas interessantes para clarificação e concentração de produtos alimentares líquidos, porque podem operar à temperatura ambiente, frequentemente apresentam menor consumo de energia, são de fácil escalonamento e têm rejeição de uma vasta gama de contaminantes alimentares. Os PSM mais empregados hoje em dia incluem micro, ultra, nanofiltração e osmose inversa (OI). Contudo, a exigência de alta pressão hidrostática, grande tendência ao *fouling* e o fato de não possibilitarem atingir alimentos com alta concentração de sólidos solúveis são limitantes técnicos importantes para a indústria.

Esforços na área de ciência, tecnologia e engenharia de alimentos têm atentado a novas tecnologias que visam maior retenção de compostos bioativos, maior rendimento, menores custos de processamento e de consumo de energia. A Osmose Direta (OD), também chamada de concentração osmótica, é um PSM, em que se utiliza uma solução como agente osmótico (AO) para criar um gradiente de pressão osmótica, através de uma membrana semi-permeável, e então remover água da solução de alimentação (SA) (CATH *et al*., 2006). Essa técnica já vem sendo utilizada na purificação de água do mar, tratamento de efluentes e concentração de alimentos líquidos (CATH *et al*., 2006). A Figura 3.11 apresenta um módulo esquemático de OD, onde a SA e o AO são bombeados em contra-corrente paralelamente a uma membrana e, devido à diferença de pressão osmótica entre as soluções, água é transportada da solução de menor pressão (SA) para a de maior pressão osmótica (AO).

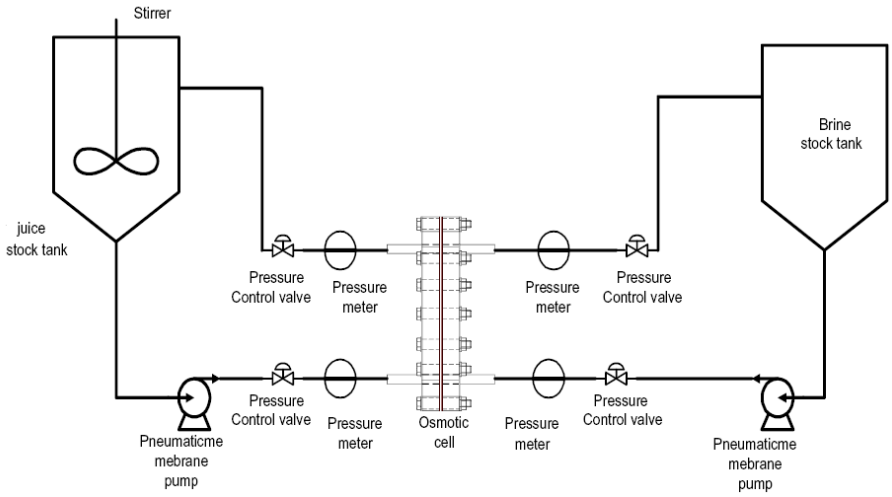


Figura 3.11 Aparato esquemático de concentração de suco por osmose direta (PETROTOS *et al.*, 2010).

As principais vantagens deste método em relação a outros métodos de PSM convencionais são:

1. uso de baixas pressões hidráulicas;
2. possibilidade de tratar soluções com alto teor de sólidos suspensos;
3. baixa incrustação das membranas e consequente baixo custo com sua reposição;
4. possibilidade de obter maior teor de sólidos solúveis no produto final;
5. facilidade de escalonamento;
6. baixo consumo de energia para o processamento do produto.

A aplicação de OD para concentração de alimentos líquidos é uma grande tendência atual, apesar de a primeira citação de tal operação ter sido na década de 60 (POPPER *et al*., 1966). Aspectos detalhados de concentração de alimentos líquidos serão apresentados em detalhes no Capítulo 5, artigo publicado pelo autor na revista Journal of Food Engineering.

A seguir, são descritos os efeitos dos principais parâmetros de OD sobre o fluxo de água transmembrana.

3.3.1 Solução de alimentação e solução osmótica

A solução concentrada de agente osmótico no lado do permeado da membrana é a fonte da força motriz do processo de OD. Termos diferentes são usados na literatura para nomear esta solução incluindo solução osmótica, agente osmótico, solução salina, salmoura, entre outros (CATH *et al*., 2006). Para fins de simplificação, neste texto ela será chamada de agente osmótico (AO). Ao selecionar um AO, o critério principal é que ele tenha uma maior pressão osmótica que a solução que se deseja concentrar (solução de alimentação). Outro aspecto importante é que o soluto que compõe o AO deve ser de fácil reconcentração, após a sua diluição ao longo do processo de osmose, a fim de minimizar os custos totais de operação (CATH *et al*., 2006).

A força motriz do processo de OD é a diferença de potencial químico da água, expresso em termos de pressão osmótica (π), entre AO e SA. A pressão osmótica de soluções diluídas pode ser calculada pela equação de Van't Hoff como mostra a Equação 1:

$$\pi = NRTi \tag{1}$$

onde, N é a concentração molar da solução (mol L^{-1}), R é a constante universal dos gases (8.314 J K mol^{-1}), T é a temperatura absoluta (K) e i é o fator de correção de Van't Hoff, relacionado com a ionização do soluto que compõe a solução.

Porém, para alimentos líquidos, o uso da relação de Van't Hoff se torna ineficaz. Assim, como sugerido por Babu e colaboradores (2006), pode-se relacionar π de soluções diversas com a atividade de água da solução, como mostra Equação 2, descrita em detalhes por Toledo (1991):

$$\pi = -\frac{RT}{V}\ln a_w \tag{2}$$

onde *R* é a constante universal dos gases (8.314 J K mol-1), *T* é a temperatura absoluta (K), *V* é o volume molar da água (18 mL mol^{-1}) e a_w é a atividade de água da solução.

A Figura 3.12 mostra a pressão osmótica de diferentes solutos em função da sua concentração. É possível observar que cloretos de magnésio e de cálcio apresentam maior pressão osmótica entre os sais apresentados. Contudo, esses solutos devem ser evitados em processos de OD, pois apresentam maior tendência ao *fouling*. Isso porque quando o AO for reconcentrado, geralmente usando OI, os cátions Mg^{+2} e Ca^{+2} formam incrustrações, causando problemas de *fouling* irreversível na membrana (ACHILI *et al.*, 2010).

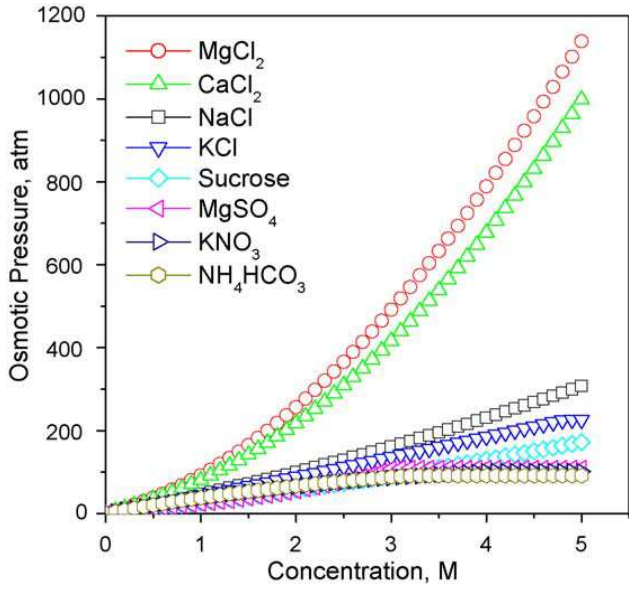


Figura 3.12 Pressão osmótica em função da concentração de diversos sais com potencial de serem usados como agente osmótico em processos de osmose direta (CATH *et al.*, 2006)

O impacto das características do AO em processos osmóticos pode ser explicado pela equação de Wilke-Chang (Wilke e Chang, 1955), no qual o coeficiente de difusão de massa é inversamente proporcional à viscosidade do AO utilizado. Petrotos e colaboradores (1998) avaliaram vários solutos para compor o AO para concentrar suco de tomate. Os autores mostraram que soluções de cloreto de sódio tiveram melhor desempenho entre soluções de glicose, sacarose, cloreto de cálcio e polietileno glicol testadas. Tal fato foi creditado à menor viscosidade da solução de cloreto de sódio, fato este confirmado por You

e colaboradores (2012), que verificaram que a viscosidade tem grande impacto no aumento do fluxo de água em OD. AO menos viscosos apresentam maior desempenho de fluxo, devido à diminuição da resistência à transferência de massa através da camada limite de polarização por concentração, implicando em maior difusividade mássica (BABU *et al*., 206). Por isso, cloreto de sódio tem sido o principal soluto escolhido para compor o AO, devido à sua alta pressão osmótica e solubilidade em água, não toxicidade, baixa viscosidade e sua relativa simplicidade de reconcentração (CATH *et al*., 2006).

Em relação à SA, alimentos mais concentrados tendem a ter maior viscosidade, o que prejudica o desempenho do processo. Em trabalho realizado por Garcia-Castello e colaboradores (2009), os autores observaram queda no fluxo de água transmembrana com o aumento da concentração da solução de sacarose, usada como SA. Durante a concentração de suco de tomate de 4,3 para 11,8 °Brix, Petrotos e colaboradores (1998) verificaram que o fluxo de água diminuiu linearmente, alcançando redução de 53% do fluxo de água inicial no final do processo.

Garcia-Castello e McCutcheon (2011) verificaram que, em um sistema modelo de liquor de laranja como SA, a presença de pectina teve efeito dominante na redução do fluxo de água, devido à intensa formação de *fouling* na membrana. Petrotos e colaboradores (1999) estudaram a microfiltração e da ultrafiltração do suco de tomate como um pré-tratamento para a concentração de suco de tomate por OD. Seus resultados mostraram que o fluxo de água no processo de OD aumentou 2,4 vezes após o uso de filtrações prévias, fato este relacionado à menor viscosidade e da separação parcial da pectina no suco de tomate devido à microfiltração e ultrafiltração.

3.3.2 Membranas de OD

Geralmente, qualquer membrana densa, seletivamente permeável, pode ser usada em processos de OD. Popper *et al*. (1966) utilizaram a primeira geração de membranas para OI para concentrar sucos de frutas. Os autores utilizaram membranas tubulares e planas para tal operação, porém verificaram uma forte difusão de sal para o suco concentrado, o que desencorajou pesquisas na área de alimentos com o uso de OD por longo tempo.

Beaudry e Lampi (1990) utilizaram membranas para OI de poliamida aromática, reduzindo a espessura da camada suporte (o que acarretou em um aumento do fluxo permeado) e a camada seletiva, possuindo uma massa molar de corte de 100 Da, o que impediu a passagem 99,9% de solutos através da membrana. Esse tipo de membrana ainda é utilizada em experimentos para concentração de alimentos líquidos (DOVA *et al*., 2007a, 2007b; PETROTOS *et al*., 1998; PETROTOS *et al*., 1999; PETROTOS *et al*., 2010).

Atualmente, membrana de acetato de celulose, é atualmente a mais utilizada para processos de osmose direto, apresentando uma espessura da camada seletiva (50 μm) mais fina do que a de poliamida aromática (160 μm) utilizada para OI e por isso apresenta rendimento de concentração mais elevado (GARCIA-CASTELLO *et al*., 2009). Ainda, o acetato de celulose apresenta características mais hidrofílicas do que a poliamida, o que é essencial para o fenômeno de molhabilidade da membrana no processo de osmose, impactando diretamente no fluxo de água transmembrana (GARCIA-CASTELLO *et al*., 2009).

Vários trabalhos têm apresentado estudos sobre síntese de membranas para investigar o incremento do desempenho da OD. Poliamida, polietersulfona, polibenzimedazola entre outros polímeros têm sido estudados para a fabricação de membranas planas, tubulares e de fibras ocas com incrementos de até 150% em relação às membranas de acetato de celulose comercial (CHOU *et al*., 2001; WANG *et al.*, 2007; WEI *et al*., 2010; YU *et al*., 2011). Estes trabalhos, contudo, visam à purificação de águas salinas e informações sobre suas eficiências em sistemas alimentares são escassos na literatura.

3.3.3 Polarização por concentração

O processo de osmose através da membrana ocorre após a solução que flui contra a camada suporte se difundir pelos poros e atingir a interface da camada seletiva. Então, por diferença de potencial químico, expresso em termos de pressão osmótica, água é transportada da SA para AO. Com isso, a principal limitação tecnológica deste processo é a criação de um gradiente de concentração próximo à superfície ou dentro da membrana, fenômeno conhecido como polarização por concentração (PC).

A Figura 3.13 apresenta esquematicamente o fenômeno de PC, sendo água como alimentação e o AO como sendo uma solução de cloreto de sódio. Na Figura 3.13A é mostrado o AO escoando contra a camada densa e a alimentação contra a camada suporte. Nessas condições, no lado do AO, o fenômeno de polarização por concentração extrena (PCE) é considerada negligenciável, uma vez que a solução é composta por um composto de baixa massa molar (NAYAK e RASTOGI, 2010). Já no lado da alimentação, como é água que está escoando, não ocorrem fenômenos de PC, pois ela é uma solução com compostos de baixa massa molar e em pequenas concentrações. Já na Figura 3.13B, a solução de cloreto de sódio escoa contra a camada suporte. Como o AO deve escoar até a interface da camada densa para que ocorra a osmose, ele é gradativamente diluído pela água que é transportada pela camada densa, ocasionando um gradiente de concentração dentro da camada suporte, fenômeno esse chamado de polarização por concentração interna (PCI) dilutiva. Esse fato reduz a pressão osmótica do AO de π_{AO} para π_{PCI}, reduzindo a força motriz do processo de $\Delta\pi_{aparente}$ para $\Delta\pi_{real}$. Nessas condições, a PCE no lado do AO é considerado negligenciável. $\Delta\pi_{aparente}$ é a força motriz esperada, sendo calculada a partir da pressão osmótica da solução *bulk*, e $\Delta\pi_{real}$ é a força motriz real que rege o fenômeno de osmose, prejudicado pela PCI.

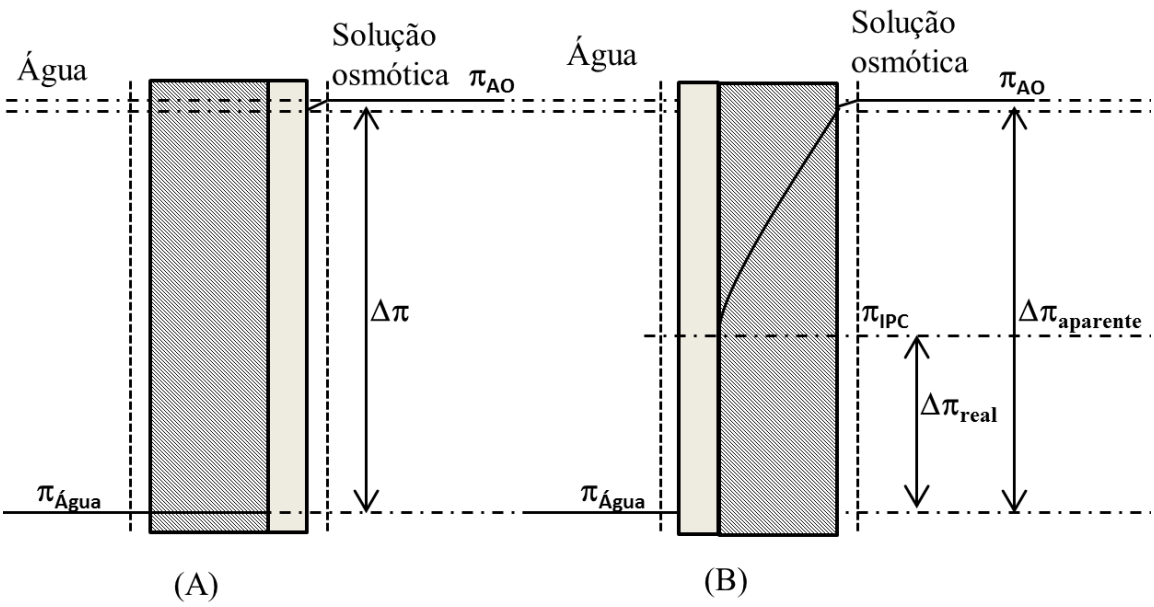


Figura 3.13 Mecanismo de osmose direta indicando o transporte de água da solução de menor pressão osmótica para a de maior pressão osmótica e as polarizações por concentração. Água é utilizada como alimentação e solução de cloreto de sódio é utilizado como agente osmótico (A) alimentação escoando contra a camada suporte da membrana; (B) alimentação fluindo contra a camada densa da membrana. Na figura, $\pi_{Água}$ e π_{AO} são as pressões osmóticas da água e do agente osmótico, respecitvamente. $\Delta\pi$ e $\Delta\pi_{apparent}$ é a diferença de pressão osmótica calculada como $\pi_{AO} - \pi_{Água}$. $\Delta\pi_{real}$ é a diferença de pressão osmótica real do processo de OD, menor que $\Delta\pi_{aparente}$, devido à redução da pressão osmótica da solução de sal de π_{AO} para π_{IPC}.

Os fenômenos de PC são mais intensos quanto maior for a massa molar dos compostos que compõe as soluções (NAYAK *et al*., 2011). A Figura 3.14 mostra, agora, a SA como sendo um alimento líquido. Quando ele escoa contra a camada suporte (Figura 3.14A) ocorre PCE, devido ao depósito de compostos de alta massa molar na superfície da membrana. A concentração do suco na entrada da camada porosa acarreta o aumento da pressão osmótica da solução de π_{suco} para π_{PCE}^{Suco}. Ainda, há intensa PCI à membrana, ocasionando maior aumento da sua pressão osmótica na interface da camada densa (de π_{PCE}^{Suco} para π_{PCI}^{Suco}), onde ocorre o transporte de água. Nesse modo de operação, ainda, há grande tendência de efeitos abrasivos à membrana devido a compostos que existem no alimento.

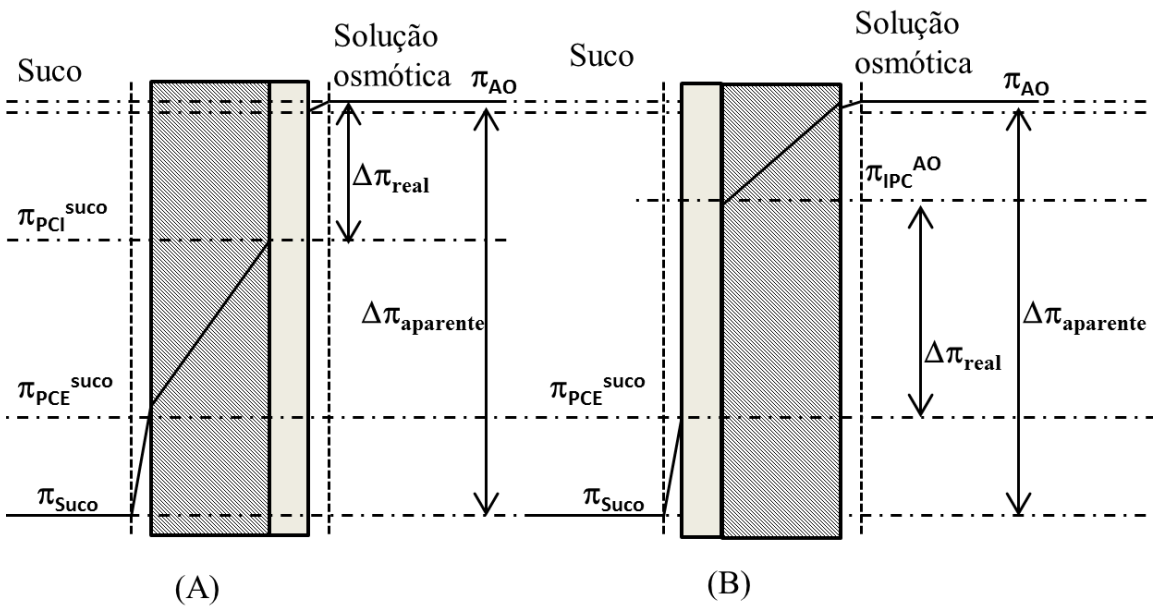


Figura 3.14 Mecanismo de osmose direta indicando o transporte de água da solução de menor pressão osmótica para a de maior pressão osmótica e as polarizações por concentração. Suco é utilizado como alimentação e solução de cloreto de sódio é utilizado como agente osmótico. (A) alimentação escoando contra a camada suporte da membrana; (B) alimentação fluindo contra a camada densa da membrana. Na figura, π_{Suco} e π_{AO} são as pressões osmóticas do suco e do agente osmótico, respecitvamente. $\Delta\pi$ e $\Delta\pi_{apparent}$ é a diferença de pressão osmótica calculada como $\pi_{AO} - \pi_{Suco}$. $\Delta\pi_{real}$ é a diferença de pressão osmótica real do processo de OD, menor que $\Delta\pi_{aparente}$, devido à redução da pressão osmótica devido aos fenômenos de polarização. π_{PCE} e π_{PCI} são as pressões osmóticas das soluções devido à polarização por concentração extrena e interna, respectivamente.

Como mostra a Figura 3.14B, a PCI no lado do AO, quando ele escoa contra a camada porosa, é menos intensa do que se escoasse suco, pois ele é composto por solutos de menor massa molar, não acarretando em prejuízos tão intensos quanto o outro caso. Assim, $\Delta\pi_{real}$ quando suco escoa contra a camada densa é maior que quando ele escoa contra a camada suporte, como mostra a Figura 3.14. Com isso, é indicado que, para a concentração de alimentos líquidos por OD, a SA escoe contra a camada densa e o AO contra a camada suporte, com a finalidade de minimizar os efeitos de PC (NAYAK e RASTOGI, 2010).

O aumento da vazão de alimentação de SA e AO tende a aumentar o fluxo de água transmembrana em OD. Issto é atribuído à redução na espessura da camada limite

hidrodinâmica de PC, aumentando a força motriz real do processo. O aumento da vazão de alimentação ainda reduz a resistência à transferência de massa da camada polarizada adjacente à superfície da membrana e, consequentemente, aumentando o fluxo transmembrana (DOVA *et al*., 2007b). Dova e colaboradores (2007b) verificaram que o transporte de água pela membrana é proporcional à vazão de alimentação da SA e exponencialmente proporcional à vazão de alimentação do AO. Além disso, o aumento da vazão de alimentação da SA pode aumentar as forças de cisalhamento na superfície da membrana, resultando em menor espessura torta depositada sobre a superfície da membrana, principalmente de pectina (LEE *et al*., 2010).

O aumento da temperatura das soluções também afeta positivamente o fluxo osmótico. A Equação Wilke-Chang descreve que o coeficiente de difusão de massa é proporcional à temperatura absoluta (Wilke e Chang, 1955). Além disso, aumentando a temperatura do processo, há redução da viscosidade das soluções, o que resulta em aumento do fluxo de água pela membrana.

Uma alternativa para acelerar o desempenho de filtração em OD é promover a turbulência das soluções dentro do módulo de membrana. Por isso, espaçadores são geralmente utilizados como promotores de turbulência dentro do módulo de membranas. No trabalho de Dova e colaboradores (2007a) e Petrotos e colaboradores (2010), uma nova configuração de módulo de membrana para OD foi apresentado. A parte do módulo que entra em contato com a SA foi equipada com defletores ortogonais, proporcionando distribuição mais uniforme da solução na superfície da membrana e promovendo grande turbulência no sistema. Contudo, tanto o uso de espaçadores quanto de outros promotores de turbulência prejudica a comparação de resultados de diferentes trabalhos ao redor do mundo, sendo sugerido por Cath e colaboradores (2012) que não sejam usados promotores de turbulência em experimentos laboratorias com a finalidade de padronização de experimentos.

3.4 Bagaço de uva

O bagaço de uva, subproduto mais importante da vinificação e da produção do suco de uva, se apresenta como fonte de compostos antioxidantes e fibras. Isto, aliado ao baixo custo, confere a este material ampla possibilidade de sua aplicação como ingrediente funcional na indústria de alimentos e farmacêutica.

O cenário hoje visto no Brasil é a utilização deste resíduo de forma "não nobre", ao usarem o subproduto da vinificação e da produção do suco de uva como adubo ou como alimentação animal sem processamento prévio. Com isso, esforços na área de alimentos, pecuária, biotecnologia e fármacos têm sido realizados com vistas ao aproveitamento de bagaço de uva (BOTELL *et al.*, 2005, 2007; ROTIVA, 2007; FARINELLA *et al*., 2008; BASALAN *et al*., 2011).

Contudo, a principal aplicação que o bagaço de uva tem sido alvo é para a extração dos compostos fenólicos remanescentes tanto na casca quanto nas sementes e nos caules (MANTELL *et al*., 2002). Propriedades fisiológicas relacionadas ao consumo de extratos de casca, sementes ou a combinação destes têm sido descritas por diversos autores nos últimos anos, sempre correlacionado o conteúdo fenólico ao efeito nutricional e/ou biológico gerado ao consumidor (SHANMUGANAYAGAM *et al*., 2002).

Amico e colaboradores (2004), ao analisarem bagaço de *V. vinífera* cv. "Torrevechia" verificaram que o grupo das antocianinas representa 1,1% da massa do resíduo, enquanto 4,3% são constituídos pelo grupo dos flavonóides. A antocianidina em maior quantidade no bagaço foi a malvinidina, enquanto a quercitina foi responsável pela maior presença no grupo dos flavonóides. Sagdic e colaboradores (2011) verificaram que 24,75% dos compostos fenólicos presentes em *V. vinífera* cv. "Gamay" são flavonóides, enquanto cerca de 9% são ácidos fenólicos. Nesta variedade, o ácido gálico foi o ácido fenólico encontrado em maior quantidade, enquanto, no grupo dos flavonóides, a catequina foi o principal composto da classe. Além de mostrar grande correlação entre a atividade antioxidante dos extratos de bagaço de uva com o conteúdo de fenólicos totais, os autores ainda observaram grande inibição de *Zygosaccharomyces rouxii* e *Zygosaccharomyces bailii*, importantes fungos fitopatogênicos. Atividade antimicrobiana já vista em trabalho de

Baydar e colaboradores (2004), que observaram poder antibacteriano de extratos de *V. vinífera* cv. “Narince” contra uma vasta gama de bactérias como *Aeromonas hydrophila, Bacillus amyloliquefaciens, Bacillus, Bacillus cereus, Enterococcus feacalis, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus*, entre outros. Negro e colaboradores (2003) observaram que o extrato de bagaço de *V. vinífera* cv. “Negro Amaro” apresenta, em base seca, 4,2% de fenólicos totais, 4% de flavonóides, 1% de antocianinas, 2,2% de taninos condensados e 1,3% de proantocianidinas. Os autores observaram também que o extrato apresenta atividade antioxidante equivalente ao di-terc-butil metil fenol (BHT), protegendo cerca de 90% da oxidação de β-caroteno para o extrato contendo 160 ppm de compostos fenólicos. Os resultados mostram grande potencial para o extrato ser utilizado como alternativa na indústria de alimentos.

Outro aspecto interessante de resíduos de frutas é sua alta concentração de fibras, representando cerca de 60% de sua massa seca (VALIENTE *et al.,* 1995). Saura-Calixto (1998) identificou fibras no bagaço de uva *V. vinífera* cv. “El Granero” com características estruturais diferentes daquelas já conhecidas. Estas diferenças estão intimamente ligadas ao fato de estarem associadas a compostos fenólicos, sendo denominadas pelo autor como fibra alimentar antioxidante. Este aspecto tem valorizado muito o perfil nutricional do bagaço de uva, tendo já produtos à base de bagaço de uva sendo comercializados em todo o mundo (MONAGAS *et al*., 2006). Shanmuganayagam e colaboradores (2002) observaram um efeito sinérgico entre substâncias bioativas presentes na casca e na semente de uva, como por exemplo, a agregação plaquetária de humanos, mostrando que o uso individual de casca ou semente, como algumas pesquisas avaliam, não é a alternativa mais interessante para efeitos fisiológicos.

Ishimoto (2008) patenteou no Brasil *sorbet* e picolé adicionado de bagaço de uva com boa aceitação sensorial, além de significativo incremento de fibras e atividade antioxidante do produto. A referida autora ainda observou que bagaço de uva provindo da fabricação de vinho (*V. vinífera* cv. Bordô) apresentou menores teores de compostos fenólicos totais que aquele vindo da produção de suco (*V. vinífera* cv. “Isabel”). Isso se deve à diferença de processamento que passam as duas variedades de uva, uma vez que o

tempo de contato do mosto de uvas Bordô durante a produção de vinho é maior do que durante a produção de suco de uva, permitindo um transporte de compostos do bagaço para o produto líquido mais intenso. Com isso, o uso bagaço de suco de uva para aplicações industriais é mais interessante que o de vinho devido ao seu maior teor de polifenóis na matriz vegetal (ISHIMOTO, 2008).

3.5 Extração sólido-líquido de compostos fenólicos de matrizes vegetais

Inúmeros estudos têm sido publicados nos últimos anos avaliando diferentes formas de extrair compostos fenólicos de matrizes vegetais (BORGES *et al*., 2011; CLADERA-OLIVERA, 2008; GUERRERO *et al*., 2008; KECHINSKI, 2011; MANTELL *et al*., 2002; MUÑOZ *et al*., 2004), a fim de aproveitá-los como ingrediente funcional para composição de alimentos e fármacos.

Entre os métodos de extração de compostos fenólicos de plantas, a extração sólido-líquido é, sem dúvida, o mais estudado. Ele consiste em manter a matriz vegetal desidratada em contato com o solvente, sob agitação, causando o transporte de compostos fenólicos dos sólidos para o líquido (CACACE e MAZZA, 2003). Os principais fatores que influenciam o rendimento de extração desses compostos através desta forma de operação são: tempo, temperatura, natureza do solvente, composição do solvente, razão entre volume de solvente e massa de sólidos (*ratio*), pH e tamanho das partículas da matriz vegetal (BORGES *et al.*, 2011; CLADERA-OLIVERA, 2008; GUERRERO *et al*., 2008; KECHINSKI, 2011; MANTELL *et al*., 2002; MUÑOZ *et al.*, 2004).

Não há consenso na literatura sobre o melhor solvente a ser utilizado para extrair compostos fenólicos. Muitos autores utilizam apenas um tipo de solvente para extrair compostos fenólicos de matrizes alimentares. Denev e colaboradores (2010) utilizaram solução aquosa de ácido cítrico (1%) para extrair antocianinas de amora, mirtilo e groselha preta, justificando a escolha pelo fato de que metanol e acetona, apesar de apresentarem melhor rendimento de extração, são tóxicos, e que a remoção completa do etanol para posterior utilização em alimentos é difícil. Cladera-Olivera (2008), ao estudar extração de compostos fenólicos de casca de pinhão, verificou que metanol apresentou melhor

rendimento de extração, aumentando em 25 e 80% o rendimento de extração em relação ao uso de etanol ou água, respectivamente. Lafka e colaboradoress (2007) verificaram que etanol, entre metanol, acetona, isopropanol e etil-acetato, foi o melhor solvente para extrair compostos com atividade antioxidante de resíduos de vinho. Guerrero e colaboradores (2008), comparando rendimento de extração de compostos fenólicos de bagaço de uva em estado semi-contínuo, verificaram que água apresentou melhor rendimento e taxas de extração do que etanol. Gan e Latiff (2011), ao extrair compostos bioativos de feijão de Petai (*Parkia speciosa*), observaram que acetona apresentou melhores condições de remoção dos compostos alvo em comparação com metanol, etanol, hexano, água e etil-acetato. Borges e colaboradores (2011) avaliaram água, metanol, etanol e acetona para extrair flavonóis e antocianinas de açaí. Os autores identificaram metanol como sendo o solvente a ser usado para melhor rendimento de remoção destes compostos da matriz da fruta. Contudo, etanol é o solvente mais utilizado para aplicação do extrato na indústria de alimentos e farmacêutica, por não ser tóxico e apresentar em geral maior rendimento de extração que o uso de água (KECHINSKI, 2011; MANTELL *et al*., 2002). A combinação de água e solventes orgânicos é geralmente a melhor opção para extração de compostos bioativos. O aumento da concentração de solvente reduz a constante dielétrica do líquido extrator, diminuindo a solvatação das moléculas e, com isso, aumenta sua difusão através da redução da interação com o solvente (CACACE e MAZZA, 2003). No entanto, não é indicado o uso de solvente orgânico puro, provavelmente devido à rápida desidratação da célula vegetal, não permitindo que o líquido extrator seja transportado de forma eficaz para dentro da célula e extrair os compostos bioativos da matriz sólida. Solventes com concentrações na faixa entre 50 e 80% são, em geral, os que apresentam melhores rendimentos na extração de compostos bioativos de matrizes vegetais (BALLARD *et al*., 2009; CLADERA-OLIVERA, 2008; KARACABEY e MAZZA, 2010; KIM *et al*., 2010; SUN *et al*., 2011).

Consenso também não é encontrado entre os diversos estudos quanto à temperatura de extração. Cacace e Mazza (2003) verificaram 30 °C como melhor temperatura de extração de compostos fenólicos de frutas vermelhas trituradas, creditando a baixa extração em temperaturas maiores à degradação térmica. Mantell e colaboradores (2002) verificaram

aumento da taxa e do rendimento de remoção de antocianinas do bagaço de uva na faixa de temperatura de 30-60 °C. Bucic-Kojic e colaboradores (2007) verificaram que a temperatura de 70 °C foi a melhor opção para extração de compostos fenólicos de sementes de uva. Comportamento semelhante foi observado por Cladera-Olivera (2008) ao extrair compostos fenólicos de casca de pinhão e por Sun e colaboradores (2011) ao extrairem compostos bioativos de *Ilex kudingcha*. Já quando foi utilizada água pura como líquido extrator, os últimos autores verificaram 92 °C como sendo a temperatura ótima de extração (SUN *et al*., 2011).

Quanto ao tamanho de partícula, é possível verificar que quanto menor é a dimensão da matriz vegetal utilizada como fonte de compostos fenólicos, maiores são o rendimento e a taxa de extração (BUCIC-KOJIC *et al*., 2007; CACACE e MAZZA, 2002; PINELO *et al*., 2006). Este fenômeno está ligado à maior superfície de contato dos sólidos com o líquido, além dos processos difusivos que ocorrem dentro da célula, que são inversamente proporcionais ao diâmetro das partículas.

O aumento da razão entre o volume de solvente e a massa de matriz vegetal – *ratio* – acompanha, em geral, o aumento do rendimento de extração (BORGES *et al*., 2011; CACACE e MAZZA, 2003). Este fenômeno é consistente com os princípios de transferência de massa, uma vez que a força motriz durante o transporte dos polifenóis dentro do sólido é considerada o gradiente de concentração e, quanto maior o volume de solvente em relação à massa de sólido, mais lenta é a diminuição do gradiente de concentração, resultando em um aumento da difusão. Cacace e Mazza (2003) verificaram que o aumento do *ratio* na faixa de 6 a 74 mL g^{-1} aumentou significativamente o rendimento de extração de compostos fenólicos totais e antocianinas de frutas vermelhas trituradas. Comportamento semelhante foi observado por Borges e colaboradores (2009) ao utilizarem solvente na faixa de 20 a 120 mL g^{-1} para extrair antocianinas, polifenóis totais e compostos com atividade antioxidante de açaí. Contudo, existe um limite para o coeficiente de partição dos fenólicos no solvente (CACACE e MAZZA, 2003), ocasionando que o excesso de solvente não influencia no rendimento de extração ou, em alguns casos, pode reduzir a eficiência de extração. Cladera-Olivera (2008) verificou que o aumento do *ratio* de 25 para 75 mL g^{-1} teve um efeito negativo para extração de compostos com atividade

antioxidante de casca de pinhão. Karabacey e Mazza (2010) observaram que o aumento do volume de etanol de 50 para 100 mL para extrair compostos bioativos de 1 g de cana de uva não afetou o rendimento da extração.

A redução do pH do meio extrator, de forma geral, aumenta o rendimento de extração de compostos fenólicos. Bravo (1998) classificou os polifenóis de alimentos em dois grupos: extraíveís e não-extraíveis. Os polifenóis extraíveis são fenólicos de baixas e intermediárias massas molares que podem ser extraídos usando diferentes solventes misturados à água. Polifenóis não-extraíveis são compostos de alta massa molar (taninos condensados e compostos fenólicos hidrolisável) ou polifenóis fortemente ligados a fibras dietéticas e proteínas e que podem ser encontrados nos resíduos da extração extrato aquoso-alcóolico. A diminuição do pH contribui positivamente na força iônica do extrato a fim de romper as ligações entre as fibras e os compostos fenólicos (BRAVO e SAURA-CALIXTO, 1998). Kapakasalidis e colaboradores (2006) verificaram grandes concentrações de antocianinas, de polifenóis e de compostos com atividade sequestrante do radical ABTS fortemente ligados a matriz de bagaço de amora preta e que não eram extraíveis com solvente hidroalcoólico, apenas após tratamento em baixo pH e alta temperatura. Revilla e colaboradores (1998) verificaram que antocianinas não-aciladas (delfinidina, cianidina, petunidina, peonidina e malvinidina) de bagaço de uva são extraíveis em grande quantidade em condições ácidas, possivelmente devido à hidrólise parcial desses compostos gerando antocianidinas no extrato. A extração de antocianinas e compostos fenólicos de bagaço de uva foi significativamente aumentada com a adição de 1% de HCl 12 mol L^{-1} (REVILLA *et al*., 1998).

Estudos recentes utilizaram a metodologia de superfície de resposta para investigar o efeito do tempo sobre o rendimento de extração de compostos fenólicos de material vegetal (BORGES *et al*., 2011; KECHINSKI, 2011; PINELO *et al*., 2006; SUN *et al*., 2011). No entanto, o uso de modelos matemáticos de cinética de extração facilita consideravelmente a otimização, o projeto, a simulação e o controle de processos, além de contribuir para melhor utilização de tempo e energia (AMENDOLA *et al*., 2010). O objetivo da modelagem matemática é avaliar o efeito de diferentes tratamentos sobre a taxa de remoção desses compostos da planta para o solvente, podendo, então, se estimar a

extração de compostos bioativos em diferentes condições de processamento (VAN BOEKEL, 2008).

Os principais modelos utilizados para avaliar a cinética de extração de compostos bioativos em publicações recentes são mostrados na Tabela 3.1.

Tabela 3.1. Modelos cinéticos de extração de compostos bioativos de materiais vegetais.

Modelo (nº)	Equação	Referência
Primeira ordem (3)	$C = kt^{-n}$	OTHMER e JAATINEN (1959)
Tipo Weibull (4)	$C = C_0 \exp\left(kt^n\right)$	AMENDOLA *et al*. (2010)
Sorção/desorção (5)	$C = {t}/{K_1 + K_2 t}$	PELEG (1988)
Duas razões (6)	$C = A\left[1-\exp\left(-k_1 t\right)\right] + C\left[1-\exp\left(-k_2 t\right)\right]$	CACACE e MAZZA (2003)
Molhamento/Difusão (7)	$C = \frac{C_\infty^W t}{t_{1/2} + t} + C_\infty^d \left[1-\exp\left(-k_d t\right)\right]$	LINARES *et al*., (2010)
Pseudo primeira ordem (8)	$C = C_\infty - {C_\infty}/{\exp\left(kt + a\right)}$	SPIRO e JAGO (1982)
Minchev e Minkov (9)	$C = A - B\exp\left(-kt\right)\right)$	(MINCHEV e MINKOV, 1984)

Nas equações apresentadas na Tabela 3.1, C_0 e C representam a concentração de composto bioativo extraído (presente no extrato) no tempo zero e t (min), respectivamente, e k (min^{-1}) é a taxa de extração, constante, a uma dada temperatura.

As equações 3 a 6 assumem que a extração ocorre indefinidamente. Valores de n, nas equações 3 e 4, representam um fator de escala da curva de distribuição. Franco e colaboradores (2007) utilizaram a equação proposta por Othmer e Jaatinen (1959) para modelar a cinética de extração de óleo e compostos com atividade antioxidante de rosa mosqueta em diferentes *ratios*, com ótima adequação dos dados experimentais à curva de tendência. Amendola e colaboradores (2010) verificaram que a equação tipo-Weibull se adequou de forma satisfatória à cinética de extração de compostos fenólicos de bagaço de uva a uma temperatura de 60 ºC.

O modelo de Peleg (Equação 5) é baseado no mecanismo de adsorção/dessorção para remover compostos de material vegetal, sendo K_1 a taxa constante de extração e K_2 a constante de capacidade de Peleg. Bucic-Kojic e colaboradores (2007) utilizaram esta equação para modelar a extração de compostos fenólicos de semente de uva e avaliar o efeito da temperatura e tamanho de partículas sobre o rendimento da extração. Os autores verificaram que quanto menor o tamanho da partícula maior é o redimento e que, na faixa de 25-80 ºC, a maior temperatura rendeu melhores resultados de extração, apresentando o processo energia de ativação entre 7,7 e 1,1 kJ mol^{-1} para a semente com tamanho de partícula entre 0,63 e 0,125 mm. Qu e colaboradores (2010) também utilizaram o modelo de Peleg (Equação 5) para estudar a extração de compostos fenólicos de bagaço de uva. Os autores verificaram que menores tamanhos de partícula e *ratio*, além de maiores temperaturas, apresentaram melhores condições de extração, com energia de ativação de 14,54 kJ mol^{-1}.

O modelo de duas taxas (Equação 6) considera dois períodos de extração, relacionadas com compostos acessíveis e inacessíveis (dentro e fora das células das plantas, respectivamente), sendo k_1 e k_2 as taxas de extração de duas classes diferentes de compostos, respectivamente, e A e C são constantes. Cacace e Mazza (2003) utilizaram esta equação para adequar dados de extração de compostos fenólicos e antocianinas de frutas vermelhas trituradas.

No modelo proposto por Linares e colaboradores (2010) (Equação 7), C_w^{∞} é a concentração de equilíbrio e $t_{1/2}$ o tempo de meia extração devido ao processo de lavagem/inchaço do material. C_d^{∞} e k_d são a concentração de equilíbrio e a taxa de extração, respectivamente, do processo acontecendo devido ao mecanismo de difusão. Os autores utilizaram esta equação para modelar a extração de sólidos solúveis de erva mate, sugerindo ao final do trabalho que este modelo pode ser utilizado para extração de óleos e biocompostos de matrizes vegetais.

O modelo de pseudo-primeira ordem (Equação 8) considera que uma concentração de equilíbrio é alcançada ao longo do processo de extração e a constante "*a*" é uma constante de integração do modelo. Amendola e colaboradores (2010) verificaram que este modelo apresentou melhor adequação dos dados experiementais de extração de compostos

fenólicos de bagaço de uva em relação aos outros cinco modelos cinéticos. Os autores avaliaram a extração para uvas de duas diferentes safras e com o processo ocorrendo a 60 ºC, não investigando outros fatores significativos na modelagem de extração. O extrato foi estável por um período de armazenamento de 1 ano, armazenado ao abrigo da luz, em solução hidroalcoólica a 4 ºC ou em pó a 25 ºC. O conteúdo de polifenóis totais permaneceu estável em valores de pH entre 3 e 7 por até 400 dias, enquanto o poder antioxidante foi prejudicado em valores de pH maiores que 5 (AMENDOLA *et al*., 2010).

O modelo de Minchev e Minkov (MINCHEV e MINKOV, 1984) é uma equação analítica que tem *k* como a taxa constante de extração e *A* e *B* como constantes. Simeonov e Minchev (1999) avaliaram a extração de taninos de folhas de tabaco e de orak com ótima adequação dos valores observados.

Contudo, a maximização dos valores de concentração de etanol e do *ratio* (volume de solvente/massa de bagaço) e a avaliação mais rigorosa dos modelos cinéticos, além de estimação de parâmetros de processo que o modelo escolhido possibilita obter, são dados que ainda faltam na literatura.

3.6 Características de secagem de alimentos

A desidratação de alimentos é uma operação muito importante em nível industrial, uma vez que impacta em maior estabilidade bioquímica e microbiológica do produto ao reduzir sua atividade de água, além de reduzir seu volume, resultando em menores custos em embalagem, transporte e de armazenamento (DOVA *et al*., 2007a). Apesar do avanço de novas tecnologias para tal fim, a secagem por ar quente convectivo permanece como o principal método de secagem de alimentos por apresentar menores custos de operação, por sua simplicidade e por inativação de microrganismos patogênicos e enzimas deteriorantes (VALISHT *et al*., 2011).

Apesar do avanço de novas tecnologias para a secagem de alimentos visando minimizar a degradação térmica de compostos bioativos, como a liofilização e atomização, a secagem por ar quente convectivo continua sendo o método mais amplamente utilizado na indústria para a desidratação de alimentos. O binômio tempo-temperatura é o parâmetro

principal desta técnica, uma vez que, além de impactar diretamente na velocidade de secagem, ele também é responsável por inativar microrganismos patogênicos e enzimas deteriorantes.

Xiao e colaboradores (2010) avaliaram a influência da velocidade e a temperatura do ar de secagem no desempenho da operação, na textura e na degradação de vitamina C durante a desidratação de uvas sem semente. Os autores verificaram que velocidades de secagem entre 3 e 9 m s^{-1} não apresentaram diferença na taxa de secagem do produto a 60 °C. Utilizando velocidade do ar de 5 m s^{-1}, o aumento da temperatura de 50 para 65 °C reduziu sensivelmente o tempo de secagem da fruta. Utilizando os conceitos de difusividade efetiva em esferas, os autores estimaram a difusividade entre 1,82 e 5,84 10^{-10} m^2 s^{-1} na faixa de temperatura de 50-65 °C, com energia de ativação de 67,29 kJ mol^{-1}. A textura da uva desidratada não foi afetada pelo aumento da velocidade do ar de secagem, porém houve aumento da sua dureza com o aumento da temperatura de operação. Isso se deve, possivelmente, ao fato de que a taxa de remoção de água da superfície da fruta seja mais rápida que a velocidade que ela migra do interior para a superfície do produto, e como consequência uma camada dura, contendo solutos previamente dissolvidos, é formada. A degradação de vitamina C não foi afetada pela velocidade de secagem, mas fortemente pela temperatura. A maior retenção obtida pelos autores foi de cerca de 40% para a desidratação da uva acontecendo a 50 °C com velocidade do ar de secagem de 5 m s^{-1}.

Larrauri e colaboradores (1997) avaliaram a influência de temperaturas de 60, 100 e 140 °C na degradação de compostos fenólicos, taninos condensados, atividade antioxidante e cor ao final da secagem de bagaço de uva. Em comparação com o resíduo desidratado por liofilização (utilizado no estudo como padrão de secagem com mínima degradação de compostos bioativos), os autores verificaram que não houve diferença significativa na degradação de polifenóis totais, taninos condensados e de compostos com atividade antioxidante quando o bagaço foi seco a 60 °C. O aumento da temperatura, contudo afetou negativamente a composição do produto seco. A intensidade da cor vermelha não foi diminuída com o tratamento ocorrendo a 60 e 100 °C, porém a luminosidade sim, em relação à amostra liofilizada. Dorta e colaboradores (2012) verificaram que a secagem da

casca de manga a 70 °C afetou significativamente a degradação de compostos fenólicos, antocianinas e compostos com atividade antioxidante.

Torres e colaboradores (2010) observaram forte perda de compostos voláteis (terpenos, aldeídos, álcoois, ácidos, éteres, derivados de benzenos, furanos) de bagaço de uva quando este foi seco a 60 °C em comparação com o resíduo liofilizado. Em relação à degradação de compostos fenólicos, os autores verificaram que, dentro do grupo das antocianinas, a peonidina 3-glucosídeo não foi afetada pela alta temperatura de secagem. Contudo, a malvinidina 3-glucosídeo, em maior concentração entre os pigmentos analisados, teve sua concentração reduzida quando o processo foi realizado a 60 °C. A concentração total de antocianinas no bagaço *in natura* foi de 405 mg kg^{-1}, 343 mg kg^{-1} para o bagaço liofilizado e 265 mg kg^{-1} para o resíduo seco a 60 °C. Para o grupo dos flavonóis, a quercitina, em maior quantidade no bagaço de uva, foi afetada pela utilização de temperaturas elevadas (60 °C). A concentração total de flavonóis no bagaço *in natura* foi de 31 mg kg^{-1}, 20 mg kg^{-1} para o bagaço liofilizado e 18 mg kg^{-1} para o resíduo seco a 60 °C.

Valisht e colaboradores (2011), ao avaliarem a secagem de muscadine, observaram degradação térmica de compostos fenólicos totais devido ao uso de altas temperaturas em comparação ao produto liofilizado. Os autores verificaram, contudo, que compostos com atividade antioxidante extraídos do produto seco aumentaram com a utilização de altas temperaturas de secagem. Esse fato foi creditado ao fato da formação de novos compostos no resíduo a altas temperaturas, incrementando o potencial antioxidante do produto. Como resíduos de frutas são ricas em açúcares e proteínas, quando sob altas temperaturas, a formação de compostos da reação de Maillard é inevitável, sendo que esses compostos apresentam alta atividade antioxidante, como mostram os resultados de Sant'Anna e colaboradores (2011). Com isso, a extração de compostos com atividade antioxidante em resíduos secos com ar quente pode não ser reduzida, mas sim incrementada.

Vega-Gálvez e colaboradores (2012) observaram resultados similares ao secar pedaços de maçãs. Produtos com maior coloração escura apresentram maior atividade antioxidante do que aqueles liofilizados. No mesmo sentido de Valisht e colaboradores (2011), Vega-Gálvez e colaboradores (2012) verificaram degradação térmica de compostos

fenólicos na fruta submetida à secagem com ar quente. Os autores também verificaram que as frutas submetidas à secagem com altas velocidade do ar apresentaram maior retenção de compostos fenólicos devido à mais rápida desidratação do produto. Com isso, as maçãs foram submetidas às altas temperaturas por menores tempos, uma vez que a secagem ocorre em taxas mais elevadas quando utilizadas altas velocidades do ar durante a secagem.

Com isso, os fatores que afetam a taxa de secagem e a retenção de compostos bioativos são diversos e devem ser estudados criteriosamente. Dados sobre a influência dos parâmetros de secagem de bagaço de uva são pouco encontrados na literatura, apesar de serem fundamentais para a sua utilização como ingrediente em alimentos.

CAPÍTULO 4

INTRODUÇÃO AOS CAPÍTULOS 5 A 12

Os capítulos 5, 6, 7, 8, 9, 10, 11 e 12 estão apresentados em forma de artigos científicos. Em cada um são apresentados introdução ao assunto abordado, materiais e métodos detalhados, resultados, discussões e referências bibliográficas.

No capítulo 5 é apresentado o artigo intitulado **"Membrane concentration of liquid foods by forward osmosis: process and quality view".** Artigo de revisão sobre concentração de alimentos por osmose direta, publicado na revista **Journal of Food Engineering**, volume 111, páginas 483-489 no ano de 2012.

No capítulo 6, são apresentados dados da influência de parâmetros do processo sobre o fluxo de água transmembrana em dois modos de operação. Com isso, foi possível avaliar o efeito da diferença de pressão osmótica, temperatura e velocidade de escoamento das soluções sobre a polarização por concentração interna à membana.

No capítulo 7, foi estudada a influência da diferença de pressão osmótica, temperatura e velocidade de escoamento das soluções sobre o desempenho da concentração de suco de uva por OD. Ao final, o suco reconstituído, após a concentração osmótica, foi comparado com o suco inicial quanto a propriedades nutricionais, como teor de compostos fenólicos e atividade antioxidante.

O capítulo 8 mostra o artigo, intitulado **"Influence of drying temperature and air velocity on characteristics of grape marc using response surface methodology**". O trabalho traz um estudo, através de planejamento fatorial, da influência da temperatura e da

velocidade do ar de secagem sobre a taxa de desidratação do bagaço de uva, sobre a retenção de compostos fenólicos totais, taninos condensados e flavonóis.

Os capítulos 9 e 10 apresentadam os artigos "**Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts**" e **"Kinetic modeling of anthocyanin extraction from grape marc"**, respectivamente, em que foram avaliadas condições de extração de compostos fenólicos totais e antocianinas monoméricas, assim como o potencial antioxidante dos extratos obtidos de bagaço de uva.

No capítulo 11, é abordado o estudo da estabilidade bioquímica e microbiológica da farinha de bagaço de uva ao longo de 6 meses de armazenamento. Também é apresentado o estudo de isotermas para esse produto, com a finalidade de se avaliar a estabilidade da farinha do bagaço de uva como um ingrediente a ser utilizado na indústria de alimentos. O trabalho é intitulado **"Grape marc poder: physicochemical and microbiological stability during storage and moisture sorption isotherm"**.

Finalmente, no capítulo 12, é apresentado o artigo **"Incorporation of grape marc powder in the production of fettuccini pasta"**, onde foi estudado o impacto da substituição da farinha de trigo por farinha de bagaço de uva sobre propriedades tecnológicas e nutricionais de massa tipo fettuccini.

CAPÍTULO 5

Membrane concentration of liquid foods by forward osmosis: process and quality view

Neste capítulo, é apresentado o artigo de revisão sobre concentração de alimentos por osmose direta, publicado na revista **Journal of Food Engineering**, volume 111, páginas 483-489, em 2012. Nele, aspectos quanto à influência de parâmetros de processo e dos componentes dos alimentos no desepenho da técnica são abordados, assim como uma visão geral dos trabalhos mais recentes publicados na área.

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Review

Membrane concentration of liquid foods by forward osmosis: Process and quality view

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ABSTRACT

The industrial thermal processing of foods may have a severe impact on the sensorial and nutritional properties of the final product. Membrane technologies have been extensively studied as alternative processes. Forward osmosis (FO) is a promising membrane technology to be used in food industries. The only driving force of the process is the osmotic pressure difference between the two solutions that flow in counter-current mode on opposite sides of a permeable membrane. Thus, the main advantages of FO, compared to both thermal and conventional membrane processing, include low hydraulic pressure, low treatment temperature, low fouling tendency, high solids content processing capability and easy scale-up. A detailed, up-to-date summary of potential FO applications for concentrating liquid foods is presented in this review article. The effect of the main process parameters on the filtration performance and their impact on the sensorial and nutritional factors of the final product are described and discussed for a broad spectrum of foods.

Contents

1. Introduction

In recent decades, the consumption of high quality foods has grown due to the consumers' constantly increasing of concern about human health and nutrition. It has motivated industries and research groups to advance the development of new products and technologies to meet this demand. The consumer demand for minimally and naturally processed, nutritious foods has led to food scientists and technologists to address the challenge of (1) minimizing the impact of industrial treatments on the nutritional, organoleptic and technological factors with low-cost processes and (2) reducing financial losses due to food spoilage.

Thermal processing remains the most widely employed method for shelf-life extension and food preservation and concentration. However, industrial thermal treatments may have negative impacts on nutritious components (such as anthocyanins, carotenoids, vitamins and bioactive proteins (Van den Hout et al., 1999; Kechinski et al., 2010; Provesi et al., 2011; Barros et al., 2011)), sensory parameters (such as color, aroma, flavor (Timoumi et al., 2007; Nisha et al., 2009)) and technological properties (Singh

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and Fox, 1985; Singh and Creamer, 1992; Zavareze and Dias, 2011), among others. To meet the current market demand, recent developments in the food industry have focused on non-thermal technologies. Membrane processing is an interesting alternative for the clarification and concentration of liquid foods because it operates at room temperature, exhibits low energy consumption and high performance, scales up easily and rejects a wide range of food contaminants. The membrane treatments most employed currently include microfiltration, ultrafiltration, nanofiltration and reverse osmosis (RO). However, high hydraulic pressures, limited maximum attainable concentrations, concentration polarizations and high organic fouling environments are several operational limitations of these techniques for use in food industry (Petrotos et al., 2010).

Forward osmosis (FO), also known as direct osmosis, is a promising membrane technology. FO as a concept for concentrating foods dates back to the 1960's (Popper et al., 1966), and its only driven force is a difference in osmotic pressure. The water transfer occurs from the feed side (low concentration) to the draw solution side (high concentration) across a semi-permeable membrane till the osmotic pressure difference between both sides is close to zero. In this sense, FO has several advantages compared to conventional food industrialization techniques, such as:

(1) Low hydraulic pressure for operation, which reduces the cost for electrical energy.
(2) Low processing temperature, which avoids the thermal degradation of food quality factors and conserves electrical energy.
(3) High product recovery and low discharge of brine to the environment.
(4) Low irreversible fouling, which leads to low costs on membrane cleaning and replacement.
(5) Efficient treatment of feeds with a high solids content.
(6) Modularity and ease of scale-up.

Reviews on the osmotic concentration of liquid foods have been published (Wong and Winger, 1999; Petrotos and Lazarides, 2001; Jiao et al., 2004). However they do not focus on the impact of the operational parameters on the process performance and on the final product quality. Moreover, recently, many studies have demonstrated FO to be a suitable alternative for concentrating liquid foods. Therefore, the aim of the present paper is to describe and discuss FO for concentrating a broad spectrum of liquid foods and to compare the impact of this technique to conventional ones on the sensorial and nutritional factors of food. Special focus will be given to the operational process parameters and final product quality, and future trends and challenges on osmotic membrane processing will be considered.

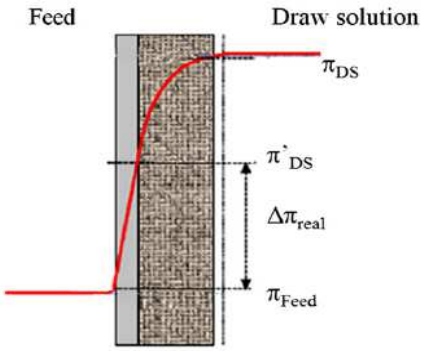

Fig. 2. Schematic mechanism of FO in a food model system indicating water transport through membrane from draw to feed solution, which is placed towards active layer. π_{feed} is the osmotic pressure of feed, $\pi_{DrawSolution}$ is the osmotic pressure of draw solution, $\pi'_{DrawSolution}$ is the actual osmotic pressure of the draw solution and $\Delta\pi_{real}$ is the actual osmotic pressure difference in FO (Adapted from Nayak and Rastogi, 2010a).

2. Theoretical background

The phenomenon of water passage across a selectively permeable membrane from a high chemical potential region to a low chemical potential region is known as osmosis. Osmotic pressure (π) is the pressure that needs to be applied to the more concentrated solution to prevent the water transport across the membrane. FO uses the osmotic pressure differential ($\Delta\pi$) across the membrane as the only driving force for water transport from the feed side (concentrating the low concentration stream) to draw solution (diluting the osmotic agent stream) (Cath et al., 2006).

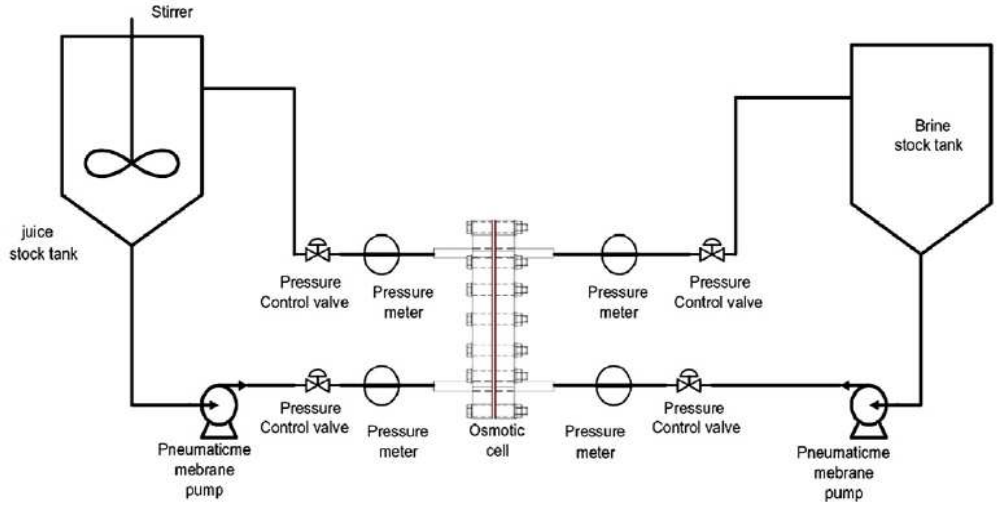

Fig. 1. Apparatus for forward osmosis process (Petrotos et al., 2010).

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Fig. 1 presents a FO apparatus, where the feed and draw solutions are pumped in a counter current, closed loop. In osmotic membrane concentration, the salt solution from the draw solution stream diffuses through the porous support layer until it reaches the interface with the active layer. Then due to a difference in chemical potential (osmotic pressure forces), water partitions into the active layer from feed to draw solution, which dewaters the liquid food.

FO uses composite, asymmetric membranes composed of two layers: one is the porous support layer and the other is the dense, active membrane layer, as shown in Fig. 2. The membrane can be placed between the feed and the osmotic agent solutions in two different methods: the feed can flow towards the support layer or towards the active layer. The first method is more common for solutions with low molecular weight compounds such as water. For the filtration of liquid foods that are composed of a complex mixture of substances, pumping the feed towards the active layer is the correct configuration due to the low external polarization, which results in a higher osmotic pressure difference. This leads to a high water flux through the membrane (Nayak and Rastogi, 2010a) and minimal damage to the membrane layers.

The general equation describing water transport in the FO process is:

$$J_w = A(\pi_{\text{Draw Solution}} - \pi_{\text{Feed}} - \Delta P) \quad (1)$$

where J_w is the water flux, A is the water permeability constant of the membrane, $\pi_{\text{DrawSolution}}$ is the osmotic pressure of the draw solution, π_{feed} is the osmotic pressure of the feed solution and ΔP is the difference pressure between the two solutions (which is close to zero, but not negligible, in FO processes,). Eq. 1 assumes that the solutes from the osmotic agent solution are totally impermeable to the membrane, and Eq. 1 does not consider the concentration polarization phenomenon. These are typical assumptions for osmotic concentration.

Fig. 2 schematically shows the concentration gradient of the draw solution in the FO membrane during liquid food processing. Because the solute that serves as the osmotic agent is preferentially of low molecular weight, it is diffused through the support layer to the interior surface of the active layer and is diluted due to convection by water diffused from the feed. Concentration polarization is the excess of solute close to the membrane surface due to diffusion of the draw solute in support layer. It results in a concentration gradient of the osmotic agent concentration within the membrane, which characterizes the internal concentration polarization (Fig. 2). Then, the actual osmotic pressure of the draw solution is $\pi'_{\text{DrawSolution}}$, instead of $\pi_{\text{DrawSolution}}$. These phenomena significantly decrease the draw solution's osmotic pressure and, consequently, the FO performance ($\Delta\pi_{\text{real}}$ is lower than $\Delta\pi$). Complete approach of modeling FO is given elsewhere (Dova et al., 2007b; Tan and Ng, 2008; Phillip et al., 2010).

3. Food concentration

The first record of the utilization of FO for liquid food processing dates of 1966. Table 1 lists the subsequent FO technology studies for liquid food processing. Popper et al. (1966) used tubular and flat sheet cellulose acetate polymeric RO membranes to concentrate the total soluble solids (TSS) of grape juice from 16 to 60 °Brix and achieved an average water flux of 2.5 kg m^{-2} h^{-1} using saturated sodium chloride (NaCl) solution as the draw solution. However, there was high salt diffusion to the product, which damaged the juice's taste and discouraged further investigation of this membrane technique for food applications. In the mid-1990s, to minimize this problem, Beaudry and Lampi (1990a) utilized a modified thin film composite RO membrane to increase salt rejection during orange juice filtration. The authors achieved a water flux of 4.0 kg m^{-2} h^{-1} and more than 99.9% rejection of salt and total organic acids, thus impeding the passage of feed solution solutes through the membrane and migration of sodium chloride to the feed.

Beaudry and Lampi (1990b) concentrated orange juice up to 42 °Brix using a 72 °Brix sugar syrup as the draw solution, and they achieved an average water flux of 1.3 kg m^{-2} h^{-1} at a process temperature of 30 °C. Authors refrigerated the feed stream in each concentration stage, which resulted in a high retention of the flavor and color of the juice concentrate. The low temperature and pressure applied in the system were critical to the maintenance of sensorial characteristics of the beverage (Beaudry and Lampi, 1990b).

Sensorial and centesimal analysis of raspberry juice concentrated by FO and by vacuum evaporation were conducted. The direct osmotic concentration from 10 to 45 °Brix was performed with 69 °Brix corn syrup as the osmotic agent at 25 °C. The treatment by both processes resulted in small anthocyanin losses, low polymer-

Table 1
Summary of concentration of liquid foods by FO.

Reference	Food	Draw solution	Temp. (°C)	Average flux (kg m^{-2} h^{-1})	Main food parameter evaluated
Popper et al. (1966)	Grape	8 mol L^{-1} NaCl	n.m.	2.5	Organoleptic
Wrolstad et al. (1993)	Raspberry	69 °Brix corn syrup	25	1.4	Anthocyanin and organoleptic
Herron et al. (1994)	Coffee	74 °Brix fructose/glucose	n.m.	4.0	Organoleptic and TSS
	Orange			3.0	
Petrotos et al. (1999)	Tomato	4 mol L^{-1} NaCl	25	3.1	n.m.
Dova et al. (2007a,b)	Sucrose/glucose solution		26	4.5	
Petrotos et al. (2010)	Tomato		Room	3.5	Color and TSS
Rodriguez-Saona et al. (2001)	Red Radish	60 °Brix corn Syrup	Room	1.2	Anthocyanin, color and organoleptic
Babu et al. (2006)	Pineapple	4 mol L^{-1} NaCl	25	1.6	Organoleptic and TSS
		2.75 mol L^{-1} NaCl + 0.9 mol L^{-1} sucrose		1.15	
Garcia-Castello et al. (2009)	Sucrose	4 mol L^{-1} NaCl	30	5.8	TSS
Nayak and Rastogi (2010a,b)	Kokum	6 mol L^{-1} NaCl	30	15.0	Anthocyanin and hidroxicitric acid
Garcia-Castello and McCutcheon (2011)	Orange liquor	4 mol L^{-1} NaCl	30	8.0	TSS
Valluri (2010), Nayak et al. (2011)	Beetroot	6 mol L^{-1} NaCl	25	7.5	Betalain, color, pH, density, viscosity
	Pineapple			6.5	TSS, color, pH, density, viscosity
	Grape			4.0	Anthocyanin, color, pH, density, viscosity

n.m. not mentioned.

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ized pigment enhance and no flavor difference, which showed that both juices were of good quality and were comparable to commercial samples (Wrolstad et al., 1993). The FO-concentrate juice exhibited a strong raspberry aroma and flavor, compared well to some commercial samples. In 1994, Herron and co-workers used a fructose/glucose solution (74 °Brix) for the concentrations of orange juice and coffee. The researchers obtained a maximum osmotic flux of 4 and 3 kg m^{-2} h^{-1}, respectively, and provided products with superior quality in comparison to that produced by a conventional vacuum evaporator.

Petrotos et al. (1998, 1999) studied the effect of different membranes and process parameters on the FO performance during the concentration of tomato juice in a tubular module with an aromatic polyamide thin film composite RO membrane. In these studies, the authors obtained higher average values for the transmembrane water flux (3.10 kg m^{-2} h^{-1}) using 4 mol L^{-1} of NaCl as the draw solution and a 400 μm thick membrane at 26 °C. The pretreatments of the juice by plain filtration, microfiltration or ultrafiltration were also found to have a positive effect on the value of the observed osmotic fluxes. The ultrafiltered juice had the best performance with a 135% increase of the direct osmosis flux. Dova et al. (2007a,b) treated sucrose/glucose solution by FO in a flat sheet module with an aromatic polyamide RO membrane at room temperature and obtained an average water flux of 4.5 kg m^{-2} h^{-1} using the same osmotic solution. Using a modified FO module at ambient temperature and low pressure (~4 bar), with NaCl as the osmotic medium (4 mol L^{-1}), fresh tomato juice was concentrated from 5.5 °Brix to 16 °Brix with an osmotic flux of 3.26 kg m^{-2} h^{-1} (Petrotos et al., 2010). The authors also verified that the red color of the tomato paste was much more intense in the concentrate produced by FO than by vacuum evaporation and that the processed product by FO was microbiologically stable.

Rodriguez-Saona et al. (2001) evaluated FO, evaporation in a Centritherm evaporator and the combination of these methods to concentrate red radish extracts. The authors used 60 °Brix corn syrup as osmotic agent to concentrate the extract from 1.1 to 5.5 °Brix at room temperature. The anthocyanins were concentrated from 11 to 55 mg per 100 g of extract. After a high temperature operation, the extract reached a concentration of 15.5 °Brix and contained 170 mg of anthocyanins per 100 g of extract; however, it exhibited a burnt flavor and a large loss of volatile aroma. Because FO is a highly time consuming process, the authors combined FO and thermal evaporation. The chroma and lightness of both extracts (produced by the Centritherm evaporator and by the combined process) were similar. A sensory evaluation of the concentrated radish extracts showed that the FO processing was more effective than thermal evaporation in reducing the characteristic radish aroma compounds in the juices added to the radish extracts. Juices colored with the extract concentrated by the combination of thermal evaporation and FO had the closest overall intensity to that of fresh extract, which showed that coupling membrane and thermal treatments is an interesting approach to concentrate liquid foods.

The processing of pineapple juice was also evaluated (Babu et al., 2006). Using a specific membrane for FO, the authors achieved 1.59 kg m^{-2} h^{-1} of water flux and a TSS concentration increase from 12 to 60 °Brix at 25 °C with a 4 mol L^{-1} NaCl solution as the osmotic agent. Because there is a diffusion of salt solutes from the draw solution to the feed, it is of public concern to minimize the salt content in the juice. Therefore, the authors also evaluated the combination of NaCl and sucrose as the osmotic agent. Among others, the combination of 2.75 mol L^{-1} of NaCl and 0.9 mol L^{-1} of sucrose yielded an average water flux of 1.15 kg m^{-2} h^{-1}, with low rate of NaCl transport to the pineapple juice. This sample exhibited less saltiness, more sweetness and better overall acceptability in the sensorial evaluation compared to other sugar–salt combinations as the osmotic agent, and it exhibited a high score on the acceptability analysis of the juice. The authors also showed that the reconstituted juice characteristics were similar to those of the fresh juice. Similar results were reported by Nayak and Rastogi (2010a) when they reconstituted kokum extract, which supports FO as a potential alternative to conventional industrial treatments for concentrating food beverages.

Garcia-Castello et al. (2009) concentrated a sucrose solution by FO using a flat sheet membrane specific for this propose and a 4 mol L^{-1} NaCl solution, and they achieved a water flux of 5.84 kg m^{-2} h^{-1} and an increase in TSS concentration from 10 to 56.4 °Brix at 30 °C. The feed concentration factor by FO was twice that by RO. The performances could have been equalized if more energy and stronger pumps were used, but energy costs, combined with the limitations on pump size and the strength of the membrane modules, prohibited this strategy (Garcia-Castello et al., 2009). In another study (Garcia-Castello and McCutcheon, 2011), these authors observed a water flux of approximately 8 kg m^{-2} h^{-1} at 30 °C with 4 mol L^{-1} NaCl as the draw solution when treating orange liquor solution. The TSS increased from 8 to 10.5 °Brix. The results showed that pectin is the critical feed component for reducing the dewatering flux due to a thick cake formation on the membrane during food filtration, which enhanced the water transport resistance and negatively affected the process rate.

Nayak and Rastogi (2010a) concentrated anthocyanin extract from kokum by FO with 6 mol L^{-1} NaCl as the osmotic solution at 30 °C and obtained an average water flux of 15 kg m^{-2} h^{-1}. The TSS content increased 25-fold, from 2 to 52 °Brix. The authors showed that the extract treated by FO exhibited better nutritional and sensorial properties, less non-enzymatic browning and a higher retention of hidroxicitric acid and anthocyanins compared to that concentrated by a thermal treatment. In another study (Nayak et al., 2011), the authors concentrated beetroot, pineapple and grape juice using the same osmotic agent at 25 °C, and obtained average water fluxes of 7.5, 6.5 and 4 kg m^{-2} h^{-1}, respectively. The betalain and TSS concentrations in beetroot juice were found to increase from 51 to 3000 mg L^{-1} and TSS from 2.3 to 52 °Brix, respectively (Nayak et al., 2011). Reconstituted beetroot juice density, pH and color purity (based on Hunter colorimeter) were of 1071 kg m^{-3}, 4.5 and 25, respectively. Those values did not differ significantly from the fresh juice (Valluri, 2010). For the grape juice, anthocyanins and TSS were concentrated from 105 to 715 mg L^{-1} and from 4.4 to 54 °Brix, respectively (Nayak et al., 2011). Reconstituted grape juice viscosity, pH and color purity were of 1.68 m Pas, 3.4 and 27, respectively. Those values did not differ significantly from the fresh juice (Valluri, 2010). In the pineapple juice, the TSS concentration increased from 4.4 to 54 °Brix (Nayak et al., 2011), which demonstrated that the treatment of high solid content liquids is not a technological limitation of FO, in contrast to RO and ultrafiltration processes. Similar behavior was found for reconstituted pineapple juice in relation to the crude product, in which no significant changes on physicochemical characteristics were observed (Valluri, 2010).

The same research group verified that concentration of kokum extract by FO achieved 2.69 g L^{-1} of anthocyanins after 18 h of process, meanwhile, in the same conditions, using osmotic membrane distillation (OMD), anthocyanins were concentrated up to 72 m L^{-1}. Moreover, transmembrane flux in OMD method was lower as compared to FO, presenting higher color stability, lower browning index and less degradation of hydroxyxitric acid (Nayak and Rastogi, 2010b).

These recent records clearly show that FO exhibits several advantages, which mainly include better preservation of bioactive compounds compared to processed foods and less operational limitations compared to thermal and conventional membrane technologies.

4. Process parameters

FO performance is affected by several process parameters such as the membrane material and module, temperature, characteristics of feed and draw solutions and the hydrodynamic conditions. Full explanations and exemplifications of the FO process parameters are provided next.

4.1. Membrane and module conditions

In FO, fruit juice can be configured to flow towards either the active or support layer of the membrane. Due to the presence of low and high molecular weight compounds in the food solution and the low concentration polarization phenomenon, the first cycle yields a higher concentration performance. Nayak and Rastogi (2010a) obtained an increase of almost 180% on filtration flux of kokum extract by placing the feed against the active layer instead of the support layer. Additionally, due to the membrane damage caused by some abrasive food components against the support layer material, flowing liquid foods towards active layer seems to be the only option to concentrate the feed in FO process.

Aromatic polyamide RO membranes are typically used for FO concentration (Petrotos et al., 1998, 1999, 2010; Dova et al., 2007a,b). However, when compared to the commercial FO membrane made of cellulose acetate, the differences in the overall support layer thickness, structure, and hydrophilicity of the RO membranes critically impact the severity of the internal concentration polarization, a phenomenon prevalent in anisotropic membranes that is seriously detrimental to FO membrane performance (McCutcheon and Elimelech, 2008; Garcia-Castello et al., 2009). The water fluxes achieved using the RO membrane in direct osmosis tests were only 5% of the specific FO membrane fluxes in the study conducted by McCutcheon and Elimelech (2008). Similar results were obtained by Garcia-Castello and coworkers (2009), where the permeate water flux obtained for the aromatic polyamide membrane was approximately 5% of that for the cellulose acetate membrane.

The commercially available FO membranes are the asymmetric cellulose acetate membranes from Hydration Technologies Inc. (HTI, Albany, OR). However, rejection layers of these membranes tends to exhibit low water permeability and limited solute retention (Tang et al., 2010; Xiao et al., 2011; Zou et al., 2011), which have created opportunities to further improve the FO performance. In this context, several works have been published on the synthesis FO membranes made of polyamide, polyethersulfone, polybenzimidazole among other polymers that exhibit better performances than the commercially available, cellulose acetate membrane (Wang et al., 2007; Chou et al., 2010; Yu et al., 2011; Wei et al., 2011). These studies were conducted mainly using deionized water, and membrane performance information regarding food system is still scarce.

Another alternative to enhance FO filtration performance is by increasing turbulence of the treated solutions. In the work of Dova et al. (2007a) and Petrotos et al. (2010), a new design for a FO module with flat configuration was presented to enhance liquid food filtration by increasing turbulence of the juice flowing through the apparatus (Fig. 3). The part labeled B was equipped with orthogonal baffles in the path of the feed solution flow, which evenly distributed the liquid food and to promoted turbulence. The achievement of high fluxes in FO is partially due to the high turbulence achieved in their filtration module. The membrane assembly of FO apparatus allowed (1) a rapid, turbulent flow, without excessive pressure drop and (2) a relatively long region of contact with the membrane without fouling or concentration polarization, even with a high solute content in the feed (Herron et al., 1994; Dova et al., 2007a; Petrotos et al., 2010).

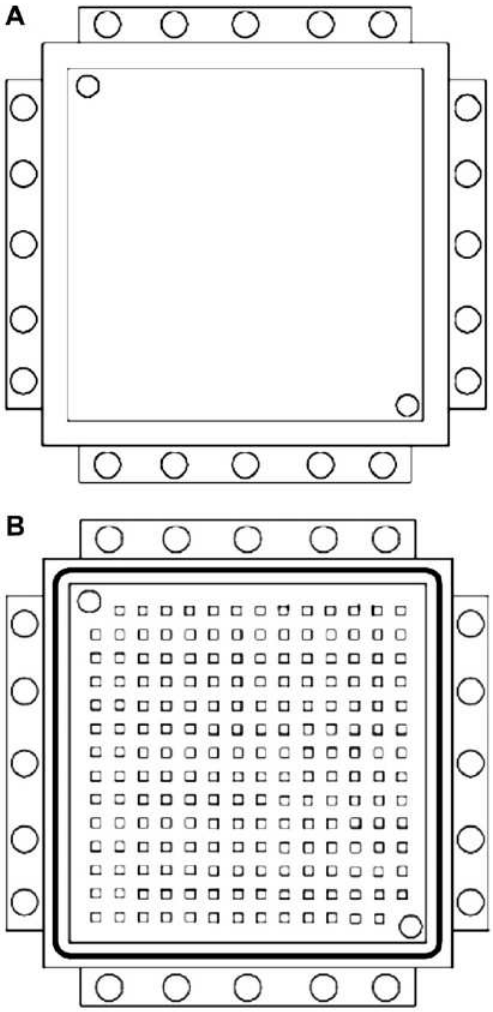

Fig. 3. Geometry of a forward osmosis module with turbulence promoters (Petrotos et al., 2010). (A) upside of FO module, where draw solution flows; (B) downside with flanges to promote turbulence in the feed side.

4.2. Draw solution

The osmotic agent solution is the source of the driving force in the FO process, and its selection for the concentration of liquid foods may be based on several criteria. For example, the solution should have a higher osmotic pressure than the feed solution and be recoverable, non-toxic and inexpensive.

The impact of draw solution characteristics on osmotic driven processes can be explained by the Wilke–Chang equation (Wilke and Chang, 1955), in which the mass diffusion coefficient is inversely proportional to the viscosity of the solution involved. Petrotos et al. (1998) evaluated several solutes to comprise a draw solution for concentrating tomato juice. The authors showed that 4 mol L^{-1} of NaCl as an osmotic agent yielded the best performance of water transmembrane flux (3.10 kg m^{-2} h^{-1}), compared to 3.5 mol L^{-1} of

glucose (0.37 kg m^{-2} h^{-1}), 1.7 mol L^{-1} of sucrose (0.55 kg m^{-2} h^{-1}), 2.65 mol L^{-1} of calcium chloride (2.33 kg m^{-2} h^{-1}), and 1.2 mol L^{-1} of polyethylene glycol (0.70 kg m^{-2} h^{-1}) tested. These results were strongly credited to the viscous characteristics of the brine: agents with lower viscosity yielded higher process performances due to higher diffusivity and the smaller resistance to mass transfer through the polarized layer of the osmotic medium.

The utilization of more concentrated draw solutions enhances the water flux because of increasing of osmotic pressure of the brine and, consequently, the difference in osmotic pressure relative to the feed. However, the solute ions diffuse from the osmotic agent to the feed solution due to the osmosis process. In this sense, NaCl utilization must be carefully studied due to an increase in the public health diseases conveyed by the high consumption of sodium. In a study of pineapple concentration, Babu et al. (2006) verified that the combination of NaCl and sucrose (2.75 mol L^{-1} of NaCl and 0.9 mol L^{-1} of sucrose) in the draw solution is an alternative for fruit juice concentration because it can overcome the drawback of sucrose (low flux) and sodium chloride (salt migration) as osmotic agents during the direct osmosis process. In another approach, Achilli and co-workers (2010) evaluated over 500 inorganic compounds and determined the water and salt fluxes through the membrane. After considering costs and other characteristics, the authors suggested that the best choice for food concentration would be potassium or sodium bicarbonate.

4.3. Temperature

Increasing the process temperature positively affects the osmotic flux. Wilke–Chang equation states that the mass diffusion coefficient is proportional to the absolute temperature (Wilke and Chang, 1955). Additionally, increasing process temperature reduces the solution viscosity and positively impacts the diffusion coefficients, which results in an enhanced transmembrane flux and concentration rate.

The osmotic flux in tomato juice concentration increased 64% by increasing the process temperature from 26 to 60 °C (Petrotos et al., 1998). Babu et al. (2006) verified that increasing the temperature from 25 to 45 °C enhances water flux by 78% when processing pineapple juice. Similar results were obtained by Nayak and Rastogi (2010a), who showed that increasing the temperature from 25 to 40 °C resulted in an increase of more than 2-fold in the concentration of anthocyanin extract from kokum. The time required to concentrate raspberry juice to 45 °Brix approximately doubled (5 vs. 10 h) when the processing temperature decreased from 26 to 8 °C (Wrolstad et al., 1993).

4.4. Hydrodynamic conditions

Increasing the feed or draw solution flow rate improves the water transmembrane flux. This may be attributed to the reduction in the hydrodynamic boundary layer thickness, which results in a larger Reynolds number (flow rate), affects the mass transfer resistance of the polarized layer adjacent to the membrane surface and consequently increases the transmembrane flux. Dova et al. (2007b) modeled the resistance of water passage through membrane to be proportional to the feed flow rate and exponentially proportional to the draw solution flow rate. Moreover, hydrodynamic shear forces may increase with increasing feed flow rates, which leads to less cake formation of flocculation of foulants (mainly pectin) on the membrane surface. This phenomenon results in less fouling and consequently a better process performance (Lee et al., 2010).

In this sense, many authors evaluated the influence of solutions flow rate on the osmotic flux in FO and verified that increasing the fruit juice or draw solution flow rate more than 3-fold increases the osmotic flux by approximately 35% (Petrotos et al., 1998; Babu et al., 2006; Nayak and Rastogi, 2010a).

4.5. Feed composition

Because the driving force of FO is the differential osmotic pressure between the feed and osmotic agent solutions, a high concentration of liquid food negatively impacts the process performance. In addition, it is also linked to an increasing in feed viscosity during the food concentration, which affects the overall mass transfer coefficient and severely impacts the FO performance. The osmotic flux decreased from 15 to 3 kg m^{-2} h^{-1} when the sucrose solution concentration was increased from 0.5 to 1.5 mol L^{-1} in a model system (Garcia-Castello et al., 2009). During tomato juice concentration, when the TSS concentration was increased from 4.3 to 11.8 °Brix, Petrotos et al. (1998) found that the water flux decreased linearly, reaching a reduction of 53% by the end of the process.

Using a juice model system, Garcia-Castello and McCutcheon (2011) verified that calcium and citric acid, which are normally critical components of organic fouling, were found to have little effect on fouling behavior. The presence of pectin, however, had a significant effect by contributing to organic fouling, which was found to reduce the permeate flux by as much as 50%. Petrotos et al. (1999) studied the effect of tomato juice filtration as a pretreatment for a FO process and showed that there was a 2.4-fold increase in the water flux due to the lower feed viscosity and the partial separation of pectin. Foods are complex matrices of low and high molecular weight compounds, which results in large variations in processing conditions. In this sense, further studies may be conducted that focus on the effect of FO parameters on the process performance and final product quality on a larger spectrum of foods.

5. Conclusions and future perspectives

Despite the several advantages that FO brings to the membrane engineering for food applications, it mainly lacks optimal process performance, osmotic solute rejection and draw solution recovery. Developments in the areas of engineering and membrane technology are crucial for improving the dewatering rate of liquid foods. Studies should focus on the design of new membranes configurations (e.g., flat, tubular, hollow fiber), with high water permeability, reduced tendency for concentration polarization, high solute rejection rates, high chemical stability and high mechanical stability. Additionally, studies on draw solutions with a low affinity for polymeric membranes, a low rate of diffusion across polymeric membranes and ability to produce high osmotic pressures are essential for developing FO for food applications.

Because the draw solution is diluted during the FO process, the osmotic reinforcement of the medium is an essential point to be evaluated to minimize industrial waste and energy consumption and to allow for the large-scale industrialization of FO. Electrodialysis has been suggested for re-concentrating post-processed NaCl brines in FO; however, more studies are needed to evaluate this technology as a solution to this challenge in FO.

Microorganisms and enzymes are important public health issues in the food industry and are generally inactivated by high temperature short time (HTST) process. Therefore, because FO applications are low temperature processes, a critical evaluation must be conducted on the microbiological and biochemical stability of FO-concentrated foods, e.g., through shelf-life studies.

Additionally, more information on the energy consumption of FO in large-scale processes is needed because little data exist that

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compare osmotic concentration to other technologies as a whole process for industrialized foods.

FO has the potential to be utilized as an alternative process in the food industry, and recent publications have shown clear evidence that it has several advantages compared to conventional techniques regarding in process and product quality. Nevertheless, more studies on concentration techniques using osmotic membranes are needed to enable their proper large-scale utilization in the food industry.

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CAPÍTULO 6

Effect of process parameters on water flux in forward osmosis using response surface methodology

Neste capítulo, é apresentado o estudo sobre o efeito da diferença de pressão osmótica, velocidade de escoamento das soluções no módulo de membrana e temperatura sobre o fluxo de água tranmembrana em sistema de OD é apresentado em forma de artigo científico. Para isso, foram utilizados água pura como alimentação e soluções de cloreto de sódio como agente osmótico, escoando de dois modos: contra a camada densa da membrana ou contra a camada suporte. A metodologia de superfície de resposta foi utilizada a fim de verificar a significância estatística dos parâmetros e as regiões ótimas de operação.

6.1 Artigo: Effect of process parameters on water flux in forward osmosis using response surface methodology

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CAPÍTULO 6 - EFFECT OF PROCESS PARAMETERS ON WATER TRANSMEMBRANE FLUXES IN FORWARD OSMOSIS USING RESPONSE SURFACE METHODOLOGY

Abstract

In the present work, a 2^3 factorial design was performed in order to evaluate the influence of solutions' flow rate, the difference of osmotic pressure ($\Delta\pi$) and temperature on the transmembrane flux of water in two modes of operation: feed flowing against the membrane porous layer (mode I) or against the dense layer (mode II). Pure water was used as feed and sodium chloride solutions as osmotic agents. Internal polarization concentration, which happens in mode II of operation, affected negatively the performance of FO. Statistical results showed that solution's flow rate, $\Delta\pi$ and temperature affected positively ($p < 0.05$) on enhance of water flux for both modes of operation. Response surface methodology presented to be a powerful tool in order to study the FO performance. The highest water flux in mode of operation I was 10 L m^{-2} h^{-1} in flow rate range of 100 and 170 mL min^{-1}, $\Delta\pi$ between 200 and 302.20 atm and temperatures between 24 and 40 °C. In mode II, the highest values water transport was 5 L m^{-2} h^{-1}, which was observed in flow rates between 100 and 170 mL min^{-1}, $\Delta\pi$ between 245 and 302.20 atm and temperatures between 30 and 40 °C. Statistical analysis show that interaction of the FO operational parameters are significant ($p < 0.05$), possibility influencing in the internal polarization concentration phenomena.

Keywords: forward osmosis; process parameters; water flux; response surface methodology.

6.1.1 Introduction

The increase of global concern about clean water, renewable energy and environmental friendly industrial processes has led many studies to develop and improve technologies to attend this demand. In this context, forward osmosis (FO) has been shown up as a promising alternative. FO is a membrane separation process, in which the only driven force is the difference of the water chemical potential, expressed in terms of osmotic pressure (π), between two solutions (feed and draw solution), separated by a semi-

permeable membrane (Cath et al., 2006; Sant'Anna et al., 2012; Zhao et al., 2012). Thus, FO presents several advantages compared to traditional pressure-driven membrane processes, such as: very low request of hydraulic pressure for operation, cost saving energy; to work at low temperature of processing, economizing electrical energy; high recovery, resulting in less brine discharge to the environment; cost saving on membrane replacement, due to low irreversible fouling; possibility of more efficiently treat feeds with high content of solids, in the liquid food field (Sant'Anna et al., 2012). FO has been used to concentrate liquid foods (Petrotos et al., 2010; Garcia-Castello et al., 2011; Nayak et al., 2011), generating energy (Achili et al., 2009; Fu et al., 2013), desalination of seawater (Yangali-Quintanilla et al., 2011; Chanukya et al., 2013), among others.

Concentration polarization (CP) is the main technological limitation of FO. Figure 6.3 shows schematically the main phenomena during the FO process.

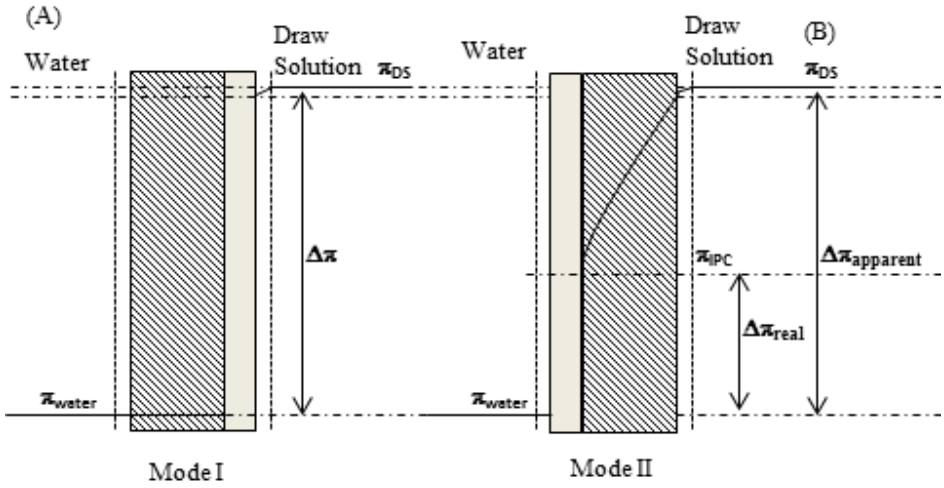

Figure 6.1 Mechanism of water transport in FO procedures. (A) feed is water, flowing against the membrane support layer and osmotic agent is sodium chloride solution flowing against the membrane dense surface; (B) feed flows against the dense layer and draw solution against the support layer, indicating schematically the occurrence of internal polarization concentration (ICP). $\Delta\pi_{real}$ and $\Delta\pi_{apparent}$ are the corresponding real and the apparent driving forces, respectively. π_{feed} and π_{DS} are the osmotic pressures of feed and osmotic agent solution, and π_{IPC} is the effective osmotic pressure of draw solution due to IPC.

Mode I (Figure 6.3A) shows the membrane osmotic behavior when pure water is used as the feed solution, flowing against the porous support layer, and a sodium chloride

as the draw solution, flowing against the dense selective layer. In this condition, the brine is composed by a low molecular weight component, thus external concentration polarization (ECP) is very low, and considered negligible (Nayak et al., 2010). Since the feed is pure water, there is no internal polarization concentration (ICP). In the mode II, when water flows against the dense layer and the osmotic agent, against the support layer (Figure 6.3B), a significant internal concentration polarization (ICP) shows up into the porous part. Since the osmotic water transfers happen in the interface of the active (dense) layer, the diffusion of water through the active layer will result in dilution of the brine, setting up of an ICP. The osmotic pressure of the solution, thus, decreases from π_{DS} to π_{ICP}, damaging the driving force and consequently the concentration performance. Thus, the apparent driving force ($\Delta\pi_{apparent} = \pi_{DS} - \pi_{Feed}$) will be lower than the actual osmotic process ($\Delta\pi_{real} = \pi_{ICP} - \pi_{Feed}$), leading to an intense prejudice on the FO performance. Thus the increase of the $\Delta\pi$ do not imply the increase of the water permeate flux, possibly due to the increase of ICP that happens in the brine solution side, causing $\Delta\pi_{real} < \Delta\pi_{apparent}$.

Temperature, solutions' flow rate into the process module and $\Delta\pi$ are important parameters in FO. There is little information about the influence of them over the CP phenomena. The response surface methodology (RSM) is a set of statistical tools for assessing the influence of processing parameters, as well as the interaction between them, on process responses (Myers and Montgomery, 2002). This methodology has already been used in the field of membrane separation successfully (Ahmad et al., 2009; Wang et al., 2010; Ngang et al., 2012), but it has not been applied to FO procedures.

Thus, the objective of this study is to evaluate the influence of $\Delta\pi$, the solutions' flow rate and the temperature on the transmembrane water flux using on response surface methodology. On this basis, it was used mode I and mode II in order to evaluate the influence of the process parameters in CP phenomena.

6.1.2 Material and Methods

6.1.2.1 Chemicals and membrane

Ultrapure water was used as feed and draw solution was prepared with sodium chloride of analytical grade (Signh, Brazil) diluted in ultrapure water. Membranes used in the FO procedures were kindly provided by Hydration Technologies Innovation (Albany, OR). They are composed by a selective layer made of cellulose triacetate cast onto a non-woven backing consisting of polyester fibers individually coated with polyethylene, presenting sodium chloride rejection is in the range 95-97%. An acrylic bench-scale laboratory membrane unit with 77 mm long × 26 mm wide × 3 mm deep channels was used for the concentration experiments.

6.1.2.2 Experimental setup

Figure 6.4 shows schematically the experimental equipment used in the FO procedures.

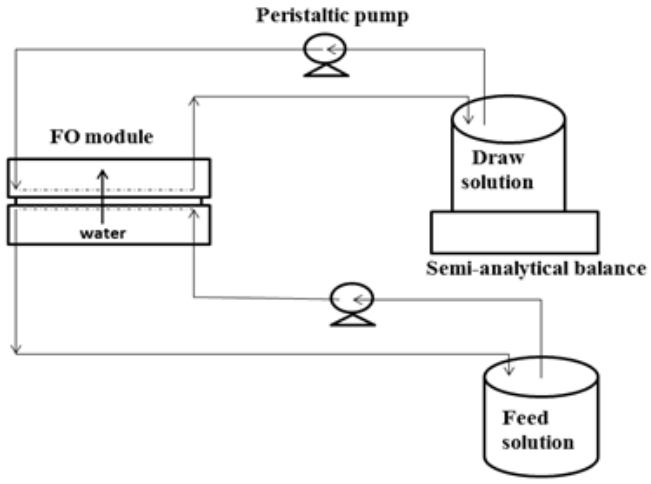

Figure 6.2 FO experimental apparatus.

Feed solution and osmotic agent solution were circulated on either side of the membrane (mode I and II) in a closed loop using peristaltic pump with adjustable speed (Masterflex, L/S, USA). Both temperature solutions were controlled within ±0.1 ºC by a

water bath (Q214M, Quimis, Brazil). Average water flux crossing the membrane to the draw solution was measured based on average weight gain in the first hour of the procedure by a semi-analytical balance (BL3200H, Shimadzu, Japan). Initial draw solution volume was 1 L while the feed solution was 200 mL (Garcia-Castello et al., 2009).

6.1.2.3 Response Surface Methodology

The study of the process parameters over FO performance was evaluated by RSM. To describe the response surface, a central composite design with five coded levels and three variables was used to study the combined influence of solutions' flow rate (x_1), $\Delta\pi$ (x_2) and temperature (x_3). $\Delta\pi$ was calculated based on the osmotic pressure (π) of the draw solutions, according to the Van't Hoff equation, mathematically expressed by Equation 6.1.

$$\pi = NRTi \tag{6.1}$$

where N is the sodium chloride concentration (mol L^{-1}), R is the gas constant (8.314 J $Kmol^{-1}$), T is the absolute temperature (K) and i is the Van't Hoff correction factor.

For the three factors, this design was made up of a full 2^3 factorial design with its eight points augmented with four replications of the center points (all factors at level 0) and the six star points, that is, points having for one factor an axial distance to the center of $\pm\alpha$, whereas the other two factors are at level 0. The axial distance α was chosen to be 1.68 to make this design orthogonal. Ranges of variables investigated with respect to their values in real and coded form are listened in Table 6.1.

Table 6.1 Experimental design ranges and levels of the independent variables in terms of actual and coded factors.

Variables	Symbol	Coded values				
		-1,68	-1	0	1	1,68
Flow rate (mL min^{-1})	x_1	30	58	100	142	170
$\Delta\pi$ (atm)	x_2	25.20	81.20	163.70	246.10	302.20
Temperature (ºC)	x_3	20	24	30	36	40

A set of 18 experiments was carried out. For three factors the equation model is:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \qquad (6.2)$$

where *Y*, water transmembrane flux (L m^{-2} h^{-1}), b_0, intercept; b_1, b_2, b_3, linear coefficients; b_{11}, b_{22}, b_{33}, squared coefficients and b_{12}, b_{13}, b_{23},interaction coefficients.

The results were analyzed by the Experimental Design Module of the *Statistica* 11 software (Statsoft, Tulsa, OK, USA). The model (Equation 2) shows the influence, linear, quadratic or interaction of each factor on the value of the dependent variable (water flux).Three-dimensional surface plots were drawn to illustrate the main and interactive effects of the independent variables on water flux.

6.1.3 Results and Discussion

The experimental design and results are shown in Table 6.2. Water fluxes in mode I (draw solution flowing against the selective layer) were higher than those for mode II (draw solution flowing against the support layer) in the same conditions. This results show that ICP, like shown in Figure 6.3, has negative impact on FO performance. In general, the increase of the three process parameters increased the permeate water flux for both modes.

Table 6.2 Experimental design and results of the 2^3 factorial design.

Flow rate (mL min^{-1})	$\Delta\pi$ (atm)	Temperature (ºC)	Water flux (L m^{-2} h^{-1})	
			Mode I	Mode II
58	81.20	24	4.87±0.28	2.81±0.08
142	81.20	24	5.19±0.37	2.72±0.10
58	246.10	24	9.76±0.35	5.71±0.15
142	246.10	24	11.49±0.47	6.18±0.28
58	81.20	36	5.53±0.16	2.54±0.15
142	81.20	36	7.34±0.22	2.60±0.11
58	246.10	36	10.77±0.33	5.65±0.21
142	246.10	36	12.08±0.58	6.54±0.27
30	163.70	30	7.44±0.38	4.26±0.23
170	163.70	30	9.46±0.48	5.24±0.10
100	25.20	30	2.17±0.15	1.19±0.07
100	302.20	30	14.64±0.68	6.64±0.09
100	163.70	20	9.72±0.42	4.80±0.27
100	163.70	40	11.68±0.55	4.69±0.19
100	163.70	30	10.63	5.05
100	163.70	30	10.48	4.91
100	163.70	30	11.18	4.79
100	163.70	30	11.24	4.86

Analysis of variance (ANOVA) was then applied in order to evaluate the statistical of the independent variables and the interaction of them. Table 6.3 shows the ANOVA for mode I, where pure water flows against the membrane support layer and the osmotic agent against the dense layer, and there is no significant PC (Figure 6.3A).

Results show that linear and quadratic effect of solutions' flow rate and $\Delta\pi$, additionally of the linear effect of the temperature affected significantly ($p < 0.05$) the water flux. The interaction of the parameters was not significant ($p > 0.05$). The calculated model *F*-value was 23.89, which is higher than the *F*-value in statistic tables at 95% of confidence ($F_{t5,6}$=4.3).

Table 6.3 Analysis of variance for the water permeate flux in mode I of FO operation.

Source	Sum of square	Degree of freedom	Mean square	*F*-value	*p*-value
Flow rate (L)	5.38	1	5.38	36.83	0.0089*
Flow rate (Q)	12.96	1	12.96	88.62	0.0025*
$\Delta\pi$ (L)	130.11	1	130.11	889.58	0.000083*
$\Delta\pi$ (Q)	13.39	1	13.39	91.57	0.0024*
Temperature (L)	4.35	1	4.35	29.76	0.012*
Temperature (Q)	0.59	1	0.59	4.07	0.13
Flow rate by $\Delta\pi$	0.10	1	0.10	0.73	0.45
Flow rate by Temperature	0.14	1	0.14	0.98	0.39
$\Delta\pi$ by Temperature	0.18	1	0.18	1.23	0.34
Error	5.78	8	1.15	7.91	0.059
Total	0.43	17	0.14		

L: linear effect; Q: quadratic effect.
* Statistically significant at 95% of confidence.

The determination coefficient (r^2) of 0.963 and the lack of fit of experimental data to the model not being significant ($p > 0.05$) demonstrate significance for the regression model (Myers and Montgomery, 2002). The equation obtained by the statistical regression was as follow:

$$Y = 10{,}91 + 1{,}56x_1 + 6{,}18x_2 + 1{,}13x_3 - 2{,}03x_1^2 - 2{,}06x_2^2 \quad (6.3)$$

The three-dimensional response surface curves were then plotted (Fig. 6.3).

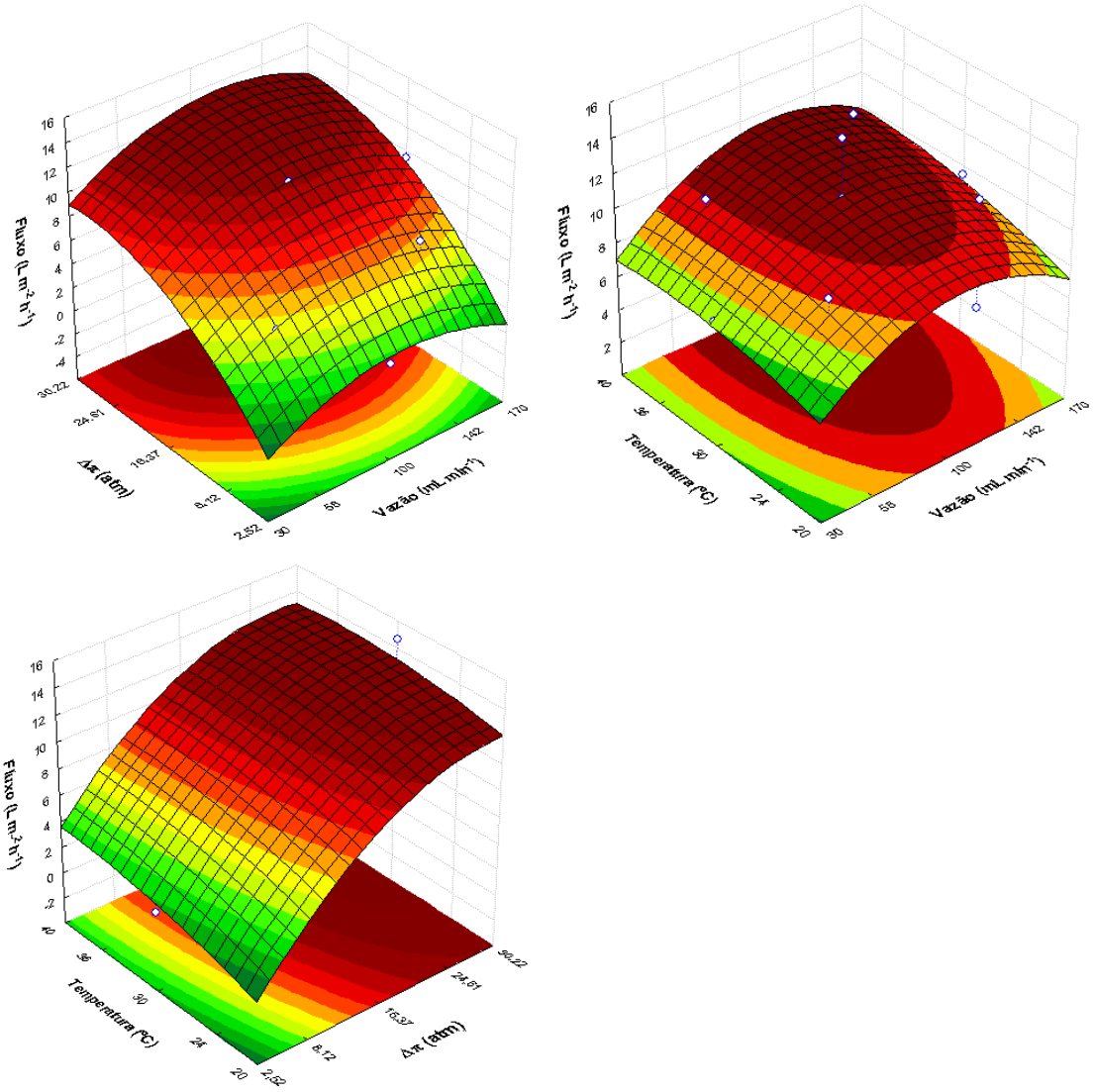

Figure 6.3 Response surface of water flux as function of Δπ, temperature and solutions' flow rate for mode I of FO operation.

Results show that higher values of flow rate, Δπ and temperature lead to higher values of water transport through the membrane. The highest water permeate flux, about 10 L m^{-2} h^{-1}, was observed in flow rate range of 100 and 170 mL min^{-1}, Δπ between 20 and 30.22 atm and temperatures between 24 and 40 °C. In mode I, the diffusion of water through the membrane is the only phenomenon that happens in FO. Since Δπ is the driven

force of the osmotic process, it presented greatest effect on the process performance. The effect of temperature and flow rate is more sensitively in higher $\Delta\pi$ ranges. The increase of the solutions' flow rate as well as the temperature increases the mass diffusion coefficient on the dense layer interface, implying in higher water transmembrane flux. Increasing process temperature also reduces the solution viscosity impacting positively in the diffusion coefficients, which results in enhancing transmembrane flux of concentration process (You et al., 2012). Moreover, higher temperatures can influence on the membrane structure, like changing the mobility of the polymeric chain allowing higher transport of water through the membrane.

Table 6.4 shows the ANOVA for mode II, where the ICP affects negatively the water flux in FO (Table 6.2). Since ICP reduces the concentration of the osmotic agent on the membrane dense layer, there is prominent decrease of the driven force, like shown in Figure 6.3B, reducing the water flux. Optimization by a conventional "one-at-a-time-approach" does not lead to a critical analysis of the operational parameter effects on the FO performance; moreover, this approach is not only massive and time consuming, but also has the limitation of ignoring the importance of interaction of process parameters.

Table 6.4 Analysis of variance for the water permeate flux in mode II of FO operation.

Source	Sum of square	Degree of freedom	Mean square	*F*-value	*p*-value
Flow rate (L)	1.40	1	1.40	114.06	0.0017*
Flow rate (Q)	0.059	1	0.059	4.84	0.11
$\Delta\pi$ (L)	33.38	1	33.38	2716.41	0.000016*
$\Delta\pi$ (Q)	1.68	1	1.68	136.89	0.0013*
Temperature (L)	0.86	1	0.86	70.72	0.0035*
Temperature (Q)	0.11	1	0.11	9.15	0.056*
Flow rate by $\Delta\pi$	0.16	1	0.167	13.62	0.034*
Flow rate by Temperature	0.00079	1	0.00079	0.064	0.816
$\Delta\pi$ by Temperature	0.00088	1	0.00088	0.071	0.81
Error	0.087	8	0.017	1.42	0.41
Total	37.63	17			

L: linear effect; Q: quadratic effect.
* Statistically significant at 95% of confidence.

Analysis of variance shows that linear and quadratic effect of solutions' flow rate and $\Delta\pi$, linear effect of temperature and the interaction of flow rate and $\Delta\pi$ affected significantly ($p < 0.05$) the water flux, indicating that the interaction of the variables must be considered for the estimation of water flux in FO processes The calculated model *F*-value was 23.90, which is higher than the *F*-value in statistic tables at 95% of confidence ($F_{t5,6}$=4.3). The determination coefficient (r^2) of 0.961 and the lack of fit of experimental data to the model not being significant (p>0.05) demonstrate significance for the regression model (Myers and Montgomery, 2002). The equation obtained by the statistical regression was as follow:

$$Y = 4{,}91 + 0{,}64x_1 + 3{,}13x_2 + 0{,}51x_3 - 0{,}73x_2^2 - 0{,}19x_3^2 + 0{,}29x_1x_2 \quad (6.4)$$

The three-dimensional response surface curves for mode of operation II (Fig. 6.4) shows that the effect of temperature and flow rate is more sensitively in higher $\Delta\pi$ ranges. The highest values water transport, about 5 L m^{-2} h^{-1}, was observed in flow rates between 100 and 170 mL min^{-1}, $\Delta\pi$ between 246.10 and 302.20 atm and temperatures between 30 and 40 °C. $\Delta\pi$ and temperature act similarly to the mode I in the enhancing of the process performance. However, ANOVA shows that the interaction of solutions' flow rate and $\Delta\pi$ turned significant ($p < 0.05$) in the water transmembrane flux.

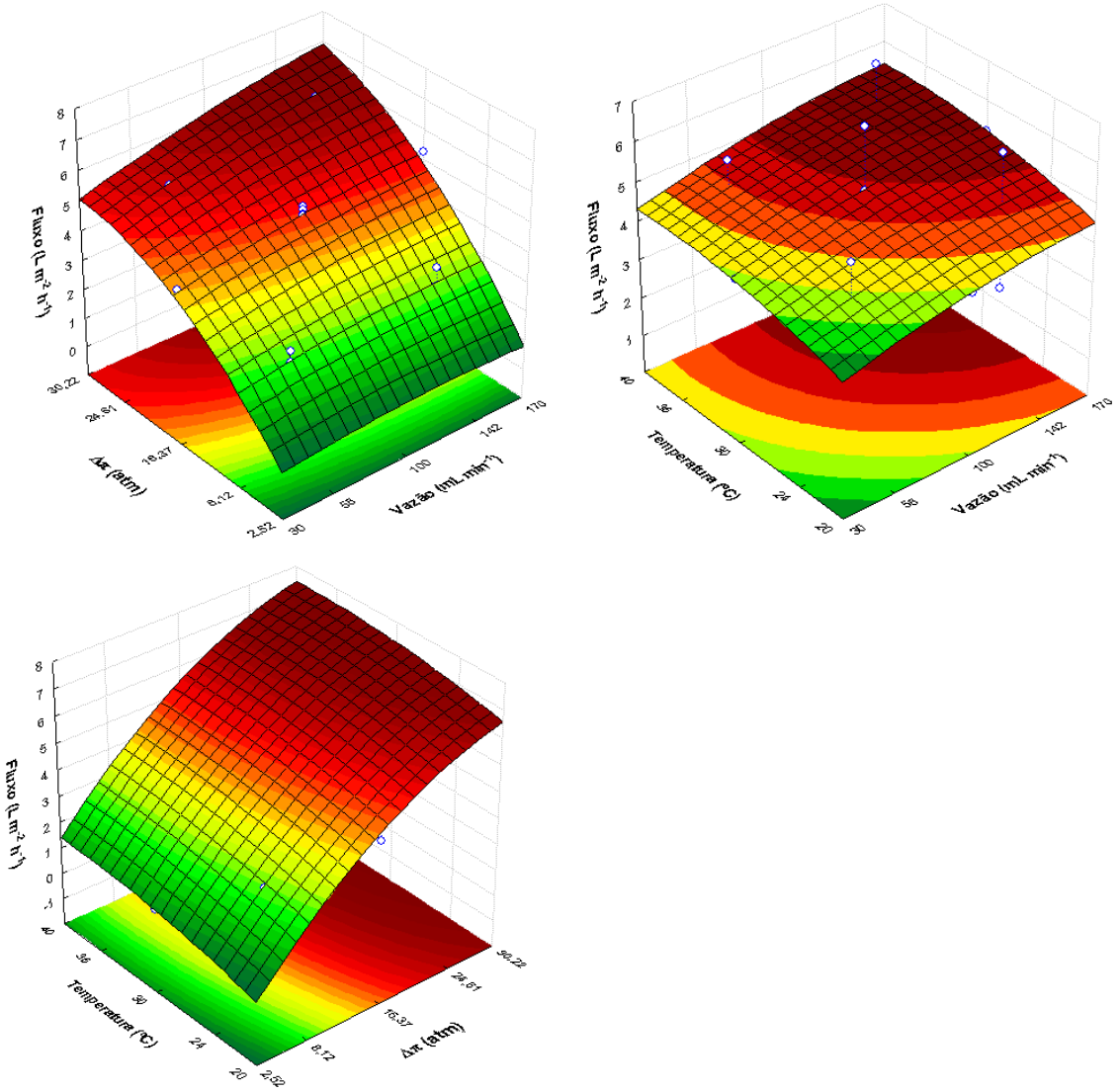

Figure 6.4 Response surface of water flux as function of Δπ, temperature and solutions' flow rate for mode II of FO operation.

The increase of the solutions' flow rate enhances the Reynolds number in the flow, reducing the hydrodynamic boundary layer thickness, turning the IPC, in the draw solution side, less accentuated. This phenomenon increases the brine concentration in the dense membrane interface, implying in higher driving force, acting positively on the FO performance (Babu et al., 2006; Sant'Anna et al., 2012).

Through phenomenological equations it would be expected the water flux to increase linearly with increasing of the process parameters studied. In the temperature range studied, this behavior was observed. However, in relation to the $\Delta\pi$, a limit water flux was obtained and the increase of the driven force presents non-significant effect on the water transport through the membrane. Similar effect was observed for the increase of solutions' flow rate. Due to the effect of ICP, high values of $\Delta\pi$ turned ICP more prominent damaging the FO performance. In the other hand, the increase of solutions' flow rate minimizes the polarization effects, generating a different behavior from linear to high $\Delta\pi$ and solutions' flow rate. These phenomena justify the statistical significance of the quadratic coefficients and the interaction of the parameters presented in the ANOVA table.

6.1.4 Conclusion

In conclusion, solution's flow rate, $\Delta\pi$ and temperature are important parameters in osmotic membrane technique, and may be controlled in order to FO present a patterned process in industrial scale. $\Delta\pi$ is the main variable, since it acts as the process driven force, although the increase of temperature and solutions' flow rate also enhances the water permeate flux. Internal polarization concentration played critical role in FO, affecting negatively the performance of the process, although the increasing of process parameters may attenuate this phenomenon. FO is an emerging membrane technique and optimization of the process relies on knowledge of the impact of parameters on water transmembrane flux. Response surface methodology showed to be a powerful tool in order to evaluate the statistical significance of the variables and their intervals of operation of FO. However, more studies are essential to warrant the adequate use of FO in industrial scale.

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CAPÍTULO 7

Effect of process parameters on water and salt transmembrane fluxes for the concentration of grape juice by forward osmosis

Neste capítulo é apresentado um estudo sobre o efeito da diferença de pressão osmótica, velocidade de escoamento das soluções no módulo de membrana e temperatura sobre o fluxo de água e sal transmembrana em sistema de osmose direta durante a concentração de suco de uva. Também foram comparadas característica (pH, acidez, teor de antocianinas, compostos fenólicos e atividade antioxidante) do suco concentrado pela técnica de membrana e reconstituído com o suco inicial.

7.1 Artigo: Effect of process parameters on water and salt transmembrane fluxes for the concentration of grape juice by forward osmosis

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CAPÍTULO 7 - EFFECT OF PROCESS PARAMETERS ON WATER AND SALT TRANSMEMBRANE FLUXES FOR THE CONCENTRATION OF GRAPE JUICE BY FORWARD OSMOSIS

Abstract

Forward osmosis is a promising membrane alternative to concentrate liquid foods and the knowledge on water and salt fluxes trough the membrane to optimize processes costs. In the present work, the influence of temperature, solutions' flow rate and osmotic pressure difference ($\Delta\pi$) were evaluated to concentrate grape juice. The increase of $\Delta\pi$ in the range of 155-370 atm implied on the improvement of salt, from 9.16 to 15.01 mg Na m^{-2} h^{-1}, and water, from 2.09 to 5.59 L m^{-2} h^{-1} fluxes. The increase of the solutions' temperature, from 15 ºC to 35 ºC, and flow rate, from 45 to 200 mL min^{-1}, in the FO system attenuated the polarization concentration phenomena leading to increase of the water and salt transmembrane fluxes. Grape juice, reconstituted after concentration by FO, presented preserved the compounds with antioxidant capability in the beverage. Sodium transport was observed to the grape juice, although it may impact slightly in the sensorial and nutritional properties of the samples.

Keywords: forward osmosis; grape juice; salt flux; water flux; sodium.

7.1.1 Introduction

Preservation and shelf-life extension are great challenges for long term stored foods due to the costs on packaging, storage and spoilage issues. Industrial processes still mostly use the heat for this aim, although it may bring severe impact on nutritious and organoleptic factors in relation to the *in natura* food. In the liquid food field, membrane separation processes, like reverse osmosis and nanofiltration, have been used to concentrate liquid foods (Versari et al., 2003; Pap et al., 2009; Gurak et al., 2010). However, these techniques present the disadvantages of presenting high tendency to the fouling phenomena and applying high hydrodynamic pressures, which may be limiting factors for their use in industrial scale.

In this context, forward osmosis (FO) has been shown up as a promising alternative. FO is a membrane separation process, in which the only driven force is the difference of the

water chemical potential, expressed in terms of osmotic pressure(π), between two solutions (feed and draw solution), separated by a semi-permeable membrane (Cath et al., 2006; Sant'Anna et al., 2012; Zhao et al., 2012). Sodium chloride brines are widely used in FO procedures as the draw solution because the solute is water-soluble, not toxic, non-expensive, meanwhile the brine presents low viscosity and high osmotic pressure (Petrotos et al., 1998). However, additionally to the water transport from the feed to the draw solution, there is the reserve transport of salt from the draw solution to the feed. Sodium is an important public health issue because it is related to hypertension diseases, and the evaluation of the effect of process parameters over the reverse sodium flux during the concentration of fruit juice is essential to the food industry, but still scarce in the literature (Sant'Anna et al., 2012).

Grape juice is a worldwide consumed beverage with great nutritional features because it is a source of polyphenols, vitamins, minerals and natural antioxidant compounds. These bioactive components present the well documented ability to scavenge free radicals, to avoid lipid oxidation, to combat cancer cell growth and other important biological activities (Sauvaget et al., 2003; Scalbert et al., 2005), potentially improving the human health. Currently, consumer's concern about human health and nutrition has growth, and thus the research for technologies that provide foods with minimal changes on their nutritious and organoleptic characteristics is an industrial trend.

In the FO field, there is little information about FO process parameters over the concentration performance and salt transport during the membrane process of grape juice. Thus, the objective of the present work is to evaluate the effect of FO process parameters (osmotic pressure difference ($\Delta\pi$), temperature and the solution's flow rate) on the water and sodium transport in the concentration of the beverage. In addition, the quality of juice concentrated by FO was compared to the fresh juice.

7.1.2 Material and Methods

7.1.2.1 Raw material

Commercial grape juice bought in the local market (Porto Alegre, RS, Brazil) was used as raw material. It was characterized by centesimal analysis according to the AOAC (1992).

7.1.2.2 Forward osmosis system and membrane

Membranes used in the grape juice concentration procedures by FO were kindly provided by Hydration Technologies Innovation (Albany, OR). They are composed by a selective layer made of cellulose triacetate cast onto a non-woven backing consisting of polyester fibers individually coated with polyethylene, presenting sodium chloride rejection in the range of 95-97%. An acrylic bench-scale laboratory membrane unit with 77 mm long × 26 mm wide × 3 mm deep channels was used for the concentration experiments.

7.1.2.3 Experimental setup

Figure 7.1 shows schematically the experimental equipment used in the FO procedures. The grape juice was flowed against the membrane dense layer and the sodium chloride solution against the membrane porous layer (in order to avoid severe internal concentration polarization and fouling phenomena of the membrane) in a closed loop. Peristaltic pump with adjustable speed (Masterflex, L/S, USA) was used to pump both solutions at the same flow rate, varying between 50 to 200 mL min^{-1}. Temperature solutions were kept at 25±1 ºC, controlled a water bath (Q214M, Quimis, Brazil), unless otherwise indicated. Initial draw solution volume was 1 L while the feed solution was 200 mL (Garcia-Castello et al., 2009). Sodium chloride at analytical grade (Synth, SP, Brazil) was used to compose the draw solution in the range of 2 to 6 mol L^{-1}, leading to $\Delta\pi$ between juice and brine in the interval of 154.82-370.51atm.

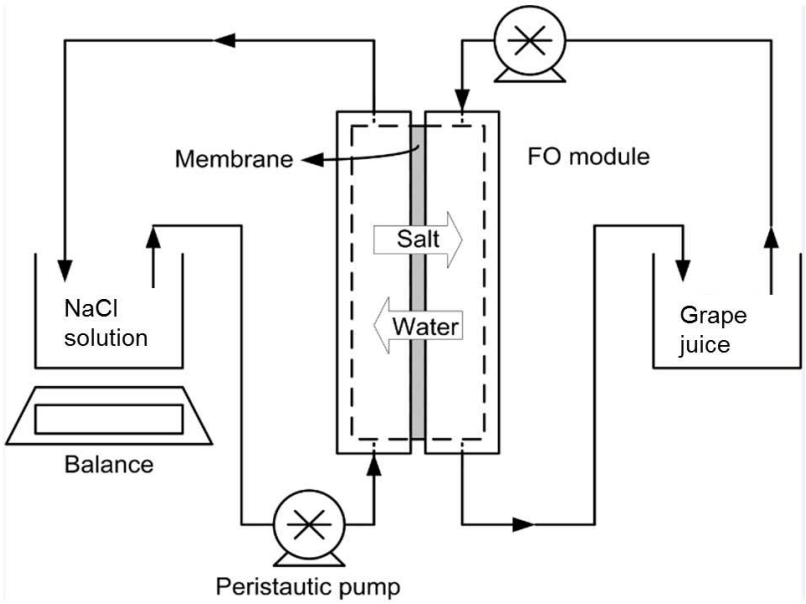

Figure 7.1 FO experimental apparatus for grape juice concentration.

The osmotic pressure (π) of the grape juice and the brine are related to water activity of the solution, as given by the following equation (Toledo, 1991):

$$\pi = -\frac{RT}{V}\ln a_w \qquad (1)$$

where R is the gas constant (8.314 J Kmol^{-1}), T is the temperature in ºK, V is the molar volume (18 ml mol^{-1} of water) and a_w is the water activity, which was measured by water activity meter (Aqualab BrasEq, S3TE, Brazil).

Average water flux crossing the membrane to the draw solution was measured based on weight gain of the draw solution in the first 5 h by a semi-analytical balance (BL3200H, Shimadzu, Japan). After the membrane concentration procedures, samples were reconstituted to the initial total soluble solid content (17 ºBrix) and salt transport was measured by analyzing the sodium content in the fresh and in the reconstituted juice by atomic absorption spectrophometer with flame atomization (Advia 1200 Siemens, USA).

Results were expressed as mg of sodium equivalent per membrane area per hour (mg Na m^{-2} h^{-1}).

7.1.2.4 Juice physicochemical quality evaluation

Samples of reconstituted juices were analyzed about their pH, using digital pHmeter (Quimis Q400M, São Paulo, Brazil) and total acidity by titration with 0.1 N sodium hydroxide (AOAC, 1997). Total soluble solid was measured using Erma's Handheld refractometer at 25 ºC.

The total phenolic content (TPC) of the beverages was determined by the Folin-Ciocalteau method by the reaction of the samples with Folin-Ciocalteau reagent (Vetec, Brazil) and sodium carbonate saturated solution (Singleton and Rossi, 1965). Results were expressed as mg gallic acid equivalent per mL of juice (mg GAE mL^{-1}). Monomeric anthocyanins (MA) were determined using the pH differential method (Wrolstad et al., 2005) by measuring the absorbance of diluted samples in potassium chloride buffer pH 1.0 and sodium carbonate buffer pH 4.5 at 520 and 700 nm and results expressed as mg of cyanidin 3-glucoside per mL of juice (mg C3G mL^{-1}).

The antioxidant capability of the grape juices was expressed by the capability scavenging ABTS radicals like proposed by Re and co-workers (1999). Briefly, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm and a 30 μL aliquot of juice was mixed with 3 mL of $ABTS^{\bullet+}$ solution and an absorbance (734 nm) reading was taken after 6 min. The percentage inhibition was calculated against a control (distilled water) and compared to a Trolox standard curve 100–2000 mM, and the results were expressed as μM of trolox equivalent per mL of juice (μM TE mL^{-1}).

7.1.2.5 Data analysis

Experiments were conducted in triplicate and average values were compared using Tukey's test by *Statistica* 11 (StatSoft, US), and differences were considered statistically significant, when $p < 0.05$.

7.1.3 Results and Discussion

7.1.3.1 C*haracterization of the juice*

According to the centesimal analysis, commercial grape juice presented 0.2 g of protein, 15.5 g of carbohydrates, 0.21 g of ashes and 84.1 g of water per 100 g of juice. The concentration of lipids was below the detection limit. The high content of water in the beverage indicates that grape juice may be suitable to biochemical and microbiological spoilage during the storage period. Thus concentration may be an interesting technique to improve the liquid food stability in long-terms periods. In this sense, the study of the effect of the parameter process on water and salt transmembrane flux for the concentration of liquid foods means an essential step for the proper utilization of FO in food industries.

7.1.3.2 Effect of $\Delta\pi$ on the salt and water fluxes

Osmotic pressure difference between the feed and draw solutions ($\Delta\pi$) is the driven force of FO processes. Figure 7.2 shows the water flux behavior through grape juice concentration time for different $\Delta\pi$. By the beginning of the process, it is observed a sharp decrease in water flux caused by initial fouling of the membrane. This first period of flux decay probably comes from the continuous deposit of high molecular weight compounds on the membrane dense surface. Grape juice showed to be a carbohydrate rich beverage, possibly due to the presence of sugars and pectin. Pectin may interact with calcium ions in the presence of acids (tartaric acid, in case of the grape juice) (Hatziantoniou and Howell, 2002), building up a gel layer on the membrane.

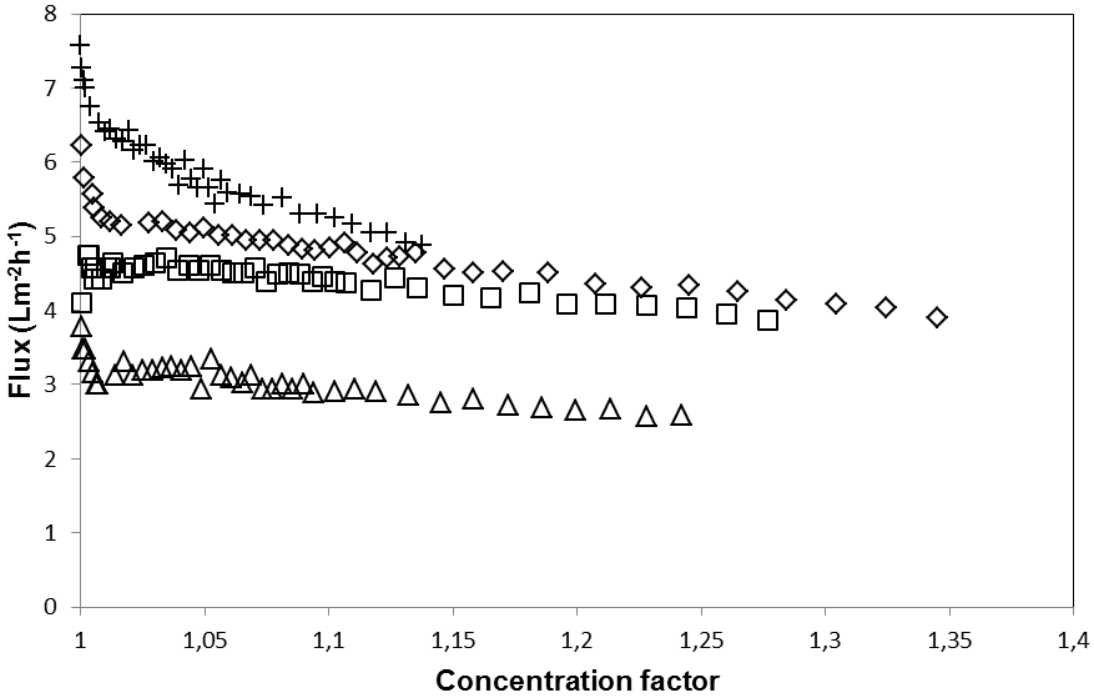

Figure 7.2 Water permeate flux versus concentration factor for the osmotic concentration of grape juice. Operation temperature was kept 25 ºC, solutions' flow rate was 200 mL min^{-1} and Δπ was (Δ) 155 atm, (□) 219 atm, (◊) 290 atm, (+) 350 atm. Data are the average of three independent experiments and standard deviation was always less than 3%.

According to Garcia-Castello and McCutcheon (2011), the fouling development phenomena in FO is mainly due to the presence of this cake layer, which causes severe fouling. After the initial fouling period, the flux decline lessens and becomes more steady, due to the juice concentration, enhancing its osmotic pressure and consequently reducing the permeate flux. The increase of the Δπ implied in higher average water fluxes, ranging from 2.91 L m^{-2} h^{-1} when Δπ was 155 atm to 5.59 L m^{-2} h^{-1} when Δπ was 350 atm. Permeate fluxes using Δπ of 219 and 290 atm did not differ significantly ($p > 0.05$) achieving values of 4.77 and 4.30 L m^{-2} h^{-1}, respectively.

Figure 7.3 shows the behavior of sodium transport across the membrane for different Δπ, due to grape juice concentration, keeping temperature at 25 ºC and solutions' flow rate at 200 mL min^{-1}.

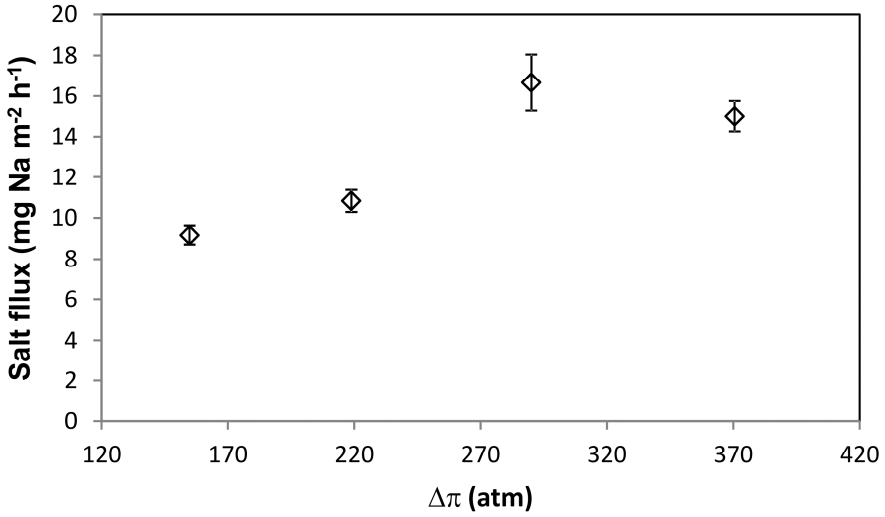

Figure 7.3 Effect of Δπ on salt reverse flux for the osmotic concentration of grape juice. Operation temperature was kept 25 ºC and solutions' flow rate was 200 mL min^{-1}. Data are the average of three independent experiments.

The increase of Δπ from 155 atm to 290 atm increased significantly ($p < 0.05$) the salt transport from 10.83 to 16.67 mg Na m^{-2} h^{-1}. In membrane osmotic processes, beyond the water transport from the feed to the draw solution, there is the reserve transport: salt from the draw solution to the feed. This phenomenon is also influenced by the difference of osmotic pressure in the interface of the active layer, and thus the increase of the Δπ may imply on the increase of the salt transport. Babu and co-workers (2006) observed similar results for the concentration of pineapple juice by FO, where it was observed the increase that the increase of sodium chloride concentration as the osmotic agent enhanced the salt transport to the juice. However, when 290 atm and 370 atm were used, there was no significant difference ($p > 0.05$) of the salt transport. This may be possible due to the concentration polarization phenomena that may happen in osmotic processes, which reduces the driving force on the interface of the dense layer (Figure 7.4).

Concentration polarization is a critical technological limitation of FO. Figure 7.4 shows schematically the main phenomena during the FO process. In this representation, a component of solution with low molecular weight, in the present work, sodium chloride, flows against the support (porous) layer. Since the osmotic water transfers happen in the

interface of the active (dense) layer, the diffusion of water through the active layer will result in dilution of the brine, setting up of an internal concentration polarization (ICP).

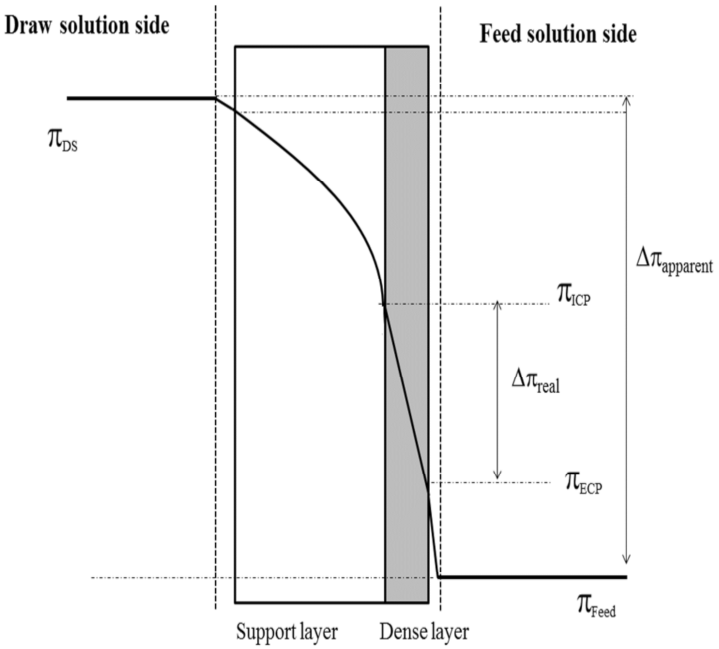

Figure 7.4 Mechanism of FO indicating schematically internal concentration polarization (ICP) and external concentration polarization (ECP) during the concentration of liquid foods using sodium chloride as draw solution. $\Delta\pi_{real}$ and $\Delta\pi_{apparent}$ are the corresponding real and the apparent driving forces, respectively. π_{feed} and π_{DS} are the osmotic pressures of feed and osmotic agent solution, and π_{ICP} and π_{ECP} are the effective osmotic pressures of feed due to ECP and the effective osmotic pressures of draw solution due to ICP.

The external concentration polarization (ECP) in this side is small and considered negligible (Nayak et al., 2011; Sant'Anna et al., 2012). The π of the osmotic agent, thus, decreases from π_{DS} to π_{ICP}, damaging the driving force and consequently the concentration performance. When a complex matrix, composed of low and high molecular weight compounds, is flowed against the porous layer, there is the deposit of these components on the dense structure of the membrane leading to a cake layer formation due to low shear forces. This phenomenon results in a buildup of intense ECP and an increase of the feed osmotic pressure from π_{Feed} to π_{ECP}. Consequently, the apparent driving force ($\Delta\pi_{apparent}$ =

$\pi_{DS} - \pi_{Feed}$) will be lower than the actual osmotic process ($\Delta\pi_{real} = \pi_{ICP} - \pi_{ECP}$), leading to an intense reduction of the FO performance. In the present work, the increase of the $\Delta\pi$ do not imply the increase of the water permeate flux, possibly due to the increase of ICP that happens in the brine solution side, masking the enhance of $\Delta\pi$.

3.3 Effect of solutions' flow rate on the salt and water fluxes

Figure 7.5 shows the results of water flux using different flow rate of the solutions into the FO system.

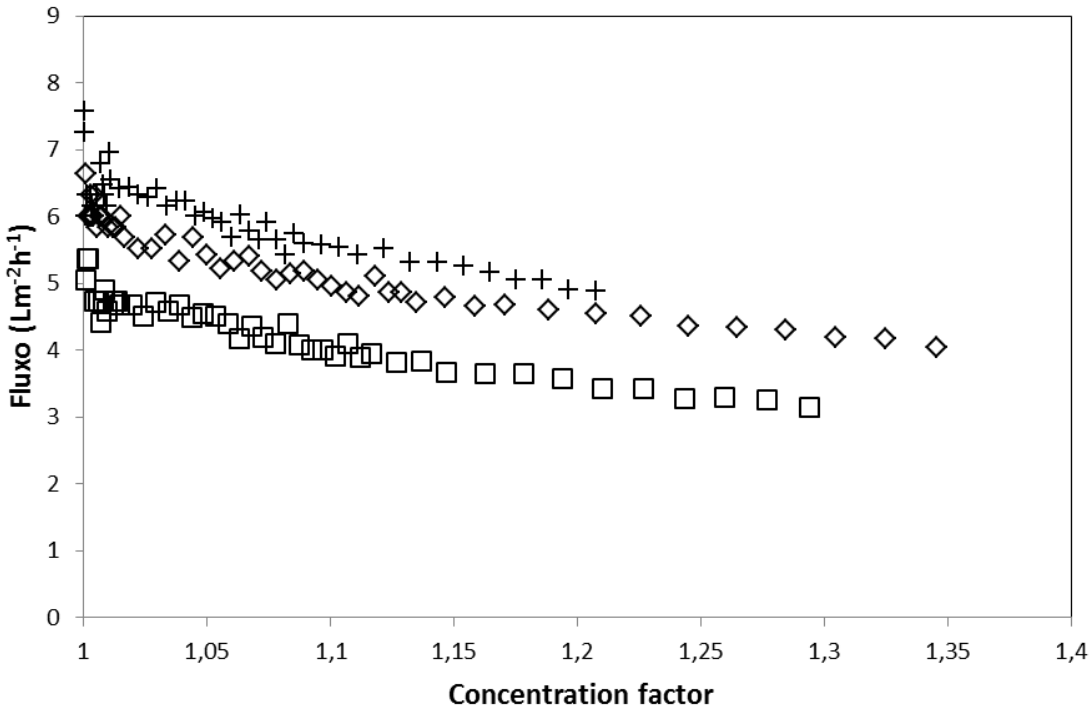

Figure 7.5 Water permeate flux through concentration factor for the osmotic concentration of grape juice. Operation temperature was kept 25 ºC, $\Delta\pi$ was 350 atm and solutions' flow rate was (□) 45 mL min^{-1}, (◊) 110 mL min^{-1}, (+) 200 mL min^{-1}. Data are the average of three independent experiments and standard deviation was always less than 6%.

Temperature was maintained at 25 ºC and the $\Delta\pi$ at 370.51 atm. The increase of the solutions' velocity implied on the significantly ($p < 0.05$) increase of the water

transmembrane flux. Responses varied from 3.86 L m^{-2} h^{-1} to 5.59 L m^{-2} h^{-1} when the flow rate increased in the range of 45-200 mL min^{-1}. An increasing on the flow rate enhances the Reynolds number in the flow, reducing the hydrodynamic boundary layer thickness, turning the ICP, in the draw solution side, less accentuated (Babu et al., 2006). These phenomena may increase the brine concentration in the dense membrane interface, implying in driving force enhance (Babu et al., 2006; Sant'Anna et al., 2012). Additionally, higher flow rate induces a reduction of ECP and higher shear strengths on the membrane surface in the feed side, reducing the fouling cake on the juice side, resulting in lower osmotic pressure. The combination of these effects seems to improve the driving force of the FO process and the transmembrane flux.

In the same context, Figure 7.6 shows the effect of the increase of the solutions' flow rate in the range of 45-200 mL min^{-1} on sodium flux through the membrane.

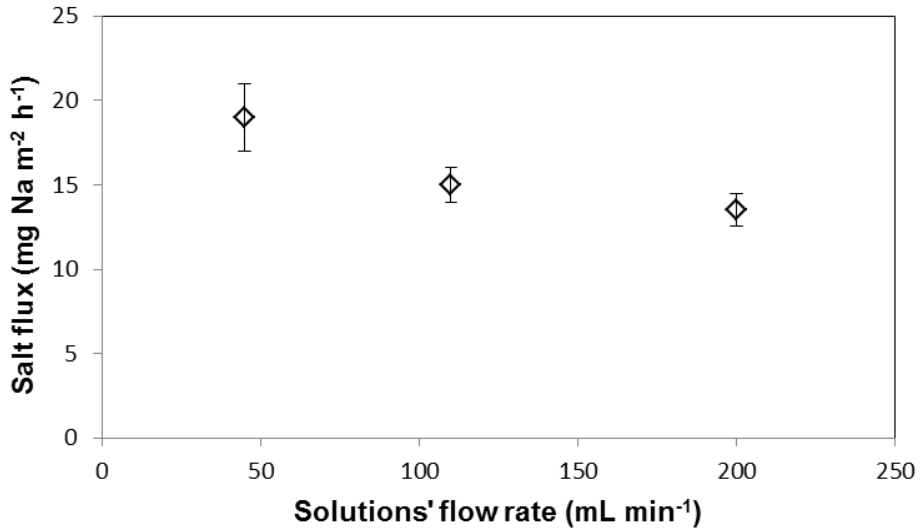

Figure 7.6. Effect of solution's flow rate on salt reverse flux for the osmotic concentration of grape juice. Operation temperature was kept 25 ºC and $\Delta\pi$ was 350 atm. Data are the average of three independent experiments.

Results show that the increase of flow rate from 110 to 200 mL min^{-1} did not differ significantly ($p > 0.05$) sodium transport. However, when the solutions' flow rate was increased from 45 mL min^{-1} to 100 or 200 mL min^{-1}, the sodium reverse flux decreased

significantly ($p < 0.05$) from 18.55 mg Na m^{-2} h^{-1} to 14.05 mg Na m^{-2} h^{-1}. The results indicate the high flow rates attenuated the PC phenomena, implying on lower transport of solutes transmembrane, in the same sense as the increase of water flux in the same conditions.

3.4 Effect of temperature on the water and salt flux

The influence of the temperature on the water flux was evaluated using the solutions' flow rate of 200 mL min^{-1} and $\Delta\pi$ of 370.51 atm. The results are due to exclusively due to the effect of the temperature, since $\Delta\pi$ was calculated based on Equation 1, which considers the influence of temperature on the solutions' osmotic pressure.

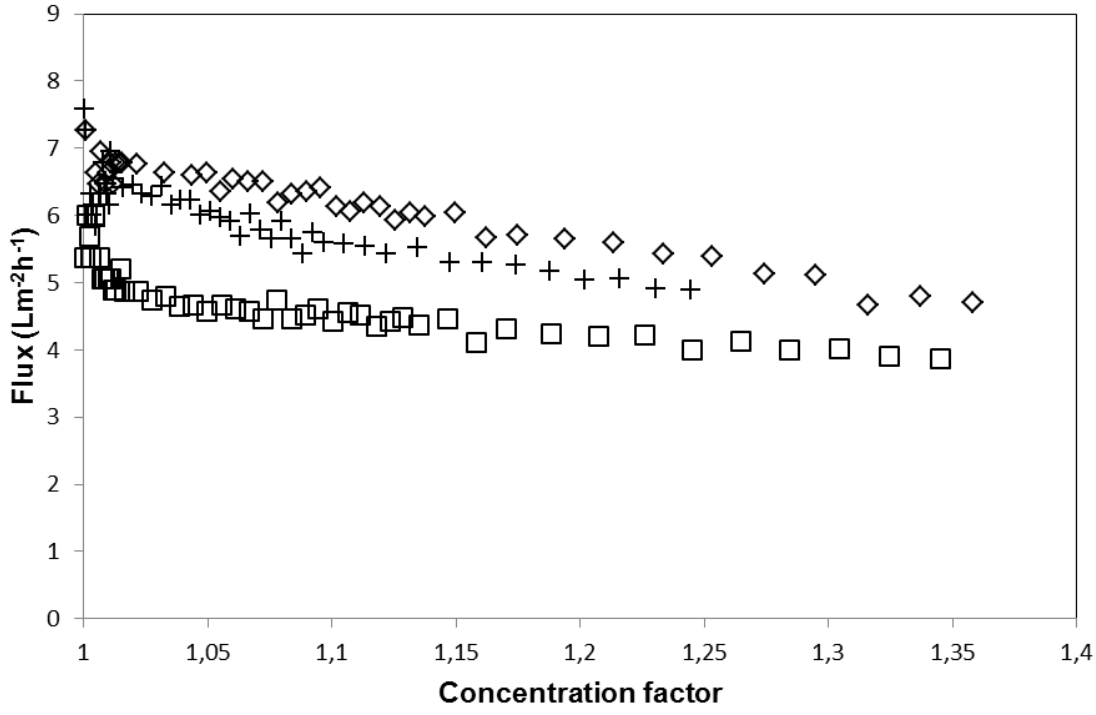

Figure 7.7 Water permeate flux through concentration factor for the osmotic concentration of grape juice. Operation solution's flow rate was kept at 200 mL min^{-1}, $\Delta\pi$ was 350 atm and solutions' temperature was (+) 15 ºC, (◊) 25 ºC, (□) 35 ºC. Data are the average of three independent experiments and standard deviation was always less than 3.5%.

Figure 7.7 shows that the increasing the temperature from 15 ºC to 25 ºC resulted on significantly ($p < 0.05$) increase of the water flux from 4.33 to 5.64 L m^{-2} h^{-1}. The salt transport increased significantly ($p < 0.05$) and varied from 6.67 to 13.17 mg Na m^{-2} h^{-1} in the same temperature range (Figure 7.8).

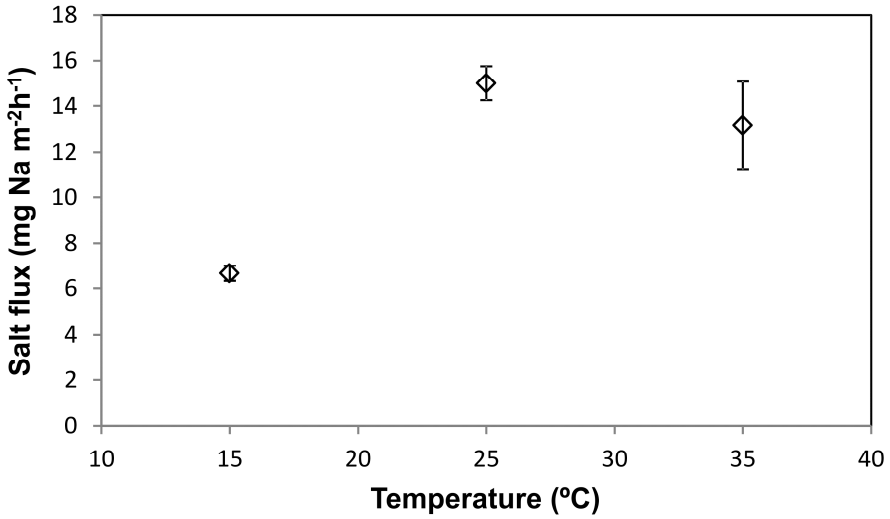

Figure 7.8 Effect of temperature on salt reverse flux for the osmotic concentration of grape juice. Solutions' flow rate was kept 200 mL min^{-1} and $\Delta\pi$ was 350 atm. Data are the average of three independent experiments.

These phenomena are related to the mass diffusion coefficient, which is proportional to the absolute temperature (Wilke and Chang, 1955). Additionally, increasing process temperature reduces the solution viscosity impacting positively in the diffusion coefficients, which results in enhancing transmembrane flux of concentration process. You and co-workers (2012) verified that the increase of the temperature presents a much more prominent effect on the viscosity of the solutions than on the osmotic pressure of them. In the present work, the effect of the temperature was accounted on the $\Delta\pi$, thus the effect of the changes on the solutions' temperature is only due to physicochemical properties of the solutions and on mass transfer phenomenon. On the other hand, the when the solutions were maintained at 35 ºC the water flux did not altered significantly ($p > 0.05$) in relation to

25 ºC, which could result from the difficult evaluation of system transport in such a small temperature variation.

3.5 Grape juice quality

Rodriguez-Saona and co-workers (2001) observed that the combination of FO and thermal concentration resulted in a fast procedure with good preservation of sensorial and nutritional characteristics of concentrated red radish extracts. Thus, FO seems to be indicated as a pre-concentration operation in liquid food field. In this sense, grape juice was concentrated by membrane direct osmosis up to 1.5-fold (up to 27ºBrix) and reconstituted with ultrapure water to the initial concentration (17ºBrix) in order to evaluate the physiochemical properties of the fruit beverage to the fresh juice. Conditions used for this aim were: 25 ºC, $\Delta\pi$ of 350 atm and solution's flow rate of 200 mL min^{-1}.

Table 7.1 presents the physicochemical properties of the fresh and reconstituted juice after FO concentration.

Table 7.1 Physicochemical properties of the fresh and reconstituted grape juice after FO concentration. Conditions: 25 ºC, $\Delta\pi$ of 350 atm and solution's flow rate of 200 mL min^{-1}.

Characteristics	Fresh	Reconstituted
ºBrix	17±0[a]	17±0[a]
pH	3.22±0.09[a]	3.30±0.07[a]
Titratable acidity (g acid/100 mL juice)	1.01±0.05[a]	1.08±0.10[a]
Total phenolic compounds (mg GAE mL^{-1})	2.19±0.14[a]	2.24±0.11[a]
Monomeric anthocyanins (mg C3G mL^{-1})	0.100±0.002[a]	0.097±0.003[a]
Antioxidant activity (μM TE mL^{-1})	10045.12±92.51[a]	10139.13±521.22[a]
Sodium content (mg mL^{-1})	0.90±0.05[a]	1.75±0.03[b]

[a,b] Different superscripts in the same column indicate statistical differences ($p < 0.05$).

The titratable acidity and pH were not significantly ($p > 0.05$) affect by the concentration procedure, presenting values of 1.08 g acid per 100 mL of juice and pH of 3.30, respectively. Babu and co-workers (2006) also observed that FO osmosis did not affect the acidity properties in pineapple juices. Additionally, Nayak and Rastogi (2010) observed that reconstituted kokum extract after FO concentration presented comparable physicochemical properties to those from the fresh extract.

TPC was not significantly affected ($p > 0.05$) by the concentration processes, being average content of 2.20 mg GAE mL^{-1} in the samples. Similar results were obtained for MA, which did not differ significantly ($p > 0.05$) to initial juice. In the same sense, the compounds with antioxidant capability, evaluated by the ABTS radical scavenging capability, were not affected significantly ($p > 0.05$) by the concentration process. Worlstad and co-workers (1993) and Nayak and Rastogi (2010) observed that FO did not affect the MA concentration of raspberry juice and kokum extract, respectively, compared to the fresh samples.

A significant ($p < 0.05$) transport of sodium was observed in the reconstituted juice when concentrated by FO. Grape juice after FO concentration presented f 17.5 mg Na L^{-1}, and the fresh juice, 0.9 mg Na L^{-1}. For sodium consumption, the Institute of Medicine (2006) set the Tolerable Upper Intake Level (UL), the highest daily component intake level that is likely to pose no risk of adverse health effects, at 2300 mg per day. Results from the present work, suggest that the transport of sodium to the grape juice do not affect the nutritional characteristics of the beverage.

Babu and co-workers (2006), concentrating pineapple juice for 18 h in FO experiments using 16% (w/w) of sodium chloride as draw solution, observed salt transport of 1.28%, which means 12800 mg L^{-1} of sodium chloride, much higher than the concentration found in the present work, where grape juice was pre-concentrated for 5 h. Thus, FO shows to be an important technique to be used for pre-concentrating liquid foods, with maintenance of antioxidant compounds and slight change the nutritional properties.

7.1.4 Conclusion

Temperature, solutions' flow rate and $\Delta\pi$ showed significant influence on the grape juice concentration performance in the FO process and on the sodium transport from the osmotic agent to the beverage. The increasing of values of the process parameters implied on increase of water and salt fluxes. Reconstituted grape juice, after concentration by FO, presented similar physicochemical characteristics to the initial juice, showing that FO preserved the compounds with antioxidant capability in the beverage. Sodium transport was

observed to the grape juice, although it may impact slightly in the sensorial and nutritional properties of the samples.

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CAPÍTULO 8

Influence of air drying temperature and velocity on the characteristics of grape marc using response surface methodology

Nesse trabalho foi avaliada a influência da temperatura e da velocidade do ar de secagem sobre a taxa de secagem e a retenção de compostos bioativos de bagaço de uva. O uso da metodologia de resposta permitiu avaliar a influência estatística dos parâmetros e da interação deles sobre aspectos nutricionais e de desempenho do processo.

8.1 Artigo: Influence of air drying temperature and velocity on the characteristics of grape marc using response surface methodology.

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Abstract

A 2^2 factorial design was performed in order to evaluate the influence of temperature (60-100 ºC) and air velocity (0.49-1.57 m s^{-1}) on the dewatering rate and nutraceutical properties of grape marc submitted to hot air drying. Both variables and the interaction between them significantly affected (P<0.01) the drying constant rate, the retention of different classes of polyphenols and the concentration of compounds with antioxidant activity. High drying performance was found at temperature range of 80-100 ºC and air

velocity between 1.30 and 1.57 m s^{-1}, while total phenolic and flavan-3-ol content was held with the process happening at temperature range of 60-70 ºC and air velocity between 1.03-1.57 m s^{-1}. Total flavonols were susceptible to oxidation due to increase of air flow. High ABTS radical scavenging activity was found in extracts of grape marc dried at high temperatures, suggesting formation of different active compounds during the drying process.

Keywords: grape marc; drying; response surface methodology; antioxidant activity; polyphenols.

8.1.1 Introduction

Agro-industrial waste management is one of the major challenges of food industries. The residue from grape juice and wine making represents approximately 15% of the fresh fruit, and it is a great source of bioactive compounds and antioxidant dietary fibers (Saura-Calixto, 1998; 2011). Dietary fibers as well as phenolic extracts from grape byproducts have shown to present the capability to reduce plasma cholesterol, adnominal aortic atherosclerosis, cardio vascular risk factors, plaque aggregation and tendency of ischemic reperfusion injury, among others beneficial effects to human health (Sato et al., 1999; Pataki et al., 2002; Auger et al., 2004; Frederiksen et al., 2007; Pérez-Jiménez et al., 2008; Saura-Calixto 2011). These features show that grape marc represents an interesting alternative as functional compound in food and pharmaceutical applications.

Dewatering is an essential step in the processing of food residues for enhancing microbial and biochemical stability and reducing costs on packaging, transport and storing (Larrauri, 1999; Dova et al., 2007). Despite of advances on the freeze drying technologies for food processing, aiming minimal degradation of labile compounds, convective hot air drying remains as the most widely industrial method for food dewatering, due to higher performance and lower costs of the equipment (Vashisht et al., 2011). Larrrauri and co-workers (1997) verified that drying grape marc at 60 ºC had no significant difference of extractable polyphenols and condensed tannins content compared to the residue dried by lyophilization. Torres and co-workers (2010) on the other hand, observed high loss of

volatile compounds, besides of high degradation of anthocyanins, comparing the dewatering of grape marc in similar conditions used by Larrauri et al. (1997).

Time, temperature and air velocity are critical parameters on the drying process. Vega-Gálvez and co-workers (2012), evaluating the effect of temperature and air velocity on drying characteristics of apple slices, observed that both process parameters were statistically significant to improve effective moisture diffusivity and the preservation of total phenolics on the dried fruit. In this context, factorial design shows up as a powerful tool in order to evaluate the influence of process parameters and mainly the interaction between them on response variables, which seems to be essential to better elucidate the effect of the drying process on the dewatering rate and on nutritional aspects. However, information in literature using this technique for drying grape byproducts is scarce.

The objective of the present work is to evaluate, by a factorial design, the influence of temperature and air velocity on the drying performance of a thin layer of grape marc and on the stability of different classes of phenolics in the dried grape residue.

8.1.2 Material and Methods

8.1.2.1 Plant material

Grape marc was obtained after juice pressing operation of *Vitis labrusca* cv. "Isabel" (Vinícola Aurora, Caxias do Sul, RS, Brazil) in 2011. Samples were kept at -40 ºC and thawed in the dark overnight at 4 ºC before drying analysis.

8.1.2.2 Drying procedure

Drying experiments were performed in an industrial pilot dryer like described elsewhere (Cassini et al., 2007). The dryer equipment was composed of a centrifugal fan (1200/3700 rpm, 15 $m^3 min^{-1}$), three electrical resistances in parallel (180 °C at 330 $m^3 min^{-1}$), a drying chamber with mobile sidewalls (that makes possible the inversion of the drying air stream between ascendant and descendant) and a recipient for product disposal (area of 0.04 m^2) connected to a digital balance (Mettler, model BB3000, Mettler-Toledo AG, Grefensee, Switzerland) with precision of 0.1 g.

The input drying air flow rate and temperature were the controlled parameters, which varied in the range of 0.49-1.57 m s^{-1} and 60-100 ºC, respectively; the first was measured through the drying air inlet channel, with a cross-sectional area of 76 cm^2, and the latter was measured at the entrance of the drying chamber. The air pumped into the dryer presented relative humidity of about 70%. Experimental procedures were organized by a 2^2 factorial design with four repetitions of the central point, like shown in Table 1. The equipment was turned on and left for 20 min at the planned condition (Table 1), when sample recipient, spread of a single layer of 230 g of the grape bagasse (2 mm of thickness), was put into the dryer. The moisture losses were recorded every 5 min with inversion of the drying air stream every 2.5 min, switched between ascendant and descendant, in order to provide a more uniform drying conditions (Cassini et al., 2007).

Experiments were stopped when drying rates were less than 0.1 g of water min^{-1}. The moisture content of the samples, before and after the drying experiment, was determined using the standard method of moisture content determination (Association of Official Analytical Chemists – AOAC, 1990).

8.1.2.3 Data adjustment

For minimizing the influence of the initial moisture content variation, the moisture rate (*MR*) concept was used:

$$MR = \frac{X - X_e}{X_0 - X_e} = \frac{X}{X_0} \quad (1)$$

where X is the relative humidity (on dry basis, gram of water per gram of solids) at time t (min), X_0 and X_e the initial and equilibrium relative moisture of the sample (on dry basis), respectively. Since, X_e is smaller than X and X_0, it can be considered negligible.

In order to analyze the influence of the studied parameters, as well as the interaction effects between them, on the drying rate performance, *MR* data through time were fitted to the classic exponential Lewis' model (Bruce, 1985) (Eq. 2), like proposed by Cassini and co-workers (2007):

$$MR = \exp(-kt) \quad (2)$$

where k (min^{-1}) is a constant drying rate, which is an indicative of how fast the dewatering process happens, and t, the time (min).

To describe the response surface, a complete factorial design with three coded levels and two variables was used to study the combined influence of temperature (x_1) and air velocity (x_2). For the two factors, this design was made up of a 2^2 factorial design with its two points augmented (-1 and +1 coded values) with four replications of the central points (all factors at level 0). A set of 12 experiments was carried out. Table 8.1 shows factorial planning, with independent variables and their real values at the different coded levels of the factorial design experiments. For two factors the equation model is:

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 \quad (3)$$

where Y is response (constant drying rates from kinetic analysis, total phenolic content (TPC), total flavonols (TF), flavan-3-ols content (F3C) and antioxidant activity (ABTS)); b_0, intercept; b_1, b_2, linear coefficients and b_{12}, interaction coefficients.

The results were analyzed by the Experimental Design Module of the *Statistica* 10.0 software (Statsoft, Tulsa, OK, USA). Three-dimensional surface plots were drawn to illustrate the main and interactive effects of the independent variables on the drying characteristics of the grape by-product.

8.1.2.4 Analysis of polyphenols

For purposes of comparison, extraction of polypehnols and compounds with antioxidant activity from dried samples was performed sequentially with 40 mL of methanol:water (50:50, v/v) and 40 mL of acetone:water (70:30, v/v) like suggested by Larrauri et al. (1997). Epicatechin, rutin, Folin-Ciocalteau reagent, *p*-dimethylaminocinnamaldehyde and 2-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid

were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were obtained from Vetec Química Fina (Duque de Caxias, RJ, Brazil).

Total phenolic content (TPC) in the extracts was determined by the Folin-Ciocalteau method described by Singleton and Rossi (1965) with few modifications. To 40 μL of diluted samples were added 3.2 mL of distilled water and 200 μL of the Folin-Ciocalteau reagent. The mixture was left in the dark for 5 min, when 600 μL of a sodium carbonate saturated solution was added and allowed to react for 1.5 h in the dark. The absorbance of the reaction mixture was measured at 765 nm by UV-1600 spectrophotometer (Pró-Análise, Brazil). A calibration curve of gallic acid was prepared and TPC of extract was standardized against gallic acid and expressed as mg gallic acid equivalent per gram of dry bagasse weight (mg GAE g^{-1}).

The flavan-3-ols content (F3C) was determined following the procedure described by Arnous et al. (2001). Briefly, a sample (500 μL) properly diluted was mixed with 1 mL of *p*-dimethylaminocinnamaldehyde (0.1% in 1 N HCl-Methanol) solution and stood for 10 min at room temperature. The absorbance was recorded at 640 nm. The concentration of FC was determined and expressed as mg epicatechin equivalents (mg of ECE/g dried sample).

The total flavonols (TF) were determined following the procedure as described by Mazza and co-workers (1999). Briefly, to 250 μL of extracts were added 250 μL of 0.1% HCl in ethanol (v/v) and 4.55 mL of 2% HCl (v/v). The solution was thoroughly mixed and allowed to stand for approximately 15 min before reading the absorbance at 360. Rutin was used as standards and results were expressed as μg of rutin equivalent (RE) per gram of dry solids.

8.1.2.5 Antioxidant activity

Antioxidant analysis were performed by the determination of 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical scavenging activity (Re et al., 1999), which involves the generation of ABTS radical chromophore by the oxidation of ABTS with potassium persulfate. The ABTS radical cation was produced by reacting 7 mmol L^{-1} ABTS stock solution with 140 mmol L^{-1} potassium persulfate, and allowing the mixture to

stand in the dark for 16 h at room temperature before use. For the assay, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. An aliquot of 30 μL of extract was mixed with 1 mL of $ABTS^{\bullet+}$ solution and an absorbance (734 nm) reading was taken after 6 min. Distilled water, instead of sample, was used as a control. The results were expressed as: scavenging activity (%) = $[1 - (A/A_0)] \times 100$, where A is the absorbance of the test and A_0 is the absorbance of the control.

8.1.2.6 Statistical analysis

Extraction of the phenolic compounds was conducted in triplicate and averages of two independent tests were calculated. Obtained values were compared using Tukey's test by *Statistica* 10.0, and differences were considered statistically significant when $p<0.05$.

8.1.3. Results and Discussion

The study of the influence of the drying temperature and air velocity was performed by their statistical combination in a factorial design. Fig. 8.1 shows the curves of *MR* loss through time for each run of the experimental design. Lewis' model (Eq. 2) showed to be a good equation to describe the drying performance, since R^2-values higher than 0.96 were obtained for the adequacy of the *MR* data through time to the model. The slowest dewatering process happened at the lowest temperature and air velocity conditions (60 ºC and 0.49 m s^{-1}) as well as the fastest drying process happened at highest temperature and air velocity applied (100 ºC and 1.57 m s^{-1}). Higher temperatures and air velocities enhance heat and mass transfer through the solids of the plant matrix, leading to faster drying performances.

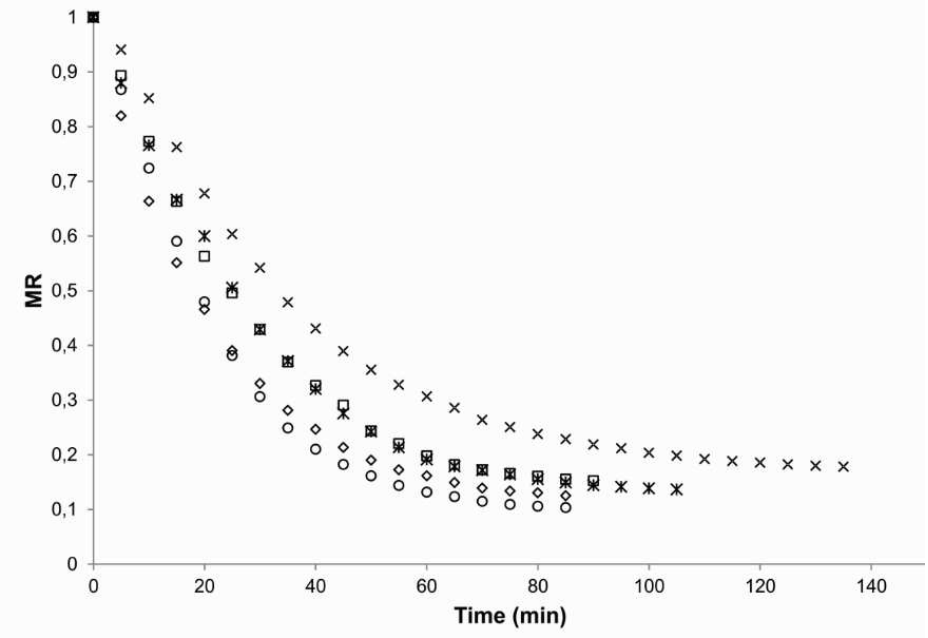

Figure 8.1 Moisture content loss through time during hot air dewatering of grape marc at 60 ºC and 0.49 m s^{-1} (◊), 60 ºC and 1.57 m s^{-1} (o), 100 ºC and 0.49 m s^{-1} (Δ), 80 ºC and 1.30 m s^{-1} (x) and 100 ºC and 1.57 m s^{-1} C (□). Data are the average values of two independent experiments with standard errors always less than 5%.

Table 8.1 presents the results of the factorial design experiments for studying the effects of drying temperature and air velocity (independent variables) on constant drying rate (estimated by non-linear regression of fitting experimental data to Eq. 2), retention of TPC, TF, F3C and compounds with antioxidant capability.

CAPÍTULO 8 - INFLUENCE OF AIR DRYING TEMPERATURE AND VELOCITY ON THE CHARACTERISTICS OF GRAPE MARC USING RESPONSE SURFACE METHODOLOGY

Table 8.1 Experimental design and results for drying grape bagasse.

	Independent variables		Drying rate		Polyphenols			Antioxidant Activity
Run	Temperature[a] (x_1)	Air velocity[b] (x_2)	*k*-values[c]	R^2	TPC[d]	TF[f]	F3C[e]	ABTS[g]
1	60 (-1)	0.49 (-1)	0.0177	0.967	22.937	48.275	4.760	20.542
2	100 (1)	0.49 (-1)	0.0363	0.979	6.020	11.786	2.880	67.313
3	60 (-1)	1.57 (1)	0.0274	0.976	25.489	11.530	5.556	71.393
4	100 (1)	1.57 (1)	0.0351	0.921	17.779	29.576	2.534	66.468
5	60 (-1)	0.49 (-1)	0.0183	0.963	24.668	47.014	4.397	19.079
6	100 (1)	0.49 (-1)	0.0355	0.994	6.002	12.058	2.911	65.759
7	60 (-1)	1.57 (1)	0.0255	0.990	26.381	11.254	5.971	69.794
8	100 (1)	1.57 (1)	0.0326	0.990	19.888	30.978	2.985	63.039
9	80 (0)	1.03 (0)	0.0316	0.982	25.269	24.884	3.221	65.174
10	80 (0)	1.03 (0)	0.0266	0.980	23.106	24.440	3.571	65.274
11	80 (0)	1.03 (0)	0.0261	0.983	20.131	23.123	3.211	61.925
12	80 (0)	1.03 (0)	0.0266	0.984	21.426	22.789	3.494	62.127
13	Lyophilized		-	-	26.973	49.018	5.904	60.111
14	Fresh		-	-	29.603	55.115	6.128	49.039

[a] real values of temperature are expressed as ºC; [b] real values of air velocity concentration are expressed as m s^{-1}; [c] values expressed as min^{-1}; [d] values expressed as mg of gallic acid equivalent per gram of dry basis; [e] values expressed as mg of epicatechin equivalent per gram of dry basis; [f] values expressed as mg of rutin equivalent per gram of dry basis; [g] values expressed as %.

The analysis of variance (ANOVA) was employed for the determination of significant parameters and to estimate response variables as a function of temperature and air velocity applied to dry the grape residue (Table 8.2).

Table 8.2 Analysis of variance for the models of grape marc drying performance (*k*-value), extractable polyphenols (TPC, TF and F3C) and antioxidant activity from dried grape residue.

Source	Sum of Square	Degree of Freedom	Mean Square	*F*-value	*P*-value	R^2
Results for *k*-values						0.9354
Temperature (L)	0.000321	1	0.000321	87.82	0.000033**	
Air velocity (L)	0.000021	1	0.000021	5.73	0.048**	
Temperature (L) by air velocity (L)	0.000055	1	0.000055	15.06	0.0061**	
Lack of Fit	0.000002	1	0.000002	0.516	0.50	
Pure Error	0.000026	7	0.000004			
Total sum of square	0.000424	11				
Results for TPC						0.8506
Temperature (L)	256.38	1	256.38	87.74	0.000033**	
Air velocity (L)	80.71	1	80.72	27.62	0.0012**	
Temperature (L) by air velocity (L)	90.33	1	90.33	30.91	0.00085**	
Lack of Fit	54.61	1	54.61	18.69	0.0035	
Pure Error	20.45	7	2.92			
Total sum of square	502.49	11				
Results for TF						0.9985
Temperature (L)	141.77	1	141.77	201.99	0.000002 [a]	
Air velocity (L)	160.157	1	160.16	228.19	0.000001**	
Temperature (L) by air velocity (L)	1490.98	1	1490.99	2124.31	0.000000**	
Lack of Fit	5.99	1	5.99	8.54	0.022276	
Pure Error	4.91	7	0.70			
Total sum of square	1803.82	11				
Results for F3C						0.8987
Temperature (L)	10.984	1	10.98	215.15	0.000002**	
Air velocity (L)	0.550	1	0.55	10.78	0.013434**	
Temperature (L) by air velocity (L)	0.873	1	0.87	17.09	0.00438**	
Lack of Fit	1.042	1	1.04	20.40	0.002742	
Pure Error	0.357	7	0.051			
Total sum of square	13.806	11				
Results for Antioxidant Activity						0.9450
Temperature (L)	835.833	1	835.83	297.29	0.000001**	
Air velocity (L)	1200.502	1	1200.50	426.99	0.000000**	
Temperature (L) by air velocity (L)	1381.601	1	1381.60	491.40	0.000000**	
Lack of Fit	179.372	1	179.37	63.80	0.000092	
Pure Error	19.681	7	2.81			
Total sum of square	3616.988	11				

For the constant drying rates (*k*-values), the computed *F*-value model (38.59) was higher than the *F*-value in the statistic table at 99% of confidence ($Ft_{8,3}$=7.59) and the regression equation obtained indicated a R^2-value of 0.9354 (a value of R^2>0.75 indicates de adequacy of the model). Thus 93.54% of the total variation is explained by the equation, demonstrating significance for the regression model (Myers and Montgomery, 2002). The following regression equation was obtained:

$$k - value = 0.02827 + 0.01266x_1 + 0.00323x_2 - 0.00524x_1x_2 \quad (4)$$

Optimization by a conventional "one-at-a-time-approach" does not lead to a critical analysis of the operational parameter effects on the food drying performance; moreover, this approach is not only massive and time consuming, but also has the limitation of ignoring the importance of interaction of process parameters. The performance of the drying process of grape marc was statistically influenced by the temperature and the air velocity, as well as the interaction between them ($P_{temperature} < 0.01$, $P_{air\ velocity} < 0.01$ and $P_{temperature\text{-}air\ velocity} < 0.01$). Vega-Gálvez et al. (2012) verified that the interaction of drying temperature and air velocity played significant role on effective moisture diffusivity during drying of apple slices, which indicates that these process parameters can act as limiting factors and variations in their values will alter the drying rate to a considerable extent. The three-dimensional response surface of temperature versus air velocity was then plotted (Fig. 8.2). As expected, lower temperatures and air velocities lead to lower *k*-values, indicating slower drying processes. However, at higher temperatures (85-100 ºC), the air velocity becomes less significant, and the temperature becomes the main parameter on the drying performance, indicating that the internal water diffusion is the major phenomena that governs the dewatering process in this temperature range.

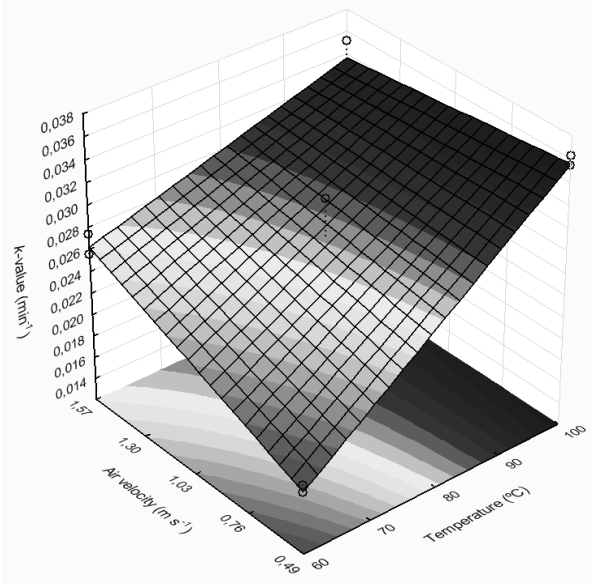

Figure 8.2 Three-dimensional surface of *k*-values as function of temperature and air velocity during forced hot air drying of grape marc.

Additionally to high dewatering rates, it is desirable to grape marc powder presents high retention of nutritional properties. In this context, the factorial design may be a powerful tool to analyze the influence of parameters on hot air dewatering process. The ANOVA employed on extractable TPC, TF and F3C from dried samples shows that the temperature, air velocity and their interaction are significant (P<0.01) on retention of these bioactive compounds on grape bagasse (Table 8.2). The computed F-value models were 15.18, 43.81 and 23.65 for TPC, TF and F3C respectively, which were higher than the F-value in statistic tables at 99% of confidence (Ft$_{8,3}$=7.59). The following regression equations were obtained:

$$TPC = 19.466 - 12.447x_1 + 7.478x_2 + 5.345x_1x_2 \quad (5)$$

$$F3C = 3.791 - 2.343x_1 + 0.525x_2 - 0.661x_1x_2 \quad (6)$$

$$TF = 24.809 - 8.419x_1 - 8.949x_2 + 27.304x_1x_2 \quad (7)$$

The three-dimensional response surface curves of temperature versus air velocity were then plotted (Fig. 8.3). Higher temperatures lead to lower retention of the

polyphenols, indicating thermal degradation of the compounds. Higher contents of TPC were found in the temperature interval of 60-80 ºC applying air velocities in the range of 1.30-1.57 m s^{-1}, being extracted approximately 24 mg GAE g^{-1} (Fig. 3A). Similar behavior was observed for the retation of F3C. When drying procedure happened in temperature range of 60-75 ºC and air velocity up to 1.30 m s^{-1}, approximately 5.0 μg ECE g^{-1} of F3C were extracted from the residue (Figure 3.3B). At high drying air velocities, heat transfer occurs at faster rates and therefore decreases the probability for the destruction and oxidation of these phenolic constituents through the process because of the shorter exposure of the bioactive compounds to the drying temperature (Michalczyk et al., 2009; Vashisht et al., 2011). Most glycosides of phenolics are localized inside vegetable cells, which provide a protective effect to the target compound when exposed to degradation factors such as high temperature and oxidation from the drying air flow (Sakihama et al., 2002; Vega-Gálvez et al., 2012).

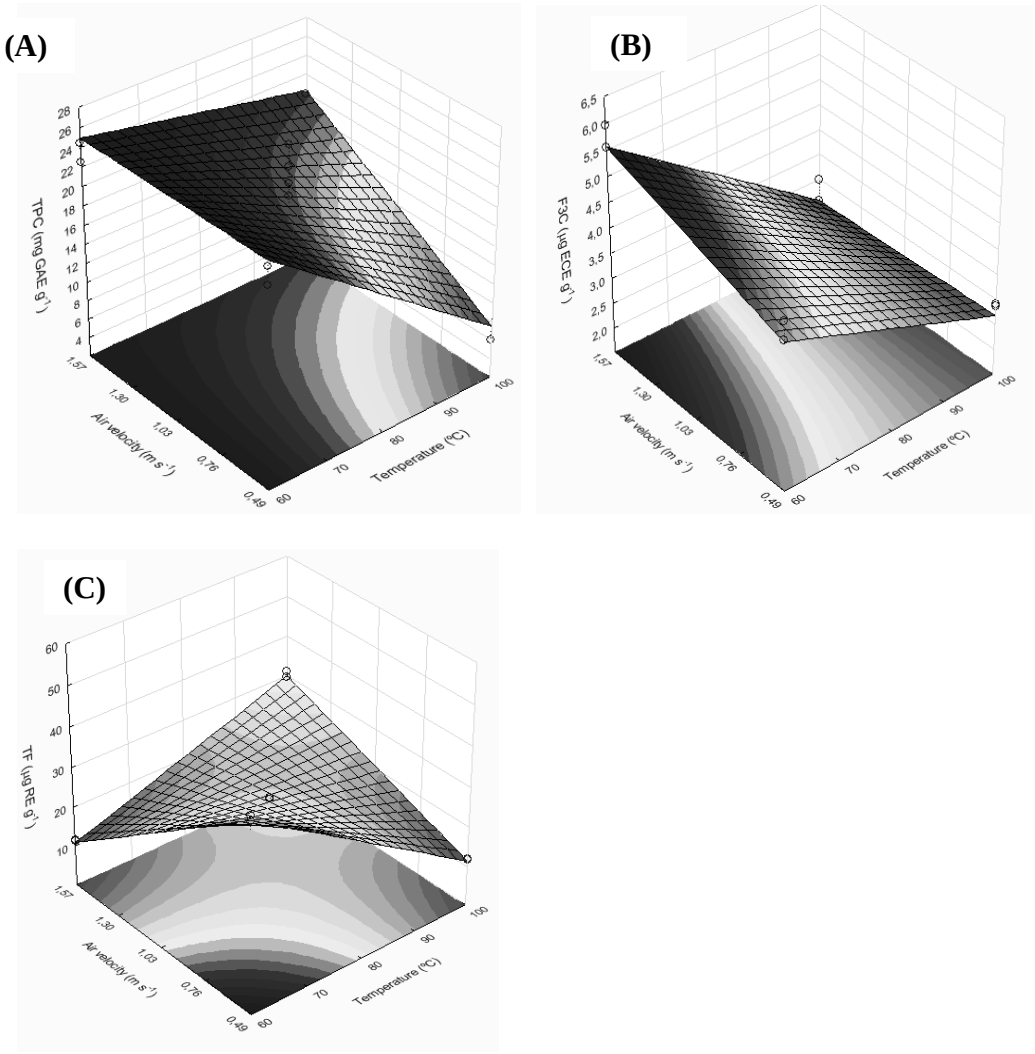

Figure 8.3 Three-dimensional surface of extractable (A) total phenolic content (TPC), (B) flavan-3-ol content (F3C), (C) total flavonols (TF) in the dried grape marc as function of drying temperature and air velocity.

The concentration of TF in the dried bagasse, on the other hand, was negatively affected by the increasing of the air velocity applied to the process (Fig. 3C). Quercetin, which belongs to the flavonol class, is a phenolic at high concentration in grape by-products (Torres et al., 2010), so the TF content in the grape derivate products is of utmost importance. Leastwise 35 µg RE g^{-1} were extracted at 60-70 ºC and 0.80-0.49 m s^{-1}. The maximum content of F3C was verified at lower temperatures and lower air velocities (Fig. 3C). Polyphenolic compounds may degrade depending upon many factors than just heat

treatment (Yousif et al., 2000; de Ancos et al., 200 Vashisht et al., 2011). For the TF, despite of a faster heat transfer at high air velocities, it is clear the sensibility of these compounds to oxidation, leading to lower retention when high air velocities were applied in the dryer. Torres and co-workers (2010) credited the decrease in the flavonol concentration of lyophilized grape skins compared to the fresh ones to the presence of flavonols outside of organelles on the plant cell, leading these compounds more exposed to degradation factors.

The results of antioxidant activity showed that the temperature, air velocity and their interaction are significant ($P<0.01$) on retention of compounds with ABTS radical scavenging capacity on the dried grape bagasse (Table 3.2). The computed F-value model was 45.78 and R^2-value of 0.9450 (Table 2). The following regression equations were obtained:

$$ABTS = 58.157 + 20.443x_1 + 24.500x_2 - 26.283x_1x_2 \quad (8)$$

Fig. 8.4 shows that higher temperatures and air velocities yielded higher content of compounds with antioxidant activity.

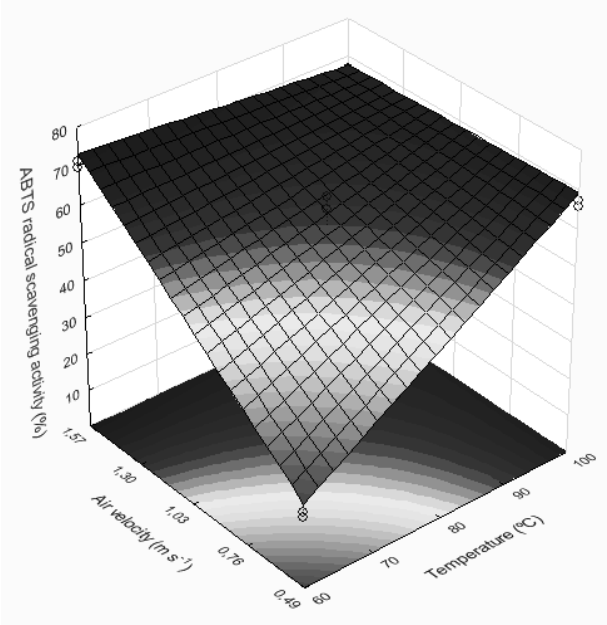

Figure 8.4 Three-dimensional surface of extractable compounds with ABTS radical scavenging activity in the dried grape marc as function of drying temperature and air velocity.

The expected results were the antioxidant activity to be associated to the concentration of polyphenols in the dried material, i.e. in lower temperature intervals (Larrauri et al., 1997; Garau et al., 2007; Vashisht et al., 2011); but this was not observed in the conditions studied. Similar results were verified by Vega-Gálvez and co-workers (2012) for DPPH scavenging capacity in drying apple slices experiments. The observed profile of antioxidant activity seemed to be associated to the generation different compounds, such as Maillard reaction products (resulted from the reaction between reducing sugars and amino acids in foods that undergo thermal processing), which presents high free radical scavenging proprieties (Rufián-Henares and Morales, 2008; Sant'Anna et al., 2011); and/or molecular structure modification of phenolics naturally presented in the fruit resulting in a wide degree of antioxidant activity (Kikugava et al., 1990; Nicoli et al., 1999).

Comparison of retention of polyphenols in fresh, lyophilized and hot air dried residues (Table 3.1) showed that lyophlization did not affect significantly ($P > 0.05$) the TPC and F3C in comparision to the fresh samples. TF, on the other hand, was significantly ($P < 0.05$) by the drying processes, possibly due to air and light exposure of samples during

forced air drying and lyophilization. TPC and F3C in lyophilized and heat dried between 80-60ºC and 1.03-0.49 m s^{-1} was not significantly ($P > 0.05$) different in the samples. Similar results were found by Larrauri and co-workers (1997) and Vega-Gálvez and co-workers (2011) when apple and grape pomace were submitted to dewatering processes and TPC in the heat dried products were analyzed com compared to the lyophilized samples . The lower ABTS radical scavenging capacity on fresh grape marc is due to low extraction yield of bioactive compounds. Most of compounds with antioxidant activity are cell wall bonded to the plant matrix (Kapasakalidis et al., 2006), and their release during hot air drying might occur due to breakdown of cellular constituents and covalent bonds (Hartley et al., 1990).

Drying performance and nutraceutical aspects seems to be in opposite sides of the process parameters. A fast drying rate is observed at high temperatures, meanwhile polyphenols are thermally degraded. Aiming a fast drying process with high retention of nutritious compounds in the dried grape bagasse, at this study, the conditions of 70 ºC and 1.03 m s^{-1} seems to be a good option to dewater grape bagasse by forced hot air. At these conditions, drying performance is approximately 75% of the highest obtained in the factorial design and there is no significant ($P > 0.05$) loss of TPC in relation to the lyophilized by-product. In this way, approximately 50% and 60% of TF and F3C remain in the product.

8.1.4 Conclusion

Factorial design showed to be an important statistical tool to enable the processors to modulate their process to achieve desirable conditions of performance and/or nutritious aspects in the forced hot air drying process of grape marc. Temperature and air velocity presented significant effect on drying rates and retention of polyphenols and compounds with antioxidant activity, acting as limiting factors in the process performance. Drying of grape bagasse at temperature of 70 ºC and air velocity of 1.03 m s^{-1} yielded a fast drying process with great preservation of polyphenols.

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CAPÍTULO 9

Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts

Neste capítulo é apresentado o artigo sobre extração de compostos fenólicos de bagaço de uva, onde foram analisadas as cinéticas de extração de compostos fenólicos totais. Também foram avaliados os compostos que não são extraíveis da matriz alimentar por extração hidroalcóolica e que estão fortemente ligados, analisando também a atividade antioxidante desses compostos. O artigo está publicado na revista **Separation and Purification Technology**, volume 100, páginas 82-87 no ano de 2012.

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Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts

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ABSTRACT

Solid–liquid extraction of total phenolic content (TPC) from grape marc was studied in the present work. Maximum TPC extraction was obtained at liquid-to-solid *ratio* of 50 mL of ethanol 50% per gram of dry marc. Extraction of TPC was, then, kinetically investigated and the applicability of several extraction models was evaluated. Pseudo-first order model was the best equation that represented extraction of TPC. Yields of extraction ranged from 11 to 22 mg GAE g^{-1}, with values of extraction rate between 0.040 and 0.1302 min^{-1} in temperature range of 25–60 °C. Acid-hydrolyzed extract presented 55% of TPC from overall TPC in the grape marc. High concentration of condensed tannins, flavonoids, anthocyanins and tartaric esters were found strongly bonded to the plant matrix. The results also show that extracts present scavenging ABTS radicals, chelating capacities and reducing power, being most of the TPC with these characteristics cell wall bonded to grape marc matrix.

1. Introduction

The consumption of fruits and derived products has increased at the same time that their beneficial effects to the human health have been studied. Among the bioactive compounds related to these effects, those with antioxidant capacities are noticeable. Grapes and grape processed products are widely consumed and present high concentration of polyphenolic compounds, the main responsible to antioxidant capacity in fruits [1]. These bioactive compounds present the ability to scavenge free radicals, avoid lipid oxidation, combat cancer cell growth and other important biological activities [1–3].

Large amounts of bagasse are generated during industrialization of grape products. This byproduct is often considered discard of industries, despite its high polyphenol contents. Recent studies have been conducted to investigate the extraction of phenolic compounds from plant material using response surface methodology [4–8]. However, the use of mathematical kinetic models considerably facilitates the optimization, the design, simulation and control of industrial projects and contributes to better use of time and energy [9].

Several equations have been proposed to model extraction of bioactive compounds from plant material (Table 1) and many works have been published focusing at modeling extraction of total phenolics and/or anthocyanins from grape byproducts, but fitting the experimental data to a single model [10–13]. Amendola and co-workers [9] evaluated four different equations to model the extraction of polyphenols from grape marc at 60 °C. However, a more rigorous assessment of the kinetic models carried out with a view to determining the best model to represent extraction of phenolic compounds from plant matrixes still lacks in the literature.

This work aimed to evaluate kinetic models for extraction of total polyphenols from grape juice marc. Specifically, it was evaluated optimal ethanol concentration and liquid-to-solid *ratio*, the influence of temperature and time on the removal of these compounds, in addition to the study of cell-bound compounds to the residue. Kinetic models described in the literature for extraction of total phenolic compounds were critically evaluated and, finally, biological activities of the extracts were investigated.

2. Materials and methods

2.1. Plant material

Grape juice marc from *Vitis labrusca* cv. "Isabel" was gently supplied by Vinícola Aurora (Caxias do Sul, RS, Brazil) in 2011. The byproduct was dried to 7.5% moisture content in a forced convection drying equipment at 70 °C, and then it was crushed in domestic mill for 1 min and passed through a 0.811 mm sieve. Samples of average particle size of 0.239 mm were obtained, as estimated by laser diffraction (Malvern Mastersizer 2000, Malverns Instruments, UK), and kept in the dark at −40 °C until used.

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Table 1
Kinetic models for extraction of biocompounds from plant material.

Model (no.)	Equation	Reference
*n*th order (1)	$C = kt^{-n}$	[14]
Weibull-type (2)	$C = C_0\exp(kt^n)$	[9]
Two-rates (3)	$C = A[1 - \exp(-Bt)] + C[1 - \exp(-Dt)]$	[11]
Swelling/diffusion (4)	$C = \frac{C^w_\infty t}{t_{1/2}+t} + C^d_\infty[1 - \exp(-k_d t)]$	[15]
Sorption/desorption (5)	$C = \frac{t}{K_1+K_2 t}$	[16]
Pseudo-first order (6)	$C = C_\infty - \frac{C_\infty}{\exp(kt+a)}$	[17]
Minchev and Minkov (7)	$C = A - B\exp(-kt)$	[18]

2.2. Selection of appropriate extraction condition: effect of ethanol concentration and ratio on total phenolic extraction

A full 2^2 composite factorial design with three replicates of central point was conducted to evaluate the effect of two independent variables on the yield extraction of total polyphenols: concentration of ethanol (%), acidified up to 0.0012 mol L^{-1} HCl, and liquid-to-solid *ratio* (mL of solvent per gram of dry bagasse). The factorial design and the independent variable intervals are shown in Table 2. The solvent volumes planned in Table 2 were stabilized at 60 °C for 15 min in well-sealed erlenmeyer, when 1 g of bagasse was added. Extraction was performed for 1 h at 225 rpm in an orbital shaker, and then the extracts were filtered through Whatman no. 1 filter paper in an ice bath. Extraction at 60 °C for 1 h was used based on initial experiments (data not shown). The extract volume was measured and concentration of total phenolics was evaluated as described in Section 2.5.

The results were analyzed by composite factorial design of the software *Statistica* 10.0 (StatSoft Inc., Tulsa, OK). The mathematical relationship between the two independent variables and the response surface can be represented by the following second-order polynomial equation:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_{12}x_1x_2 \quad (1)$$

where Y is the concentration of total phenolics extracted (mg GAE g^{-1}); x_1 and x_2, coded values of liquid-to-solid *ratio* and the ethanol concentration, respectively; b_0, b_1, b_2, b_3, b_4, b_{12} are regression coefficients.

2.3. Kinetic modeling

Under conditions maximized in Section 2.2, the extraction of total phenolic compounds was evaluated at 25, 30, 40, 50 and 60 °C up to 120 min. Experimental data were fitted to different extraction kinetic models (Table 1) by non-linear regression, minimizing the squared errors by using Gauss–Newton method from *Statistica* 10.0. The choice of the best model was based on the analysis of the highest correlation coefficient (r^2), lowest chi-square (χ^2) and standard mean error (SME) of experimental data to the equations (Table 1).

Table 2
Experimental design and results for extraction of total phenolics compounds from grape marc.

Independent variables		Extraction of TPC (mg GAE g^{-1})	
Ratio[a] (x_1)	Ethanol[b] (x_2)	Observed	Predicted
22 (−1)	18 (−1)	9.43 ± 0.32	8.78
78 (1)	18 (−1)	18.82 ± 1.01	18.54
22 (−1)	82 (1)	12.02 ± 0.25	11.58
78 (1)	82 (1)	18.91 ± 0.88	18.85
10 (−1.41)	50 (0)	4.36 ± 0.11	4.99
90 (1.41)	50 (0)	16.90 ± 0.42	16.99
50 (0)	5 (−1.41)	16.37 ± 0.12	16.88
50 (0)	95 (1.41)	18.87 ± 0.33	19.08
50 (0)	50 (0)	22.61 ± 1.28	22.05
50 (0)	50 (0)	21.97 ± 0.87	22.05
50 (0)	50 (0)	22.35 ± 0.99	22.05
50 (0)	50 (0)	21.27 ± 0.80	22.05

[a] Real values of liquid-to-solid *ratio* are expressed as mL g^{-1}.
[b] Real values of ethanol concentration are expressed as %.

2.4. Acid extraction

The residue from extraction conducted at optimal conditions evaluated in Sections 2.2 and 2.3 was submitted to successive extractions under the same condition until the extraction was ceased. Then, the residual bagasse underwent acid-extraction as described by Kapakasalidis et al. [19] and analyzed for polyphenols strongly linked to the plant matrix. For this purpose, the residue from the solvent extraction was added to 50 mL of methanol 60% acidified with HCl up to final concentration of 1.2 mol L^{-1}. Extraction was performed at 90 °C for 90 min, when it was cooled in ice bath and filtered. Extracts were passed through Sepack filters and evaluated for several classes of phenolic compounds.

2.5. Analysis of polyphenols classes

Total phenolic content (TPC) in the extracts was determined by the Folin–Ciocalteau method described by Singleton and Rossi [20], which involves the reaction of the sample, the Folin–Ciocalteau reagent (ALZ, Brazil) and sodium carbonate saturated solution. The absorbance of the reaction mixture at 765 nm was measured by UV-1600 spectrophotometer (Pró-Análise, Brazil). TPC of extracts was standardized against a gallic acid (Sigma, USA) curve and expressed as mg gallic acid equivalent per gram of dry bagasse weight (mg GAE g^{-1}).

Monomeric anthocyanins (MA) were determined using the pH differential method [21], by measuring the absorbance of diluted samples in potassium chloride buffer pH 1.0 and sodium carbonate buffer pH 4.5 at 520 and 700 nm. The units for extracted MA were expressed as mg of cyanidin 3-glucoside per gram of dry bagasse (mg C3G g^{-1}).

Total flavonoid content (TFC) was determined using a colorimetric method described previously [22]. Shortly, extracts were diluted and suffered reaction with 5% $NaNO_2$, 10% $AlCl_3{\cdot}6H_2O$ and 1 mol L^{-1} NaOH solutions. The absorbance was measured immediately at 510 nm and the results were calculated by a calibration curve with epicatechin and expressed as mg of epicatechin equivalents (mg of ECE per gram of dried sample).

Analysis of condensed tannin content (CTC) was carried out according to the method of Price et al. [23], which involves the reaction of the samples with vanillin solution. The absorbance was measured at 500 nm and results were calculated and expressed as mg epicatechin equivalents (mg of ECE/g sample).

The flavan-3-ols content (FC) was determined following the procedure described by Arnous et al. [24]. Briefly, samples were mixed with *p*-dimethylaminocinnamaldehyde solution and stood for 10 min. The absorbance was recorded at 640 nm and results expressed as mg epicatechin equivalents (mg of ECE/g sample).

The total flavonols (TF) and tartaric esters content (TEC) in the extracts were determined following the procedure described by Mazza and co-workers [25]. Briefly, extracts were thoroughly mixed sequentially with acidified ethanol and distillated water. The absorbance at 360 and 310 nm was measured for TF and TEC, and results were expressed as mg of rutin equivalent (RE) and mg of caffeic acid equivalent (CAE) per gram of dry solids for TF and TEC, respectively.

2.6. Determination of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity

ABTS assays were carried out like described by Re et al. [26], which involves the generation of ABTS radical chromophore by

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the oxidation of ABTS with potassium persulfate. The ABTS radical cation was produced by reacting 7 mmol L^{-1} ABTS stock solution with 140 mmol L^{-1} potassium persulfate, and allowing the mixture to stand in the dark for 16 h at room temperature before use. For the assay, the $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. An aliquot of 30 μL of extract was mixed with 1 mL of $ABTS^{\cdot+}$ solution and an absorbance (734 nm) reading was taken after 6 min. Distilled water, instead of sample, was used as a control. The results were expressed as: scavenging capacity (%) = $[1 - (A/A_0)] \times 100$, where A is the absorbance of the test and A_0 is the absorbance of the control.

2.7. Determination of metal chelating capacity

The chelating capacity of Fe^{2+} was measured using the method described by Chang et al. [27] with slight modifications. One milliliter of sample was mixed with 3.7 mL distilled water and then the mixture was reacted with 0.1 mL of 2 mmol L^{-1} $FeSO_4$ (Fe^{2+}) and 0.2 mL of 5 mmol L^{-1} ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine). After 10 min the absorbance was read at 562 nm. One milliliter of distilled water, instead of sample, was used as a control. The results were expressed as: chelating capacity (%) = $[1 - (A/A_0)] \times 100$, where A is the absorbance of the test and A_0 is the absorbance of the control.

2.8. Determination of reducing power

Reducing power of the extracts was measured as previously described [28]. Samples of 1 mL of extracts were mixed with 2.5 mL phosphate buffer (0.2 mol L^{-1}, pH 6.6) and 2.5 mL potassium ferricyanide (10 mg mL^{-1}), and then the mixture was incubated at 50 °C for 20 min. Then, 2.5 mL TCA (10%, v/w) was added and the mixture was centrifuged (3000*g* for 10 min). The supernatant (1 mL) was mixed with 2.5 mL distilled water and 0.2 mL ferric chloride (1 mg mL^{-1}), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

2.9. Data analysis

All experiments were conducted in triplicate and averages of two independent tests were calculated. Obtained values were compared using Tukey's test by *Statistica* 10.0, and differences were considered statistically significant, when $p < 0.05$. Graphical plots were performed using Microsoft Excel 2000 (MapInfo Corporation, Troy, NY, USA).

3. Results and discussion

In order to perform kinetic studies of extraction of TPC from grape marc, the best conditions of the liquid-to-solid *ratio* and solvent concentration to be used for extraction were initially evaluated. Thus, a 2^2 factorial design was conducted and the results are shown in Table 2.

Extraction of TPC, according to analysis of variance (Table 3), was significantly affected ($p < 0.05$) by linear and quadratic effects of liquid-to-solid *ratio* and ethanol concentration. The computed *F*-value (18.65) was higher than the *F*-value in statistic tables at 95% of confidence ($F_{t5,6} = 3.23$) and the lack of fit of experimental data to the model was not significant ($p > 0.05$), which demonstrate significance for the regression model. The regression equation obtained indicated the r^2 value of 0.9833. The following regression equation was obtained:

$$Y = 22.05 + 8.52x_1 - 11.13x_1^2 - 1.56x_2^2 - 4.10x_2 - 1.25x_1x_2 \quad (2)$$

where Y is the concentration of total phenolics extracted (mg GAE g^{-1}), x_1 and x_2 are the coded values of liquid-to-solid *ratio* and the ethanol concentration, respectively.

The three-dimensional response surface curve of TPC yield as function of the liquid-to-solid *ratio* versus ethanol concentration were then plotted (Fig. 1). Maximum yield of TPC extraction from grape juice marc was at central point of the factorial design: 50% ethanol at 50 mL per gram of sample. Combination of water and organic solvents is generally more effective for extraction of bioactive compounds from plant material. The increase of ethanol concentration reduces the dielectric constant of the solvent, decreasing solvation of molecules. Thus, an increase of solvent concentration leads to an increase in the diffusion of the molecules by reduction of the interaction with the solvent. However, highly pure organic solvent does not lead an enhancing of extraction yield, probably due to dehydration of the vegetable cell, not allowing the alcohol to be introduced effectively into the cell to diffuse TPC to the extract. The increase of TPC yields with the increase of the liquid-to-solid *ratio* is consistent with mass transfer principles. The driving force during mass transfer within the solid is considered to be the concentration gradient, which was greater when a higher solvent-to-solid ratio was used, resulting in an increase of the diffusion rate [11].

Several mathematical equations have been described in literature to model the solid-to-liquid extraction of bioactive compounds from plants. Seven models used in recent publications are presented in Table 1. Choosing the best equation to model industrial processes is essential from the engineering point of view, in order to minimize processing errors, thus improving the accuracy of the procedure and the quality of the final product. Thus, experimental data of TPC extraction in temperature and time ranges of 25–60 °C and 5–120 min, respectively, were fitted to the models and statistically analyzed.

The results of r^2, χ^2 and SEM for the different models are presented in Table 4. Equations that consider the extraction happening in one continuous step (Eqs. (1) and (2)) did not show good relation to the experimental data. Models that represent the extraction going on two different rates (Eqs. (3) and (4)) yielded

Table 3
Analysis of variance for the model for extraction of total phenolic compounds from grape bagasse.

Source	Sum of square	Degree of freedom	Mean square	*F*-value	*P*-value
Ratio (*L*)	144.663	1	144.663	421.538	0.0003[a]
Ratio (*Q*)	196.446	1	196.446	572.427	0.0002[a]
Ethanol (*L*)	4.823	1	4.823	14.055	0.033[a]
Ethanol (*Q*)	26.629	1	26.629	77.594	0.003[a]
Ratio (*L*) by ethanol (*L*)	1.559	1	1.559	4.543	0.123
Lack of fit	1.404	3	0.468	1.364	0.402
Pure error	1.029	3	0.3432		
Total sum of square	355.907	11			

L: linear effect.
Q: quadratic effect.
[a] Statistically significant at 95% of confidence.

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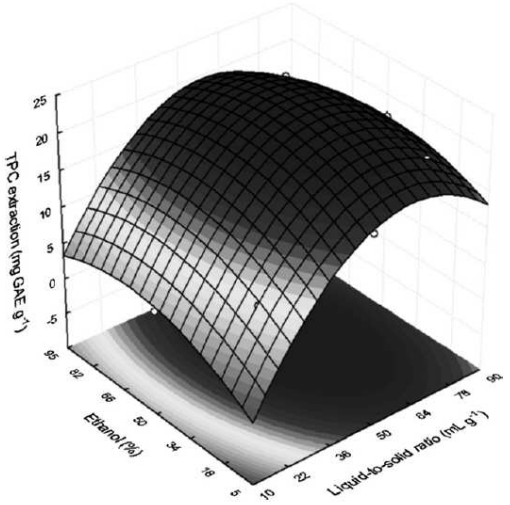

Fig. 1. Tridimensional surface of extraction of total polyphenols from grape juice bagasse as function of liquid-to-solid *ratio* and ethanol concentration.

Table 4
Error analysis for fitting experimental data to different models.

Model	r^2	χ^2	SEM
*n*th order	[0348;0.610]	[5.838;27.418]	[11.032;51.781]
Weibull-type	[0.689;0.890]	[1.644;13.083]	[3.109;24.726]
Two-rates	[0.984;0.999]	[0.011;0.396]	[0.013;0.449]
Swelling/diffusion	[0.971;0.996]	[0.109;0.135]	[0.153;0.013]
Sorption/desorption	[0.905;0.980]	[0.362;3.977]	[0.685;7.516]
Pseudo-first order	[0.984;0.999]	[0.008;0.297]	[0.012;0.449]
Minchev and Minkov	[0.984;0.999]	[0.008;0.297]	[0.012;0.449]

low r^2 values and high χ^2 and SEM values. The equation proposed by Linares et al. [15], for example, is based on the assumption that extraction occurs primarily due to the washing and swelling of the material and then due to diffusive process of polyphenols to extract. The model of Peleg [16] (Eq. (5)), used by Bucić-Kojić et al. [10] and Qu et al. [12], is based on curves of sorption/desorption and was not the model that best fitted to the experimental data as is shown in Table 4.

Pseudo-first order model (Eq. (6)) and that proposed by Minchev and Minkov [18] (Eq. (7)) yielded quite similar errors values for fitting experimental data and, thus, there was not enough information to choose between the two models reliably. Values of extraction rate (k-values) increased with increasing processing temperature for both models. k-values estimated by pseudo-first order ranged from 0.040 to 0.1302 min^{-1} in temperature interval of 25–60 °C, meanwhile, for Minchev and Minkov model, k-values ranged from 0.040 to 0.080 min^{-1} in the same temperature interval.

In industrial processes, beyond modeling concentration of bioactive compounds extracted as function of time, equating of k-values as function of temperature is very important. Some studies [10,12,15] have reported that constants estimated by kinetic treatments follow Ahrrenius equation:

$$\ln(k) = \ln(k_0) - \frac{E_a}{RT} \quad (3)$$

where k_0 is a constant (mg GAE g^{-1} min^{-1}), R the universal gas constant (8.314 J mol^{-1} K^{-1}), E_a the activation energy (J mol^{-1}) and T is the absolute temperature (K).

The results are presented in Fig. 2. Modeling k-values estimated by pseudo-first order equation yielded a r^2 of 0.900 and for extraction rates from Minchev and Minkov model a r^2 of 0.701. Thus, since the binomial time–temperature is a very important issue for industries, the pseudo-first order model is more indicated to represent the batch extraction of polyphenols from grape juice bagasse. The energetic barrier to be overcome in order to the process begins (E_a) estimated by Ahrrenius approach is 23 kJ mol^{-1}.

Graphical representation of TPC extraction through time is presented in Fig. 3. Extraction occurs exponentially until it reaches an equilibrium concentration in which the solvent is not able to remove the biocompounds from the grape byproduct. In Table 5, the kinetic parameters estimated by the non-linear regression of the experimental data to the pseudo-first order equation to TPC extraction are presented. The equilibrium concentration values were 11.55, 14 and 16.14 mg GAE g^{-1}, at 25, 30 and 40 °C, respectively ($p < 0.05$). There was no statistical difference ($p > 0.05$) of TPC extracted at 50 and 60 °C (21.66 and 22.08 mg GAE g^{-1}, respectively). However, extraction rate at 60 °C was significantly ($p < 0.05$) higher that at 50 °C, being the equilibrium concentration being reached after 45 min at 50 °C and after 30 min at 60 °C. These results can be observed in Fig. 2.

Information about kinetic parameters from pseudo-first order model for extraction of TPC from plant material is scarce. The half time extraction ($t_{1/2}$), calculated by Eq. (4) [15], decreased with increasing of temperature, varying between 17.38 and 5.32 min in the temperature interval of 25–60 °C.

$$t_{1/2} = \frac{\ln(2-a)}{k} \quad (4)$$

When the integration constant (a-value) in pseudo-first order model is equal to zero (the ideal behavior of the phenomenon), k-values are called apparent (k_{apar}), and are calculated by Eq. (5) [15]. Since a-values estimated in this study were close to zero, the standard mean error between observed and apparent k-values was about of 6%.

$$k_{apar} = \frac{\ln(2)}{t_{1/2}} \quad (5)$$

Increasing of k-values is related to the increase of the internal energy of the molecules and to the reduction of dynamic viscosity of solvent [11], enhancing also the extraction yield of TPC and decreasing time needed to remove them from the matrix. Nevertheless, the processing temperature cannot be increased indefinitely, because bioactive compounds are relatively thermo labile, being susceptible to degradation at high temperatures.

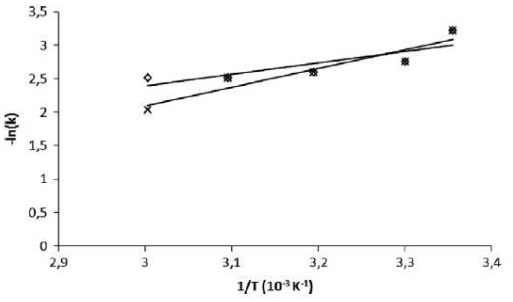

Fig. 2. Arrhenius plot of total polyphenol extraction rates from pseudo-first order (x) and from Minchev and Minkov (◇) model. The regression equation for pseudo-first order model was determined as $y = 2786.669x - 6.265$ ($r^2 = 0.900$); regression equation for Minchev and Minkov model was determined as $y = 1716.832x - 2.756$ ($r^2 = 0.701$).

CAPÍTULO 9 - KINETIC MODELING OF TOTAL POLYPHENOL EXTRACTION FROM GRAPE MARC AND CHARACTERIZATION OF THE EXTRACTS

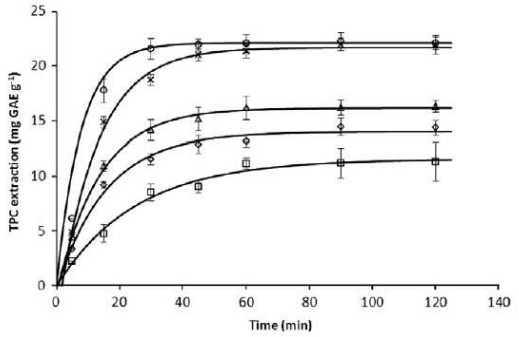

Fig. 3. Extraction of total polyphenols from grape juice bagasse at 60 (o), 50 (x), 40(Δ), 30 (◇) e 25 °C (□). Data presented, fitted to a pseudo-first order model, are average values of triplicates from two independent experiments.

Beyond of presenting high concentration of phenolic compounds in its composition, grape marc is an interesting source of fibers. Saura-Calixto [29] verified fibers in grape residue are rich of non-extractable polyphenols, which is a different characteristic in relation to white oats, apple and lemon fibers. In that work, the author denominated these fibers as antioxidant dietary fibers. In this sense, it was evaluated the effect of successive extractions and the acid extraction over the TPC, monomeric anthocyanins (MA), total flavonoid content (TFC), condensed tannin content (CTC), flavan-3-ols content (FC), total flavonols (TF), tartaric ester content (TEC) yield and their biological activities such as scavenging of ABTS radicals, iron chelating power and reducing power of the extracted compounds.

The results are summarized in Table 6. Two extractions were enough to remove all extractable TPC from the bagasse. Despite in the first extraction an equilibrium concentration was reached, 9% of water–alcohol removable TPC were not extracted. About 55% of global polyphenols presented in the grape juice marc are strongly bond to the residue matrix and are extractable only in high ionic strength conditions. This is possible due to high amounts of tannins, insoluble in both aqueous and organic solvents, present in the acid-hydrolyzed extract and representing 56% of CTC in grape bagasse (Table 6). Soluble or extractable polyphenols are low or intermediate molecular mass phenolics that are extracted using different solvents, which appear to be absorbed from the digestive tract and produce systemic effects, while non-extractable polyphenols are mainly condensed tannins of high molecular mass, quantitatively recovered in feces [29,30]. These results contributes to work of Tagliazucchi et al. [31], who demonstrated viability of polyphenols from whole grapes after passing through gastrointestinal conditions, showing clear bioavailability of these compounds to act positively in human health.

Kapakasalidis et al. [19] verified higher concentration of cell-bond polyphenols in the black currant residue than those extractable by organic solvents, as well as higher ABTS scavenging capacity. Hydroxycinnamic acids were the major phenolic compounds in acid-hydrolyzed extract; meanwhile major extraction of flavonols happened in water–alcohol mixture in black currant marc [19]. This is a probable consequence of the association of phenolic compounds to polymers of vegetable cell wall [32]. Equivalent contents of anthocyanins were found in water–alcohol and acid hydrolyzed extract in black currant marc [19], as well as in the present work in grape residue (Table 6).

Table 6
Concentration of polyphenols for different extraction steps and chelating antioxidant activities and reducing power of the extracts.

	First extraction	Second extraction	Acid extraction
TPC (mg GAE g^{-1})	21.02 ± 1.83	2.52 ± 0.117	28.96 ± 1.23
TFC (mg ECE g^{-1})	6.99 ± 0.70	0.93 ± 0.02	4.78 ± 0.22
MA (mg C3G g^{-1})	0.99 ± 0.05	0.076 ± 0.005	0.96 ± 0.08
CTC (mg ECE g^{-1})	30.89 ± 1.03	5.01 ± 0.64	32.20 ± 0.94
FC (mg ECE g^{-1})	4.71 ± 0.38	0.24 ± 0.01	0.46 ± 0.01
TF (mg RE g^{-1})	7.31 ± 0.08	1.27 ± 0.02	4.04 ± 0.01
TEC (mg CAE g^{-1})	1.86 ± 0.10	0.51 ± 0.03	1.64 ± 0.07
ABTS (%)	79.05 ± 3.25	43.81 ± 1.54	76.12 ± 2.24
Chelating activity (%)	11.10 ± 0.53	9.66 ± 0.17	79.90 ± 2.51
Reducing power (Abs 700 nm)	2.12 ± 0.03	1.50 ± 0.04	2.52 ± 0.031

Most MA, TFC, TFC, TEC and FC were removed in water–alcohol conditions, but high amount of them are cell wall-bond, which explains the high antioxidant capacities in the acid-hydrolyzed extract. Most of extractable compounds removed during the first extraction showed capacity to scavenge ABTS radicals, relative chelating capacity and high reducing power (Table 6). Polyphenols extracted in further solvent extraction present considerable amounts of biological activities. Furthermore, compounds highly bond to the plant material present equivalent antioxidant capacity and reducing power to those extracted by ethanol–water mixture. In addition, acid-hydrolyzed extract presents high iron chelating power. Iron is a catalyst for hydroxyl radical formation, potentially contributing to diseases related to oxidative stress [33]. Hydroxycinnamic acids, rarely extracted by solvents, are highly reactive antioxidant due to the double bond in their molecules, which participates of the stability of the radical by resonance displacement of odd electrons [34,35].

Results of the present work bring a great perspective to the development of new products with grape marc or its phenolic extract in food and pharmaceutical industries.

4. Conclusions

Kinetic extraction studies of total polyphenols from grape marc are essential to elucidate the phenomena involved, thereby better dimensioning equipment for industrial projects. Ethanol concentration of 50% in a relation of 50 mL per gram of bagasse resulted on the major yield of extraction. The pseudo-first order was the model that better represented the batch extraction in the temperature range evaluated, with activation energy of 23 kJ mol^{-1}. High amount of polyphenols and compounds with biological activities are strongly bond to the plant material, which shows grape marc as a potential functional component to be used in industrial applications.

Table 5
Kinetic parameters for extraction of total polyphenols from grape juice bagasse.

T (°C)	C_∞ (mg GAE g^{-1})	k (min^{-1})	a	$t_{1/2}$ (min)	k_{apar} (min^{-1})
60	22.084 ± 1.112[a]	0.130 ± 0.005[a]	−0.325 ± 0.002	5.324 ± 0.211	0.107 ± 0.002
50	21.660 ± 0.962[a]	0.081 ± 0.001[b]	−0.140 ± 0.003	8.577 ± 0.156	0.081 ± 0.001
40	16.137 ± 0.955[b]	0.075 ± 0.005[b]	−0.437 ± 0.007	9.337 ± 0.359	0.074 ± 0.005
30	13.995 ± 0.271[c]	0.064 ± 0.003[c]	−0.022 ± 0.005	11.019 ± 0.623	0.063 ± 0.003
25	11.557 ± 0.805[d]	0.040 ± 0.003[d]	−0.002 ± 0.004	17.402 ± 0.245	0.040 ± 0.003

[a,b,c,d] Different superscripts in the same column indicate statistical differences ($p < 0.05$).

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CAPÍTULO 10

Kinetic modeling of anthocyanin extraction from grape marc

Neste capítulo é apresentado o artigo sobre extração de antocianinas de bagaço de uva, onde foram analisadas as cinéticas de extração de antocianinas monoméricas. O trabalho foi aceito para publicação em 2012 na revista **Food and Bioprocess Technology**, com o doi 10.1007/s11947-012-1016-1.

CAPÍTULO 10 - KINETIC MODELING OF ANTHOCYANIN EXTRACTION FROM GRAPE MARC

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Kinetic Modeling of Anthocyanin Extraction from Grape Marc

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Abstract In the present work, kinetic studies of extraction of anthocyanins from grape marc were conducted in order to statistically evaluate several models presented in the literature. Based on a full 2^2 factorial design, extraction was performed using 50 mL of ethanol, at a concentration of 50 %, per gram of dry marc. Extraction was evaluated up to 120 min in the temperature range of 60–25 °C. A pseudo-first-order model provided the best description of extraction of anthocyanins. Yield and rate of extraction increased with increasing processing temperature, varying from 0.906 to 0.476 mg cyanidin 3-glucoside per gram of dry marc and from 0.157 to 0.034 min^{-1}, respectively, in the temperature range. Results show that the highest yield of extraction happens after 15 min at 60 °C (activation energy of 29.5 $kJmol^{-1}$), and that after 120 min, thermal degradation has started.

Keywords Grape marc · Anthocyanins · Extraction · Kinetic modeling

Nomenclature

MA	Monomeric anthocyanins
$mgC3Gg^{-1}$ db	Milligrams of cyanidin 3-glucoside per gram on dry basis
C_0	Concentration of MA extracted at time zero ($mgC3Gg^{-1}$ db)
C	Concentration of MA extracted at time t (mg $C3Gg^{-1}$ db)
t	Time (in minutes)
k	Extraction rate constant (per minute)
m	Scale factor of the distribution curve in Eq. 2
a	Integration constant in Eq. 3
B and D	Extraction rate constants in Eq. 4
A and C	Constants in Eq. 4
C^{w}_{∞}	Equilibrium concentration of the swelling–washing process ($mgC3Gg^{-1}$ db)
C^{d}_{∞}	Equilibrium concentration of the diffusive process ($mgC3Gg^{-1}$ db)
k_d	Extraction rate constant of the diffusive process (per minute)
k_{apar}	Apparent extraction rate of the diffusive process (per minute)
$t_{1/2}$	Half time of extraction of the swelling–washing process (in minutes)
K_1	Extraction rate constant (per minute)
K_2	Peleg's capacity constant
r^2	Coefficient of determination
χ^2	Chi-square
SEM	Standard error of mean
E_a	Activation energy for extraction (in kilojoules per mole)

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Introduction

The increase of consumer's demands for minimally processed and nutrient-rich foods has led many studies to be conducted on the development of natural alternatives, mainly in the dye area, since toxic effects have been associated to some chemical colorants available for food applications (Sabater-Vilar et al. 1999; Mapari et al. 2005). Utilization of agro-industrial by-products is now an increasing trend because they may represent a natural source of food and pharmaceutical ingredients (Arranz et al. 2009). Additionally, the utilization of these low-

cost wastes as source of pigments reflects on final product costs and represents a way of waste management (Silveira et al. 2011).

Anthocyanins, water-soluble natural pigments responsible to the red–purple color of most fruits and vegetables, are the main polyphenols in berries' skins, belonging to the flavonoid class (Denev et al. 2010). Anthocyanic extracts from plant materials are of great interest for food and pharmaceutical applications, due to their attractive features like natural source, anti-inflammatory effects (Ronziere et al. 1981), preservation of eyesight disorders (Ghosh and Konishi 2007), free radical scavenging (Denev et al. 2010), among others.

Despite new technologies for obtaining phenolic-rich extracts from food matrices such as supercritical, ultrasound, instant controlled pressure drop, microwave, high-hydrostatic-pressure methods (Corrales et al. 2009; Allaf et al. 2012; Bimark et al. 2012; Routray and Orsat 2012), solid–liquid extraction remains the most widely studied method for this aim. Within this context, intensive studies to elucidate the structural and functional mechanisms of separation of pigment biocompounds from plant residues have been conducted. Response surface methodology is almost widely used for evaluation of extraction of anthocyanins (Ku and Mun 2008; Pompeu et al. 2009; Nayak and Rastogi 2011; Borges et al. 2011; Sun et al. 2011). However, extraction optimization of anthocyanins relies on adequate mathematical models to warrant high extract yield and quality. Thus, the knowledge on anthocyanin extraction kinetics from grape residue is essential to allow their adequate use in industrial scale, thereby minimizing processing errors and improving the procedure precision and the product final quality (Amendola et al. 2010; Silveira et al. 2011).

Various phenomena might govern the removal of compounds from plant matrices, including sorption/desorption, washing and swelling of plant material, diffusion, among others. Table 1 shows several mathematical equations that have been used in recent publications. Nevertheless, applied research carried out with a view to determining the best model to represent extraction of anthocyanins from fruit residues is not available in the present literature.

In this context, this study presents a kinetic analysis of extraction of anthocyanins from grape marc by statistically evaluating several models to fit experimental data. On this basis, process kinetic parameters, as well as the energy needed for start of separation, were determined.

Table 1 Kinetic models for extraction of compounds from plant matrices

Equation	Model	Reference
(1)	$C = kt^n$	Othmer and Jaatinen (1959)
(2)	$C = C_0 \exp(kt^n)$	Weibull (1951)
(3)	$C = C_\infty - C_\infty / \exp(kt + a)$	Spiro e Jago (1982)
(4)	$C = A[1 - \exp(-Bt)] + C[1 - \exp(-Dt)]$	Cacace and Mazza (2003)
(5)	$C = \frac{C_\infty^W t}{t_{1/2}+t} + C_\infty^d [1 - \exp(-k_d t)]$	Linares et al. (2010)
(6)	$C = t/K_1 + K_2 t$	Peleg (1998)

Material and Methods

Plant Material

Grape marc was obtained after juice pressing operation of *Vitis labrusca* cv. "Isabel" (Vinícola Aurora, Caxias do Sul, RS, Brazil) harvested in 2011. The residue, with initial moisture content of 84±1.09 % (wet basis), was dried in pilot forced air equipment (70 °C, 8 ms^{-1}) until moisture content of 8.0± 0.61 %. Then, it was crushed in a domestic blender (RI1764, Walita, Brazil) for 1 min and passed through a 20 Taylor mesh sieve, obtaining samples of average particle size of 0.239 mm, which was estimated by laser diffraction (Malvern Mastersizer 2000, Malverns Instruments, UK). Samples were stored at −40 °C in the dark. For the solid–liquid separation procedure, samples of 1 g were weighed and immediately introduced into the extraction flasks.

Determination of Monomeric Anthocyanins

Monomeric anthocyanins (MA) were determined using the pH differential method (Lee et al. 2005), which has demonstrated high correlation to HPLC analysis (Lee et al. 2008). The absorbance of samples diluted separately in 0.025 mol L^{-1} potassium chloride buffer, pH1.0, and 0.4 molL^{-1} sodium carbonate buffer, pH4.5, were measured at 520 and 700 nm of samples diluted separately. MA concentrations were expressed as cyanidin-3-glucoside (molar extinction coefficient of 26,900 $Lcm^{-1}mol^{-1}$ and molecular weight of 449.2 $gmol^{-1}$). The units for extracted MA were expressed as milligrams of cyanidin 3-glucoside per gram on dry basis ($mgC3Gg^{-1}db$).

Evaluation of Ethanol and Ratio Conditions for Extraction of Monomeric Anthocyanins

Evaluation of solvent conditions for extraction of anthocyanins (relation of solvent volume per dry weight of material—*ratio*—and solvent concentration) is an important initial step prior to kinetic analysis of extraction of the pigment. Extraction of MA is usually more effective with organic solvents, and ethanol is the most recommended solvent for posterior use of the extract in foods due to its non-toxicity and low cost. The influence of liquid-to-solid ratio and ethanol concentration on

MA extraction yield was evaluated using a full 2^2 factorial design.

In an orbital shaker, well-sealed erlenmeyers, with the established volume of acidified ethanol (0.01 % HCl) planned in Table 2, were stabilized at 60 °C for 15 min when 1 g of dried residue was added. Extraction was performed for 1 h with orbital stirring of 225 rpm, when the extracts were filtered through filter paper (Whatman paper, no. 1) in an ice bath. Extraction at 60 °C for 1 h was used based on initial experiments (data not shown). Then, the extract volume was measured, and the concentration of MA was evaluated.

To describe the response surface, a central composite design with five coded levels and two variables was used to study the combined influence of ratio (x_1) and acidified ethanol (0.01 % HCl) concentration (x_2). For the two factors, this design was made up of a full 2^2 factorial design with its four points augmented with four replications of the central points (all factors at level 0) and the four star points. A set of 12 experiments was carried out. Table 2 shows factorial planning, with independent variables and their concentrations at the different coded levels of the factorial design experiments. For two factors, the equation model is:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (7)$$

where Y is the MA extraction yield ($mgC3Gg^{-1}db$); b_0 is the intercept; b_1 and b_2 are linear coefficients; b_{11} and b_{22} are squared coefficients, and b_{12} is the interaction coefficient.

The results were analyzed by the Experimental Design Module of the *Statistica* 10.0 software (Statsoft, Tulsa, OK, USA). Three-dimensional surface plots were drawn to illustrate the main and interactive effects of the independent variables on pigment extraction yield.

Table 2 Experimental design and results for extraction of monomeric anthocyanins from grape marc as function of liquid-to-solid ratio and ethanol concentration

Independent variables		Extraction of MA ($mgC3Gg^{-1}$)	
Ratio[a] (x_1)	Ethanol[b] (x_2)	Observed	Predicted
22 (−1)	18 (−1)	0.340±0.012	−0.213
78 (1)	18 (−1)	0.735±0.020	0.489
22 (−1)	82 (1)	0.362±0.009	−0.213
78 (1)	82 (1)	0.647±0.014	0.4885
10 (−1.41)	50 (0)	0.139±0.003	−0.469
90 (1.41)	50 (0)	0.650±0.036	0.521
50 (0)	5 (−1.41)	0.495±0.019	0.258
50 (0)	95 (1.41)	0.526±0.029	0.258
50 (0)	50 (0)	0.917±0.037	0.834
50 (0)	50 (0)	0.896±0.041	0.834
50 (0)	50 (0)	0.900±0.020	0.834
50 (0)	50 (0)	0.854±0.022	0.834

[a] Real values of liquid-to-solid ratio are expressed in milliliters per gram

[b] Real values of ethanol concentration are expressed in percent

Statistical Analysis for Kinetic Modeling Extraction

Under conditions maximized in the section before, extraction of MA was investigated at 25, 30, 40, 50, and 60 °C for 5, 10, 15, 30, 45, 60, 90, and 120 min. Experimental data (milligrams monomeric anthocyanin per gram of dry marc) as function of time were fitted to kinetic models presented in Table 1 by non-linear estimation using the Gauss–Newton method.

In the equations, C_0 and C represent the MA extracted ($mgC3Gg^{-1}db$) at time zero and t (in minutes), respectively, and k (in minutes) is the extraction rate constant at a given temperature. Equations 1 and 2 are empirical equations, assuming extraction happens in one continuous step, and m is a scale factor of the distribution curve (Weibull 1951; Othmer and Jaatinen 1959). Pseudo-first-order model (Eq. 3) considers that the compound concentration in the extract tends to "plateau," and a is an integration constant of the model. In Eq. 4, the extraction of compounds is assumed to happen in two distinct periods, related to accessible and inaccessible compounds (outside and inside plant cells, respectively) (Cacace and Mazza 2003). B and D parameters are the extraction rates of the two different classes of compounds, and A and C are constants. In the model proposed by Linares et al. (2010) (Eq. 5), C^{w}_{∞} is the equilibrium concentration and $t_{1/2}$ the half-time extraction of the swelling–washing step; C^{d}_{∞} and k_d are the equilibrium concentration and the rate of extraction, respectively, of the process happening due to the diffusive mechanism. Equation 6 is based on the sorption/desorption mechanism to remove compounds from the plant material (Peleg 1998); in the model, K_1 represents the extraction rate constant and K_2, Peleg's capacity constant.

Coefficient of determination (r^2), chi-square (χ^2), and standard error of means (SEM) were the statistical criteria evaluated.

Calculation of χ^2 is done by the equation:

$$\chi^2 = \frac{\sum \left(a_{\text{measured}} - a_{\text{predicted}}\right)^2}{n - p} \quad (8)$$

SEM is defined as:

$$\text{SEM} = \frac{\sum \left(\text{a}_{\text{measured}} - \text{a}_{\text{predicted}}\right)^2}{\sqrt{\text{n}}} \quad (9)$$

where n is the number of observations and p the number of parameters. The model with the lowest χ^2 and SEM, and higher r^2 for MA extraction, is considered as the best choice

for modeling the anthocyanin extraction behavior during processing (Sant'Anna et al. 2010).

Statistical Analysis

Statistical analysis of the data was performed using the Statistica 10.0 software (Statsoft Inc., Tulsa, OK, USA) and plots using Microsoft Excel 2000 (MapInfo Corporation, Troy, NY, USA). Obtained kinetic parameters, from triplicates of at least two independent experiments, were compared using Tukey's test, and differences were considered statistically significant at $p<0.05$.

Results and Discussion

Mathematical models consist of equations that provide an output based on a set of input data. It is a concise way to express physical behavior in mathematical terms (van Boekel 2008). In the solid–liquid extraction context, solvent concentration and liquid-to-solid ratio are significant process variables on the extraction yield aiming industrial applications. Therefore, the investigation of optimal conditions of solvent concentration and liquid-to-solid ratio was evaluated by a 2^2 factorial design, the results of which are presented in Table 2.

According to the analysis of variance (Table 3), the quadratic and linear effects of liquid-to-solid ratio influenced significantly ($p<0.05$) the extraction of MA, as well as the quadratic effect of ethanol concentration. The calculated model F value was 11.33, which is higher than the F value in statistic tables at 95 % of confidence ($F_{t5,6}=3.23$). The determination coefficient (r^2) of 0.9771 and the lack of fit of experimental data to the model not being significant ($p>0.05$) demonstrate significance for the regression model (Myers and Montgomery 2002). The equation obtained by the statistical regression was as follows:

$$Y = 0.834 + 0.017x_1 - 0.407x_1^2 - 0.290x_2^2 \quad (10)$$

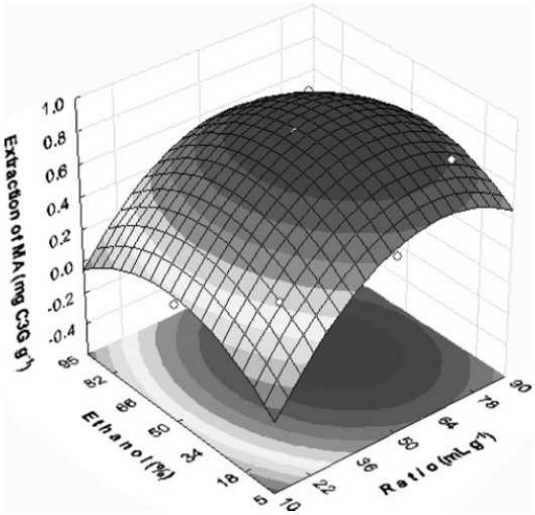

Fig. 1 Response surface of extraction of monomeric anthocyanins from grape marc as function of liquid-to-solid ratio and ethanol concentration

where Y is the concentration of MA extracted ($mgC3Gg^{-1}$ db) and x_1 and x_2 are the coded values of liquid-to-solid ratio and the ethanol concentration, respectively.

Figure 1 shows the plot of Eq. 10 for extraction of MA from grape marc as function of liquid-to-solid ratio and ethanol concentration. Extraction of bioactive compounds from plant matrices enhances with the increase of dielectric constant of the solvent (Cacace and Mazza 2003). Since organic solvents present higher ionic strength, the extraction of MA is improved by an increase in ethanol concentration of up to 50 %. Nevertheless, it is not indicated to use a highly pure organic solvent, probably due to the osmotic effects of the vegetable cell, since the water comes out very quickly, not allowing the alcohol to be introduced effectively into the cell to then diffuse the MA to the extract. Maximum liquid-to-solid ratio for extraction of MA from grape marc was 50 mLg^{-1}. Removal of compounds from

Table 3 Analysis of variance for the model for extraction of monomeric anthocyanins from grape marc

Source	Sum of square	Degree of freedom	Mean square	F value	p value
Ratio (L)	0.2454	1	0.2459	346.977	0.000338[a]
Ratio (Q)	0.3425	1	0.3420	482.662	0.000206[a]
Ethanol (L)	0.000062	1	0.000062	0.0879	0.7862
Ethanol (Q)	0.1913	1	0.1917	270.537	0.000489[a]
Ratio (L) by ethanol (L)	0.00302	1	0.00302	4.268	0.1307
Lack of fit	0.0118	3	0.00393	5.557	0.0963
Pure error	0.00212	3	0.00071		
Total sum of square	0.7132	11			

L linear effect; *Q* quadratic effect

[a]Statistically significant at 95 % of confidence

Table 4 Summary of errors performance of selected models to describe extraction of anthocyanins from grape marc

Equation	r^2	χ^2	SEM
1	[0.4322;0.7001]	[0.0042;0.0148]	[0.0280;0.0792]
2	[0.8643;0.9607]	[0.0005;0.0077]	[0.0009;0.0146]
3	[0.9853;0.9992]	[0.0001;0.0004]	[0.0001;0.0005]
4	[0.8546;0.9943]	[0.0001;0.0095]	[0.0001;0.0108]
5	[0.8901;0.9911]	[0.0004;0.0059]	[0.0004;0.0060]
6	[0.7620;0.9462]	[0.0019;0.0043]	[0.0023;0.0682]

solids has the concentration gradient between solvent and solid as the main driving force, so the increase of solvent–solid ratio increases the compound's yield (Cissé et al. 2012). This tendency is also observed for extraction of polyphenols from fruit materials in several published works (Cacace and Mazza 2003; Ku and Mun 2008; Karacabey and Mazza 2010; Sun et al. 2011; Cissé et al. 2012).

Extraction kinetics is usually expressed in terms of solute concentration extracted from the solid to the solvent per unit time (Allaf et al. 2011). Mechanism of extraction of biocompounds from plant matrices is not fully enlightened; therefore, several mathematical equations have been described in literature. Statistical errors for fitting the experimental data of extraction of MA from grape marc to models used in recent publications are shown in Table 4. Adequacy of the results to Eq. 1 yielded the lowest r^2 values, ranging from 0.432 and 0.700, meanwhile fitting to Eq. 2, which considers an exponential behavior for extraction, yielded r^2 values between 0.864 and 0.961. The model that represents the extraction going on two different rates (Eq. 4) yielded low r^2 values and high χ^2 and SEM values. Linares et al. (2010) proposed an alternative equation to model extraction of compounds from plant matrices (Eq. 5); however, for extraction of MA from grape marc, SEM and χ^2 values ranged from 0.0004 to 0.0059 and from 0.0004 and 0.0060, respectively. Bucić-Kojić et al. (2007) and Qu et al. (2010) used the sorption and desorption concept from Peleg (1998) (Eq. 6) to describe the extraction of polyphenols from fruit by-products. However, this model did not present good fitting for separation of anthocyanins from dried grape marc (lowest r^2 values of 0.762 and highest values of χ^2 and SEM of 0.043 and 0.0682, respectively).

Choosing the best mathematical model to represent processing curves is fundamental in order to minimize processing errors, maximize final product quality, and facilitate designing and simulation of industrial processes. Analysis of data presented in Table 4 clearly demonstrates that the best model to describe extraction of anthocyanins from grape marc is the pseudo-first order (Eq. 3). The r^2 values were the highest, varying between 0.985 and 0.999, while χ^2 and SEM were the lowest, ranging from 0.0001 to 0.0004 and from 0.0001 to 0.0005, respectively. Based on the statistical analysis, the anthocyanin extraction in the conditions studied is continuous, unlike in two different rates, for example, as proposed by Cacace and Mazza (2003) (Eq. 4), and MA presents a unique behavior during extraction from grape marc, suggesting that diffusion is the main mechanism in the removal of MA from grape marc.

Figure 2 shows extraction of MA at 25, 30, 40, 50, and 60 °C through time. The beginning of the removal process occurs at the external surface of the dried residue, where there is the pigment dissolution and its immediate transport into the extractor solvent. After this initial period, extraction proceeds through a network of phenomena including solvent diffusion within the solid matrix, internal solute solubilization in the solvent, and solute diffusion in the solvent within the solid matrix toward the surface to the external environment (Allaf et al. 2011). When the gradient of concentrations between the solvent and the dried grape material is quite high, extraction occurs at high rates, until the driven force tends to zero and an equilibrium concentration is reached.

Table 5 shows kinetic parameters estimated by modeling experimental data to pseudo-first-order equation. Extraction

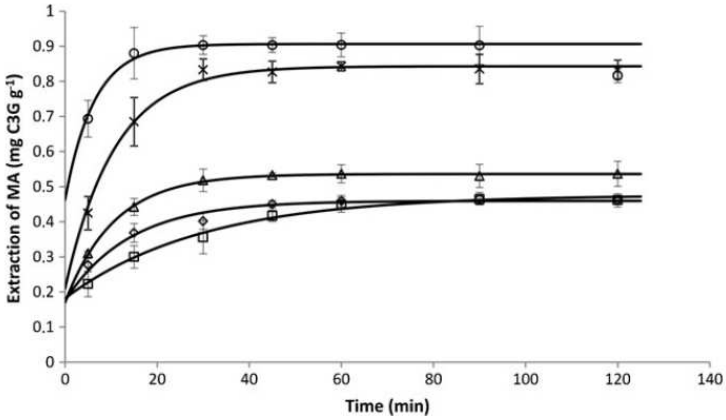

Fig. 2 Extraction of monomeric anthocyanins from grape marc at 60 (*empty circles*), 50 (*x marks*), 40 (*empty triangles*), 30 (*empty diamonds*), and 25 °C (*empty squares*). Data presented, fitted to a pseudo-first-order model, are average values of triplicates from two independent experiments

Table 5 Kinetic parameters for extraction of monomeric anthocyanins from grape marc

T (°C)	C_∞ (mgC3Gg^{-1})	k (min^{-1})	a	$t_{1/2}$ (min)	k_{apar} (min^{-1})
25	0.476±0.024a	0.034±0.002a	0.481±0.020	12.435±0.623	0.056±0.002
30	0.460±0.013a	0.071±0.003b	0.480±0.017	5.881±0.230	0.118±0.003
40	0.536±0.029b	0.094±0.003c	0.382±0.012	5.135±0.117	0.135±0.006
50	0.843±0.045c	0.092±0.003c	0.287±0.015	5.824±0.241	0.119±0.005
60	0.906±0.008d	0.157±0.008d	0.716±0.036	1.589±0.053	0.436±0.020

Different letters (a, b, c, d) in the same column indicate statistical differences ($p<0.05$)

yield enhanced with the increase of process temperature. At 60 °C, 0.906 mgC3Gg^{-1}db was extracted from grape marc, while, 0.843 and 0.536 mgC3Gg^{-1}db were extracted at 50 and 40 °C, respectively. Extraction at 25 and 30 °C had no significant difference ($p>0.05$), being extracted at about 0.476 mgC3Gg^{-1}db. Similar results were found by Negro and coworkers (2003), who extracted 0.98 mg of anthocyanins per gram of grape bagasse at 50 °C. Constant extraction rate (k value) at 60 °C was 0.157 min^{-1}, being the equilibrium concentration reached after 15 min of extraction, while at 50 °C, total removal of MA occurred at 30 min, with k value of 0.092 min^{-1}. For extraction at 25 and 30 °C, although equilibrium concentration did not differ significantly ($p>0.05$), the k value was enhanced with the increase of the temperature, as can be observed in the data presented in Table 5. Extraction of total anthocyanins from berry by-products took about of 30 min in a batch solid–liquid process (Cacace and Mazza 2002, 2003). Mantell et al. (2002), in a continuous extractor apparatus, found an effective diffusivity of 10.8 10^{-10}m^{2}s^{-1} for extraction of anthocyanins from grape marc at 60 °C, reaching the equilibrium concentration after 40 min of processing. At 25 °C, Cissé and coworkers (2012) reached maximum anthocyanin removal yield from roses after 10 min of a batch solid–liquid extraction.

Increasing of yield, as well as extraction rates (k values), are related to the increase of the internal energy of the molecules and to the reduction of dynamic viscosity of solvent (Cacace and Mazza 2003). However, extraction for 120 min at 60 °C shows clear reduction of concentration of MA in extract, probably due to initial of thermal degradation of the pigment. Cissé and coworkers (2012) also observed similar behavior during extraction of anthocyanins from *Hibiscus sabdariffa*. This fact contributes to observation of work of Cacace and Mazza (2003), where the authors suggest that processing temperature cannot be increased indefinitely because of heat sensitivity of bioactive compounds.

Integration constants (a values), which represent the intercept of tendency curve to ordered axis (when $t=0$ min), were estimated far from zero (an ideal behavior). This occurs probably because MA is not strongly bound to the plant matrix and is easily removed. Kapasakalidis et al. (2006) showed that major of anthocyanins from black currant pomace are extracted by water–alcohol, and a low amount is cell wall bound. Then, MA seems to be "instantly" removed to the solvent. Additionally, the solute solubilization at the surface of the plant matrix, immediately transporting anthocyanins into the solvent, is a common phenomenon in extraction processes (Allaf et al. 2011). Half-time extraction ($t_{1/2}$) and apparent extraction rate (k_{apar}) were calculated by Eqs. 11 and 12, respectively (Linares et al. 2010). The $t_{1/2}$ values decrease, and k_{apar} values increase with the increasing temperatures from 60 to 25 °C, indicating augmentation in both yield and rate of extraction, at higher temperatures.

$$t_{1/2} = \frac{\ln(2-a)}{k} \tag{11}$$

$$k_{apar} = \frac{\ln(2)}{t_{1/2}} \tag{12}$$

Figure 3 shows adequacy of k values against the reciprocal absolute temperature to the Arrhenius equation. This approach allows estimation of the activation energy (E_a), which can be seen as the energy barrier that MA molecules need to cross in order to be able to be removed from the grape residue. In the conditions evaluated, activation energy of MA extraction from grape marc was 29.5 kJmol^{-1}. Cacace and Mazza (2003) estimated activation energy of 77 kJmol^{-1} for extraction of anthocyanins from milled berries, and Bucić-Kojić et al. (2007) verified the necessity of 8 kJmol^{-1} to extract total polyphenols from grape seeds. E_a values of extraction may depend on the food matrix, the

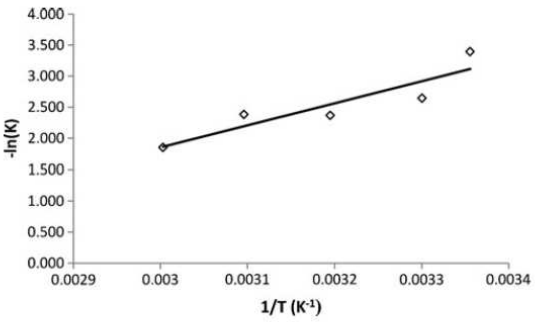

Fig. 3 Arrhenius plot of monomeric anthocyanin extraction rates from pseudo-first-order model. The regression equation was determined as $y=3{,}545.254x-8.783$ ($r^2=0.8265$)

pre-extraction procedures, the target compound, among other factors.

Conclusions

In conclusion, kinetic extraction studies of anthocyanins showed to be a fast process to remove anthocyanins from grape marc at 60 °C using 50 % ethanol–water as solvent at a liquid-to-solid ratio of 50 mL g^{-1}. Pseudo-first-order model was the equation that better represented the extraction in the temperature range of 25–60 °C, with activation energy of 29.5 kJ mol^{-1}, reaching extraction yield of 0.906 mg cyanidin-3-glucoside per gram of dried residue. Grape marc stands up as a cheap alternative to be used as source of natural pigments for industrial application, and the knowledge about kinetics extraction of anthocyanins from plant materials is essential to elucidate the phenomena involved and better dimensioning equipment for industrial plants.

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CAPÍTULO 11

Grape marc powder: physicochemical and microbiological stability during storage and moisture sorption isotherm

Neste capítulo é apresentado um estudo sobre a estabilidade microbiológica de farinha de bagaço de uva, além da estabilidade de seus compostos fenólicos e de compostos com atividade antioxidante durante a armazenagem do produto. Também, são apresentados resultados de isotermas de sorção nas mesmas condições de armazenagem. Artigo aceito no ano de 2013 na revista Food and Bioprocess Tecnology sob o doi 10.1007/s11947-013-1198-1.

CAPÍTULO 11 - GRAPE MARC POWDER: PHYSICOCHEMICAL AND MICROBIOLOGICAL STABILITY DURING STORAGE AND MOISTURE SORPTION ISOTHERM

Food Bioprocess Technol
DOI 10.1007/s11947-013-1198-1

ORIGINAL PAPER

Grape Marc Powder: Physicochemical and Microbiological Stability During Storage and Moisture Sorption Isotherm

Voltaire Sant'Anna · Alexandre Hahn Englert · Ana Paula Folmer Corrêa · Adriano Brandelli · Ligia Damasceno Ferreira Marczak · Isabel Cristina Tessaro

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Abstract In the present work, the stability of grape marc powder was evaluated during its storage at room conditions for 6 months. Grape juice marc from *Vitis labrusca* cv. "Isabel" was dried to 8.8±0.9 % moisture content (wet basis) in forced convection drying equipment at 70 °C, crushed and stored aseptically in dark polyethylene bags and kept at 25±2 °C for up to 6 months. Grape marc powder was microbiologically safe, free of *Salmonella* sp., *Bacillus cereus*, and fecal coliforms. Total phenolic compounds with 1,1-diphenyl-2-pic-ryl-hydrazyl (DPPH) scavenging capacity and ferric reducing antioxidant power (FRAP) were stable, although monomeric anthocyanins and compounds with the capability of scavenging 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals showed to be susceptible to degradation through the storage period. Moisture sorption isotherm at 25 °C for the powder material was determined by static gravimetric methodology. The sorption isotherm of the grape marc powder showed a sigmoidal shape (type II), typical of food materials. The experimental data was satisfactorily fitted by the GAB (Guggenheim–Anderson–de Boer) model, giving a monolayer moisture content (X_m) of 6.75 % (dry basis). Results indicate that grape marc powder can be considered as a potential functional ingredient with an acceptable stability.

Keywords Grape marc powder · Stability · Isotherm · Antioxidant activity · Microbiology

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Introduction

Grapes are of great economical and nutritional importance because they are world widely consumed and have high concentration of polyphenolic compounds, which present a well-documented protective effect against low density lipoprotein (LDL) oxidation, reduction of platelet aggregation, improvement of coronary blood flow, among other beneficial effects to human health (Demrow et al. 1995; Stein et al. 1999; Keevil et al. 2000; Cui et al. 2002). During the industrialization of grape products, large amounts of solid residues are generated, which are currently discarded by industries, despite of its high polyphenol contents. Thus, grape residue may be an important source of dietary fiber and phenolic compounds, presenting great potential to be used as functional ingredient (Saura-Calixto 2011).

Recent scientific efforts have focused on the investigation of potential applications of phenolic extracts as natural antioxidants in the food industry (Maier et al. 2009; Peng et al. 2010; Sant'Anna et al. 2012a). However, antioxidant dietary fibers are now becoming of great interest as a source of polyphenolic compounds, vitamins, carotenoids, fibers, among other compounds (Saura-Calixto 2011). Recently, Sant'Anna and co-workers (2012a) verified that grape marc presents compounds with high antioxidant activity, which are strongly linked to the plant matrix. In this sense, yogurt, frankfurters and bread fabricated with the addition of grape byproducts have been developed, showing to increase the concentration of beneficial compounds in the final product

(Peng et al. 2010; Mildner-Szkudlarz et al. 2011; Özvural and Vural 2011; Coda et al. 2012). Thus, dried grape marc appears as an interesting alternative to be used as functional ingredient in the food industries.

For the commercialization and application of phenolic-rich dried plant material, the stability during short-to-medium storage periods should be carefully investigated. Additionally, information about moisture sorption isotherms is of great importance to understand the interaction between water and non-aqueous food components (Kaymak-Ertekin and Gedik 2004), since water is an active component that controls biochemical reactions, determines texture properties and the overall physical and biological behavior (Doporto et al. 2012). A moisture sorption isotherm describes the relationship between water activity (a_w) and the equilibrium moisture content (X_{eq}) of a food material at constant temperature (Kaymak-Ertekin and Gedik 2004; Sahin and Sumnu 2006). Nevertheless, experimental data regarding sorption isotherm, microbiological stability and the maintenance of compounds with antioxidant activity in grape marc powder is still scarce in the literature.

Thus, the objective of the present work is to evaluate the stability of grape marc powder during storage. The physicochemical and microbiological properties of the grape byproduct powder were evaluated through 6 months of storage at 25 °C. The moisture sorption isotherm of the dried plant material was experimentally obtained at 25 °C and utilized to evaluate the fitting to common mathematical models that describe moisture sorption behavior.

Materials and Methods

Plant Material and Storage Conditions

Grape marc powder was obtained as described in Fig. 1. Grape juice marc from *Vitis labrusca* cv. "Isabel" was kindly supplied by Vinícola Garibaldi (Garibaldi, RS, Brazil), harvested in 2012. The byproduct was dried to 8.8±0.9 % moisture content (wet basis) in a forced convection drying equipment at 70 °C, and then immediately it was crushed in a domestic blender (RI1764, Walita, Brazil) for 1 min and passed through a 0.811-mm sieve. Grinder and sieve were sanitized with 70ºGL alcohol and allowed to dry previously to sample preparation. After size screening procedure, samples were immediately transferred to sterile dark polyethylene bags inside laminar chapel flow. Grape marc powders were kept at 25±2 °C for up to 6 months, collected inside laminar chapel flow at predetermined periods for microbiological and biochemical analysis (Fig. 1). Grape marc powder was analyzed for its content of proteins, ashes, carbohydrates, humidity and lipids according to the Association of Official Analytical Chemists (AOAC 1995).

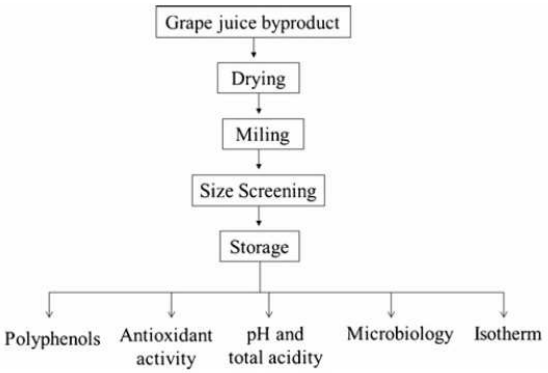

Fig. 1 Flowchart of grape marc powder production

Physicochemical Analysis

Grape marc powder was analyzed for pH and total acidity according to the AOAC (1995) within the storage period studied. For pH and total acidity, 75 ml of boiled distilled water at 25 °C was added to 10 g of sample and homogenized every 10 min for 1 h. The extract had its pH measured using a digital pH meter (Quimis Q400M, São Paulo, Brazil) and its total acidity by titration with 0.1 M sodium hydroxide solution, the latter being expressed as milligram of acid per gram of dried sample (mg acid g^{-1}).

Extraction of total phenolic content (TPC) and monomeric anthocyanins (MA) was performed according to Sant'Anna and co-workers (2012a, b). Briefly, dried samples (1 g) were added to 50 ml of 50 % ethanol aqueous solution and extraction was performed at 60 °C for 1 h. TPC in the extracts was determined by the Folin–Ciocalteau method (Singleton and Rossi 1965) using gallic acid as standard. The absorbance of the reaction mixture was measured at 765 nm by a UV-1600 spectrophotometer (Pró-Análise, Brazil), and results were expressed as mg gallic acid equivalent per gram of dry bagasse weight (mg GAE g^{-1}). MA were determined using the pH differential method (Lee et al. 2005). MA values were expressed as cyanidin-3-glucoside and results expressed as mg of cyanidin 3-glucoside per gram of dry bagasse (mg C3G g^{-1}).

Antioxidant Activities

The antioxidant properties of the grape marc powder were evaluated by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-pic-ryl-hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. For the ABTS radical scavenging, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. An aliquot of 30 μl of extract was mixed with 1 ml of the diluted $ABTS^{\bullet+}$ solution and an absorbance (734 nm) reading was taken after 6 min. Distilled water, instead of sample, was

used as a control. The results were expressed as: ABTS scavenging activity (%)=[1−(A/A_0)]×100, where A is the absorbance of the test and A_0 is the absorbance of the control (Re et al. 1999).

FRAP activity of the extracts was measured as described elsewhere (Zhu et al. 2006). Samples of 1 ml of extracts were mixed with 2.5 ml phosphate buffer (0.2 mol l^{-1}, pH 6.6) and 2.5 ml potassium ferricyanide (10 mg ml^{-1}), and the resultant mixture was subsequently incubated at 50 °C for 20 min. Then, 2.5 ml TCA (10 %, v/w) was added and the mixture was centrifuged (3,000×g for 10 min). The supernatant (1 ml) was mixed with 2.5 ml distilled water and 0.2 ml ferric chloride (1 mg ml^{-1}), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

The DPPH antioxidant activity was evaluated according to Brand-Williams et al. (1995). In the dark, aliquots of 0.1 ml sample was transferred to test tubes with 3.9 ml radical DPPH (60 μmol l^{-1} DPPH solution, diluted in methyl alcohol). After 45 min, the scavenging activity was measured spectrophotometrically by the decrease in absorbance at 517 nm. Likewise, these same proportions (0.1 ml distilled water and 3.9 ml DPPH radical) were used as a control, using methyl alcohol a blank. The results were expressed as scavenging activity (%)=[1−(A/A_0)]×100, where A is the absorbance of the test and A_0 is the absorbance of the blank.

Microbiological Analysis

Grape marc powder was aseptically collected from the storage bags every 30 days. Samples were then subjected to quantification of total viable bacteria and yeast, total and fecal coliforms, *Salmonella* spp. and *Bacillus cereus*. The methodologies used were conducted according to the Brazilian Ministry of Agriculture and Food Supply (MAPA), which follows the methods recommended by the American Public Health Association (APHA 2001).

Moisture Sorption Isotherm

The moisture sorption isotherm at 25 °C was determined by static gravimetric methodology (Al-Muhtaseb et al. 2002; Sahin and Sumnu 2006). Saturated electrolyte aqueous solutions (potassium hydroxide, potassium acetate, magnesium chloride, potassium carbonate, magnesium nitrate, potassium nitrite, sodium chloride, potassium chloride and copper (II) sulphate — all with reagent grade purity) were utilized to produce relative humidities varying between 8 % and 97 % (Young 1967). Samples (triplicate) with approximately 5 g of the grape marc powder were weighted in 25 ml glass beakers and placed inside hermetically sealed containers, each with a different electrolyte solution (and consequently relative humidity).

The containers were placed inside a temperature-controlled chamber with air circulation (CL350; ColdLab, Piracicaba, SP, Brazil) and kept at the specified temperature (25 °C). After the first week, one sample of each container was weighted every 3–5 days to check whether chemical equilibrium was attained (i.e., constant weight). With the purpose of preventing microbiological contamination and growth in the samples, thymol or toluene were added to the sealed containers along with the samples (Sahin and Sumnu 2006; Cassini et al. 2006). After equilibrium was reached (between 20 and 48 days), the moisture content of the samples was determined by measuring the weight loss after drying for 3 h in a laboratory oven (Biomatic 303, Biomatic Aparelhos Científicos Ltda., Porto Alegre, RS, Brazil) at 105 °C. In equilibrium, the water activity (a_w) of the sample is equal to the relative humidity (%) of the gaseous phase inside the container divided by 100 (Sahin and Sumnu 2006).

Data Analysis

The storage stability experiments were conducted in triplicate. Obtained values were compared using Tukey's test by *Statistica* 11, and differences were considered statistically significant for $p<0.05$.

The isotherm measurement procedure was conducted in two independent experiments. Six mathematical models were fitted to the experimental data (equilibrium moisture (X_{eq}) versus water activity [a_w]) via nonlinear regression using MATLAB®. Oswin, Halsey, BET, GAB (Guggenheim–Anderson–de Boer), Peleg and D'arcy Watt models are mathematically described by Eqs. 1–6, respectively (Cassini et al. 2006):

$$X_{eq} = A\left(\frac{a_w}{1-a_w}\right)^B \quad (1)$$

$$X_{eq} = \left(\frac{-A}{\ln a_w}\right)^{1/B} \quad (2)$$

$$X_{eq} = \frac{(X_m C a_w)\left(1-(N+1)a_M^N + N a_M^{N+1}\right)}{(1-a_w)\left(1-(C-1)a_w - C a_M^{N+1}\right)} \quad (3)$$

$$X_{eq} = \frac{X_m C K a_w}{(1-K a_w)(1-K a_w + C K a_w)} \quad (4)$$

$$X_{eq} = k_1 a \frac{n_1}{w} + K_2 a \frac{n_2}{w} \quad (5)$$

$$X_{eq} = \frac{K_1 K_2 a_w}{1+K_1 a_w} + K_5 a_w + \frac{K_3 K_4 a_w}{1-K_3 a_w} \quad (6)$$

where A, B, X_m, C, N, K, k_1, n_1, k_2, n_2, K_1, K_2, K_3, K_4, and K_5 are constants (i.e., model parameters).

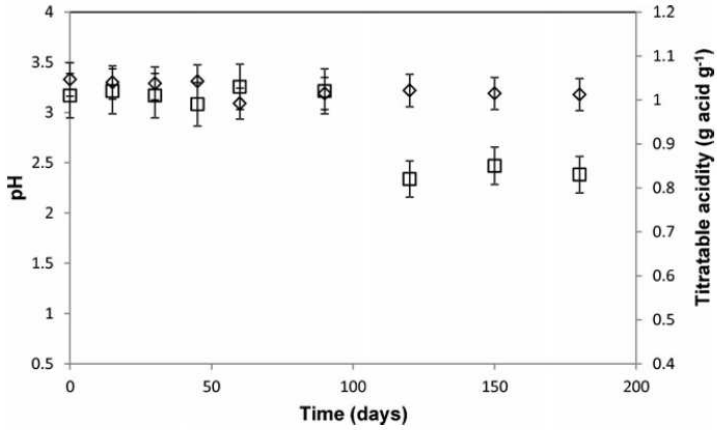

Fig. 2 Changes on total acidity (*empty square*) and pH (*empty diamond*) of grape marc powder during storage of 180 days. Data presented are average values of triplicates from three independent experiments

These isotherm models are widely studied for food materials and details are found elsewhere (Park et al. 2002; Cassini et al. 2006). Fitting quality was assessed via the coefficient of determination (R^2) and the mean relative deviation (MRD) obtained from the nonlinear regression. A good fit must show MRD values below 10 % (Park et al. 2002).

Results and Discussion

The use of dried grape marc as a functional ingredient is an increasing trend in the food and pharmaceutical industries. Thus, the behavior of biochemical and microbiological properties of the grape residue powder during its storage were evaluated in order to understand the impact over the grape marc powder stability. Grape marc powder utilized in the present work, according to the proximate analysis performed, showed 11.87 % moisture, 9.58 % crude protein, 8.15 % lipids, 1.45 % ashes and 68.95 % carbohydrates in dry basis.

Figure 2 shows the pH and the total acidity of the grape marc powder through the storage period. The pH was not significantly ($p>0.05$) altered during the storage. The total acidity, on the other hand, decreased as the time was increased. This parameter showed a significant difference ($p<0.05$) from the initial value after the third month. Organic acids are closely related to the total acidity content and they possibly were degraded during grape marc powder storage, consequently decreasing the acidity value (Margalit 1997; Sahari et al. 2004; Toit et al. 2011).

TPC and MA concentrations in grape products are related to the benefits that the consumption of the fruit may bring to the human health due to their antioxidant capabilities. Table 1 shows the TPC and MA concentrations, together with the three types of antioxidant capabilities (ABTS, DPPH and FRAP), for the grape marc powder through the 6 months of storage. As can be seen in Table 1, the TPC was not affected significantly ($p>0.05$) for the stored product. The MA concentration, on the other hand, started to decrease significantly ($p<0.05$) after the third month of storage. Polyphenolic compounds may degrade depending upon many factors such as temperature, light exposure, air oxidation phenomena, among others (Yousif et al. 2000; de Ancos et al. 2000; Vashisht et al.

Table 1 Total phenolic content (TPC), monomeric anthocyanin (MA), ABTS and DPPH scavenging activities and the FRAP of grape marc powder during 180 days of storage

Time (days)	TPC (mg GAE g^{-1})	MA (mg C3G g^{-1})	ABTS (%)	DPPH (%)	FRAP (Abs 700 nm)
0	31.53±2.30^{a}	2.01±0.03^{a}	26.76±1.34^{a}	90.88±5.22^{a}	2.15±0.12^{a}
15	31.30±1.61^{a}	2.00±0.12^{a}	25.39±0.98^{a}	92.24±3.91^{a}	2.13±0.10^{a}
30	28.98±2.71^{a}	2.14±0.21^{a}	25.76±0.80^{a}	88.34±6.98^{a}	2.11±0.09^{a}
45	28.89±1.38^{a}	2.00±0.18^{a}	26.98±1.89^{a}	90.01±4.07^{a}	2.05±0.17^{a}
60	30.56±1.30^{a}	1.88±0.03^{a}	24.16±1.76^{a}	89.87±0.91^{a}	2.11±0.11^{a}
90	31.54±1.03^{a}	1.63±0.05^{b}	25.09±0.91^{a}	93.10±5.44^{a}	2.00±0.17^{a}
120	29.53±1.10^{a}	1.55±0.09^{b}	26.03±1.05^{a}	90.33±4.90^{a}	2.40±0.19^{a}
150	30.13±0.32^{a}	1.42±0.10^{b}	24.02±0.89^{a}	90.45±3.44^{a}	2.20±0.11^{a}
180	28.29±2.20^{a}	1.29±0.01^{c}	21.79±1.11^{b}	91.03±1.98^{a}	2.11±0.07^{a}

^{a,b,c} Different superscripts letters within same column indicate statistical differences ($p<0.05$)

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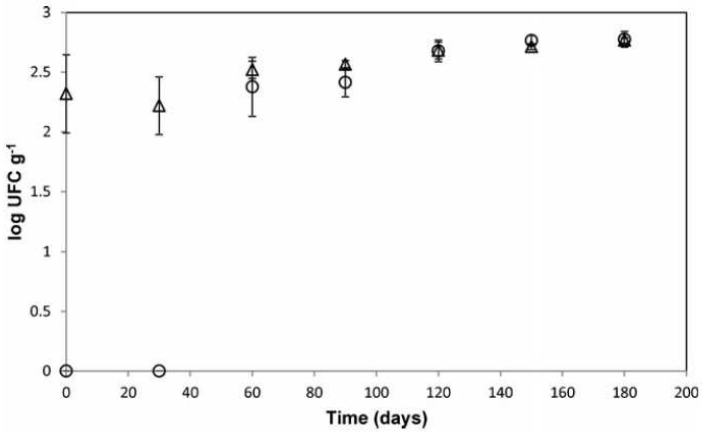

Fig. 3 Total viable bacteria (*empty square*) and yeast (*empty circle*) content in the grape marc powder during storage of 180 days. *Bacillus cereus*, *Salmonella* spp., total and fecal coliforms were not identified in the samples. Data presented are average±standard deviation values of triplicates

2011). Anthocyanins and flavonols are present outside the cellular vacuoles of vegetable cells, while most glycosides of phenolic compounds are localized inside them (Chism and Haard 1996; Torres et al. 2010; Sakihama et al. 2002; Vega-Gálvez et al. 2012). Consequently, the compounds stored outside the organelles are more sensitive to degradation, with MA being more exposed to degradation factors in the grape marc powder. TPC, on the other hand, possibly experienced a protective effect via cellular inclusion, leading to the phenolic compounds not being degraded during the storage period and therefore higher TPC values being measured.

The antioxidant capability of the grape marc powder was measured by the ABTS and DPPH scavenging activities and the FRAP capacity of the compounds extracted from the dried residue during 180 days of storage of the dried grape marc (Table 1). Results show that compounds with DPPH scavenging capability were stable during the storage period, presenting the capability of stabilizing 90 % of the free radical in the assays. DPPH is a free radical that accepts an electron or a hydrogen radical, becoming a stable molecule (Brand-Williams et al. 1995). The ABTS radical scavenging activity of the grape marc was stable up to 5 months of storage, and started a significant ($p<0.05$) decrease with 180 days of storage. The radical ABTS is reduced with concomitant conversion to a colorless product in the presence of antioxidants with hydrogen-donating or chain-breaking properties (Re et al. 1999). Iron acts as a catalyst for the generation of hydroxyl radicals, potentially contributing to diseases related to oxidative stress and stimulating lipid peroxidation in foods (Pownall et al. 2010; Zhang et al. 2010). Grape marc polyphenolic compounds showed stable iron-binding capacity through the storage period. The antioxidant activity in the dried grape byproduct seems to be related to the presence total phenolics in the residue and less with anthocyanins, since the concentration of MA decreased during the experiment period and the antioxidant activity was not affect, likewise TPC. Grape byproducts are rich in condensed tannins, flavonols, phenolic acids, which shows high antioxidant capability (Sant'Anna et al. 2012a). Results show that polyphenols are stable in grape marc powder, indicating that it may be stored at room conditions without damage of their antioxidant capability.

Additionally to the biochemical stability of the dried grape by-product, the microbiological behavior of the powder throughout the storage time is an important issue. Figure 3 shows the results for the analysis of total and fecal coliforms,

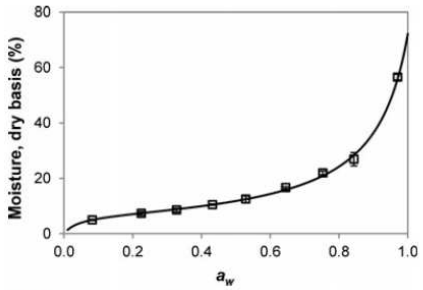

Fig. 4 Moisture sorption isotherm for grape marc powder at 25 °C. ***SYMBOLS*** represent experimental data points and the ***LINE*** corresponds to the GAB model fitting

Table 2 Performance of models (i.e., regression parameters) to describe the isotherm of grape marc powder at 25 °C

Model (eq.)	R^2	*MRD* (%)
Oswin (Eq. 1)	0.9906	7.06
Halsey (Eq. 2)	0.9770	15.02
BET (Eq. 3)	0.9792	17.25
GAB (Eq. 4)	0.9914	5.10
Peleg (Eq. 5)	0.9878	10.10
D'arcy Watt (Eq. 6)	0.9871	12.60

Salmonella spp., *B. cereus*, total viable bacteria and yeasts in the product. Microbiological analysis showed that the grape residues submitted to drying process with hot air at 70 °C was free of *Salmonella* spp. and *B. cereus*, two important pathogenic bacteria in the food. Total and fecal coliforms were also inactivated by the drying procedure and did not grow in the powder during the 180 days of storage. Total viable bacteria content in the grape marc powder grew in the dried samples significantly ($p<0.05$) after 60 days of storage and slightly multiplied throughout the time. In the beginning of the storage there was an absence of yeast growth in the product. After 30 days of storage, there was a presence of about 10^2 UFC g^{-1} of viable yeast, which kept growing up to 180 days. The results suggest that the grape marc powder was kept microbiologically safe in relation to pathogenic bacteria during the storage period in ambient conditions. However, for long storage the product may undergo degradation due to the growth of yeast and other spoilage microorganisms.

Water sorption of foodstuffs is of great interest for the food science and engineering area. Sorption isotherms of food products may provide viable information for drying, aeration and storage operations, helping on maximization of stability and quality during packaging and storage of food products (Doporto et al. 2012). Figure 4 presents experimental results obtained for the equilibrium moisture content (dry basis) versus water activity (a_w) for the grape marc powder at 25 °C. As expected, results show that equilibrium moisture increases with the increase in a_w. At higher a_w values, equilibrium moisture sharply increased, leading the isotherm to a sigmoidal shape, which is classified as type II (Sahin and Sumnu 2006). This seems to be in agreement to powder food materials, like with results obtained for *pinhão* flour (Cladera-Olivera et al. 2009), β-carotene encapsulated in *pinhão* starch (Spada et al. 2013), unpeeled banana flour (Bezerra et al. 2013), as well as ahipa and cassava flours (Doporto et al. 2012).

Table 2 shows the results for adequacy of the experimental isotherm data to Eqs. 1–6. Results show that GAB model showed the best fit to the experimental data (R^2=0.9914 and MRD=5.10 %). Additionally, the Oswin model showed also a satisfactory fitting to the experimental data (i.e., MRD<10 % and R^2>0.99). In the GAB equation, X_m represents the monolayer moisture content and C, K are adsorption constants related to the energies of interaction between the first and further adsorbed water molecules at the individual sorption sites (Sahin and Sumnu 2006). The parameters obtained from the fit were X_m=0.0675 g H_2O g^{-1} (dry basis); C=27.03 and K=0.9068. Therefore, the monolayer moisture content for the grape marc powder at 25 °C corresponds to 6.75 % (dry basis), showing that the moisture of the material studied (8.8 %, wet basis; 9.6 %, dry basis) is slightly higher than the content corresponding to a monolayer of water molecules on its surface (which would not be expected to easily vaporize upon forced convection drying at 70 °C). Labuza (1984) has stressed that the maximum monolayer moisture content for foods corresponds to 10 % (dry basis), because when this value is exceeded, the stability of the product is compromised. Moreover, a_w lower than 0.6 guarantee microbiologic stability of food systems (Jay et al. 2005). Thus, since the water activity of the grape marc powder is 0.38, calculated using the fitted GAB model for an X_{eq} value of 0.096 g H_2O g^{-1} (dry basis), it can be stored at ambient conditions with an acceptable stability. In an analogous way, an a_w value of 0.18 is obtained corresponding to the monolayer moisture content (X_m), showing that the material, if dried to such moisture, is also microbiologically stable (a_w<0.6).

Conclusions

In conclusion, grape marc powder produced by drying at 70 °C in hot air equipment presented to be microbiologically safe during the storage period in ambient conditions. Additionally, it may be stored at room conditions with minimal degradation of phenolics and compounds with antioxidant activity. The moisture sorption isotherm of the grape marc powder at 25 °C showed a sigmoidal shape (type II), typical of food materials. The data was satisfactorily fitted by the GAB model, giving a monolayer moisture content (X_m) of 6.75 % (dry basis), indicating good stability at usual conditions of storage.

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CAPÍTULO 12

The effect of the incorporation of grape marc powder in fettuccini pasta properties

Neste capítulo é apresentado o artigo sobre a incorporação de farinha de bagaço de uva na formulação de massa tipo fettuccini, cadastrado na Platarforma Brasil com o protocolo número 241.529. Foram avaliados a capacidade de absorção de água e perda de sólidos da massa, o teor de compostos fenólicos totais e antocianinas monoméricas, assim como a capacidade antioxidante das massas com a incorporação do resíduo de uva seco.

12.1 Artigo: The effect of the incorporation of grape marc powder in fettuccini pasta properties

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Abstract

Several studies have been conducted to evaluate the potentiality of grape residues to be used in the food industry. In the present work, the incorporation of grape marc powder in fettuccini pasta preparation was evaluated over its cooking, nutraceutical and sensory properties. Incorporation of the dried byproduct did not interfere in the water absorption and in the solid loss of the pasta after cooking procedure. Addition of the grape residue

increased the antioxidant capacity of the product due to the incorporation of polyphenols stemmed from grape. Sensory analysis showed that incorporation of 2.5% of grape marc power had the best acceptance by the member-panel, with lower changes of colour, according to the CIELAB method. Results show that the development of fettuccini pasta added of grape marc is an interesting alternative for using the grape juice byproduct, potentially reflecting on final product costs and representing a way of the industrial waste management.

Keywords: grape marc powder; pasta; antioxidant activity; sensorial analysis; colour.

12.1.1 Introduction

Consumers' demand for nutritional diets, rich in compounds with functional properties has increased since several researches have shown their beneficial effects to the human health, preventing and combating several diseases. Grapes have world widely economical and nutritional importance, because they have good sensorial acceptance by consumers and are phenolic-rich fruits, presenting well documented protective effect against LDL oxidation, reduction of platelet agregation, improvement of coronary blood flow, among other beneficial effects to human health (Demrow et al., 1995; Stein et al., 1999; Kevil et al., 2000; Cui et al., 2002). In the industrial processing of grapes, large amounts of solid residue are generated and often considered discard. However, several studies have shown that grape marc presents high contents of dietary fibers and polyphenolic compounds (Saura-Calixto, 2011; Sant'Anna et al., 2012a), indicating that the dried grape byproduct has great potential to be used as functional ingredient in the food area.

Antioxidant dietary fibers are now being of great interest due to their source of polyphenolic compounds, vitamins, carotenoids, fibers, among others (Saura-Calixto, 2011). In this sense, yogurt, frankfurters and bread, added of grape byproducts have been developed showing to increase the concentration of beneficial compounds in the final product (Peng et al., 2010; Mildner-Szkudlarz et al., 2011; Özvural and Vural, 2011; Coda et al., 2012). Pasta is a traditional cereal-based product which represents a good vehicle for

the addition of nutrients because it is well accepted worldwide due to the low cost, ease production and sensory attributes (Chillo et al., 2008; Mildner-Szkudlarz et al., 2011).

Thus, the objective of the present work is to evaluate the incorporation of grape marc powder in the preparation of fettuccini pasta. On this basis, nutritional, sensorial and cooking properties of the pasta were analyzed.

12.1.2 Material and Methods

12.1.2.1 Grape marc powder (GMP)

Grape juice marc from *Vitis labrusca* cv. "Isabel" was gently supplied by Vinícola Garibaldi (Garibaldi, RS, Brazil), harvested in 2012. The byproduct was dried in a forced convection drying equipment at 70 °C, and then was crushed in domestic blender (RI1764, Walita, Brazil) for 1 min and passed through a 0.811 mm sieve. Grape marc powder (GMP) utilized in the present work, according to the centesimal analysis, had 10.87% of humidity, 9.58% of crude protein, 8.15% of lipids, 2.45% of ashes and 68.95% of carbohydrates in wet basis.

12.1.2.2 Pasta preparation and cooking

Fresh pasta was prepared with wheat flour, water and different concentrations of GMP. Fettuccini pastas were coded as FP0, FP2.5, FP5, FP7.5, according to the percentage of GMP incorporation: 0%, 2.5%, 5% and 7.5%, respectively. For each formulation, wheat flour, grape byproduct powder and water (30%) were mixed using an industrial mixer (G.Paniz, Mod 90334, Brazil) for 10 minutes, to obtain homogeneous dough. The premixed dough was extruded (40 ± 2 °C), through a die, in the same equipment, to obtain the fettuccini shaped pasta. Then, samples of 10 g of pasta were, submitted to cooking in 170 mL boiling distilled water for 10 minutes.

12.1.2.3 Pasta Quality

12.1.2.3.1 Pasta cooking properties

The American Association of Cereal Chemists Official Methods 16-50 and 16-51 (AACC, 2000) were used to determine optimum cooking time, cooking loss and cooked weight (water absorption). Fettuccini (10g) was broken into pieces of 5 cm and cooked in 170 ml

of boiling tap water. The optimal cooking time was taken to be when, after squeezing the fettuccini between two glass plates, the white core from the strands disappeared. After cooking, fettuccini was drained and the cooking water collected. Cooked fettuccini was rinsed with water for 30 seconds and drained for one minute to expel the remaining water. The rinsate was combined with the cooking water and weighed after complete evaporation (reaching dryness) and expressed as a percentage of the original pasta weight as a cooking loss. At this stage, fettuccini samples were weighed to determine the cooked weight.

12.1.2.3.2 Extraction and evaluation of polyphenols

Extraction of total phenolic content (TPC) and monomeric anthocyanins (MA) was performed according to Sant'Anna and co-workers (2012a,b). Briefly, dried samples (1g) were added to 50 mL of 50% ethanol and extraction was performed at 60 ºC for 1 h. TPC in the extracts was determined by the Folin-Ciocalteau method (Singleton and Rossi, 1965) using gallic acid as standard. The absorbance of the reaction mixture was measured at 765 nm by UV-1600 spectrophotometer (Pró-Análise, Brazil), and results were expressed as mg gallic acid equivalent per gram of dry pasta (mg GAE $100g^{-1}$). Analysis of condensed tannin content (CTC) was carried out according to the method of Price et al. (1978), which involves the reaction of the samples with vanillin solution. The absorbance was measured at 500 nm and results were calculated and expressed as mg epicatechin equivalents (mg of ECE/ 100g of dried sample). Monomeric anthocyanins (MA) were determined using the pH differential method (Lee et al., 2005). Absorbance was measured at 520 and 700 nm of samples diluted separately in 0.025 mol L^{-1} potassium chloride buffer pH 1.0 and 0.4 mol L^{-1} sodium carbonate buffer pH 4.5. MA values were expressed as cyanidin-3-glucoside (molar extinction coefficient of 26,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}). The units for extracted MA were expressed as mg of cyanidin 3-glucoside per 100 gram of dry samples (mg C3G $100g^{-1}$).

Antioxidant analysis were performed by the determination of 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical scavenging activity (Re et al., 1999). The ABTS radical cation was produced by reacting 7 mmol L^{-1} ABTS stock solution with 140 mmol L^{-1} potassium persulfate, and allowing the mixture to stand in the dark for 16 h at room temperature before use. For the assay, the $ABTS^{\bullet+}$ solution was diluted with

ethanol to an absorbance of 0.7 at 734 nm. An aliquot of 30 μL of extract was mixed with 1 mL of $ABTS^{\bullet+}$ solution and an absorbance (734 nm) reading was taken after 6 min. Trolox solutions (100-2000μM) were used as standards and results were expressed as μM Trolox Equivalent per 100g of dried pasta.

12.1.2.3.3 Colour evaluation of pasta

The colour of fresh and cooked (10 minutes/ 100°C) fettuccini pasta was measured with a Hunter Lab Colorimeter (MiniScan XE Plus, Reston, VA). The samples were placed in the colorimeter and the color reading expressed by Hunter *L**, *a** and *b** values. Results were expressed as color differential (ΔE) between fresh (pasta not cooked) and cooked pasta, calculated as follows:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

Where, ΔL was calculated as : $L^*_{\text{fresh sample}} - L^*_{\text{cooked sample;}}$

Δa was calculated as : $a^*_{\text{fresh sample}} - a^*_{\text{cooked sample;}}$

Δb was calculated as : $b^*_{\text{fresh sample}} - b^*_{\text{cooked sample.}}$

Results are the means of independent duplicate determinations.

12.1.2.4 Sensorial analysis

A 50-member panel performed the sensorial profiling of the four samples, which were presented simultaneously. The panel members assigned the intensity of liking or disliking, using verbal hedonic 9-point scale (1 represented low intensity of liking and 9 represented high intensity of liking) of cooked fettuccini pasta: appearance, colour, texture, flavor, aftertaste and overall acceptance.

12.1.2.5 Data analysis

Results are shown as average standard deviation of at least triplicate measurements. Analysis of Variance (One-way ANOVA) followed by Fisher LSD post hoctest was

performed using Statistica 7.1 (StatSoft, U.S.A.), and differences were considered statistically significant when $p < 0.05$.

12.1.3 Results and Discussion

The use of dried grape marc as a functional ingredient is an increasing trend in the food industries. Thus, the study of its incorporation in new products and the effect of the addition on nutraceutical and technological properties is an important step. Grape marc power (GMP) was added to pasta formulation and changes on the cooked product characteristics were evaluated.

12.1.3.1 Water absorption and cooking loss

No differences were observed in processing for control pasta (100% wheat flour) or GMP enriched pastas. Table 11.1 shows the cooking quality characteristics of control fettuccini pasta and GMP enriched fettuccini pastas (FP2.5, FP5 and FP7.5). There was no statistically significant ($p > 0.05$) difference in optimum cooking time.

Table 11.1 Cooking characteristics of control and grape marc powder enriched pasta[a].

Samples	Percentage weight increase(%)	Solid loss (%)
Control	89.20 ± 7.54^{b}	5.45 ± 0.09^{b}
FP2.5	94.90 ± 6.98^{b}	5.38 ± 0.16^{b}
FP5	88.70 ± 0.10^{b}	6.18 ± 0.20^{c}
FP7.5	85.24 ± 0.44^{b}	6.35 ± 0.07^{c}

[a] Cooking time: 4 min.

[b,c] Different superscripts letters within same column indicate statistical differences ($p < 0.05$).

According to Borneo and Aguirre (2008) this parameter was defined as the cooking time needed for “white pasta center core” to disappear when pasta was squeezed between 2 glass plates (2.5 cm x 2.5 cm). For all samples, the optimum cooking time was around 4 min and the other cooking characteristics (solid loss and percentage weight increase) were evaluated at this standard cooking time.

Measurement of solid loss (or cooking loss) is one of the important parameters in assessing its overall quality. According to recent studies, during pasta cooking, soluble parts of starch and other soluble components including non-starch polysaccharides leach

into the water, and as a result, the cooking water becomes cloudy and thick (Tan, Li & Ta, 2008; Fu, 2008, Aravind et al., 2012). The results indicated that solid loss of the fettuccini pasta significantly went up only when the incorporation of GMP increased from 2.5 to 5.0%. No differences ($p > 0.05$) were found between control and FP2.5, as well as, between FP5 and FP7.5. Ajila et al. (2010) found similar results for dried macaroni with mango peel powder addition, though with higher values of solid loss for 5.0% and 7.5% (8.24±0.08 and 8.71±0.36, respectively) of mango peel powder incorporation.

Granito et al. (2003) reported the influence of temperature on the cooking loss, indicating that the use of higher temperatures (drying stage) in pasta manufacture produce lower cooking losses. In the present work, fresh pasta was produced without a drying step, and even so, the solid losses were lower than the results reported by Ajila et al. (2010) for semolina dried macaroni (85°C/3 hours), at all levels of mango peel powder addition (2.5%, 5.0% and 7.5%). This way, pasta can be manufactured without loss of quality, in spite of the fact that it has been made without semolina and without undergoing a drying stage.

In the particular case of this work, as reported by Aravind et al. (2012), the cooking loss could be attributed to changes in the gluten protein network because of the interference of GMP, which is rich in dietary fiber content. Indeed, several reports have shown that the addition of non-gluten flours in the production of pasta dilute the gluten strength, and interrupt and weaken the overall structure of pasta (Rayas-Duarte et al., 1996) which can result in a negative change (Gallegos-Infante et al., 2010; Sabanis et al., 2006). According to Hoseney (1999), for a good-quality pasta, cooking loss should be lower than 12%, which is reached by the pastas made with grape marc power, for all levels of addition.

At 4-min cooking time the percentage of weight increase was statistically equal for all fettuccini pasta samples, which shows that the grape marc power incorporation did not affect the product quality.

12.1.3.2 Polyphenols

TPC, CTC and the MA are related to the benefits that the consumption of the fruit may bring the human health due to their antioxidant capability. Additionally, the combined effect of dietary fibers and antioxidant compounds has shown to be more effective than

each of their effects separately (Pérez-Jiménez et al., 2008; Saura-Calixto, 2011). Grape juice marc is a phenolic-rich by-product of the food industry and presents high concentration of TPC and CTC strongly bounded to the plant matrix, indicating to be an interesting alternative as functional ingredient (Sant'Anna et al., 2012a). Table 11.2 shows the TPC, CTC, MA and the antioxidant capability extracted from the raw and cooked pasta.

Table 11.2 Total phenolic content (TPC), monomeric anthocyanin (MA), Condensed tannin content (CTC) and ABTS scavenging activities of fettuccini pasta added of grape marc powder.

Samples	TPC (mg GAE $100g^{-1}$)	CTC (mg ECE $100g^{-1}$)	MA (mg C3G $100g^{-1}$)	ABTS (μM TEC $100g^{-1}$)
RFP0	69.52 ± 6.01^{aA}	52.05 ± 3.91^{aA}	n.d.	100.05 ± 9.20^{aA}
CFP0	61.20 ± 5.15^{aA}	58.88 ± 5.07^{aA}	n.d.	92.24 ± 3.91^{aA}
RFP2.5	104.09 ± 7.39^{aB}	139.42 ± 21.55^{aB}	6.70 ± 0.45^{aA}	336.34 ± 6.98^{aB}
CFP2.5	95.45 ± 3.22^{aB}	145.07 ± 18.19^{aB}	5.25 ± 0.50^{bA}	305.57 ± 10.07^{bB}
RFP5	215.70 ± 15.75^{aC}	253.32 ± 3.54^{aC}	13.25 ± 0.80^{aB}	557.85 ± 20.91^{aC}
CFP5	194.55 ± 10.35^{aC}	242.39 ± 5.70^{aC}	10.05 ± 0.91^{bB}	453.50 ± 45.44^{bC}
RFP7.5	295.45 ± 14.00^{aD}	380.45 ± 9.04^{aD}	20.22 ± 1.60^{aA}	775.60 ± 54.90^{aD}
CFP7.5	299.06 ± 9.07^{aD}	365.41 ± 10.03^{aD}	17.94 ± 0.89^{aA}	655.99 ± 45.44^{bD}

n.d. not detected

^{a,b} Different superscripts indicate statistical differences ($p < 0.05$) between raw and cooked pasta.

^{A,B,C,D} Different superscripts indicate statistical differences ($p < 0.05$) due to the addition of grape marc powder.

Control raw fettuccini pasta presented 69.52 mg GAE $100g^{-1}$ of TPC, 52.05 mg ECE g^{-1} of CTC, antioxidant activity of 100.05 mM TEC $100g^{-1}$. MA was not detected in the pasta produced only with wheat flour. FP2.5, the pasta in which there was the incorporation of 2.5% of GMP, enhanced significantly ($p < 0.05$) an concentration of TPC to 104.09 mg GAE $100g^{-1}$, CTC to 139.42 mg ECE $100g^{-1}$, MA to 6.70 mg C3G $100g^{-1}$ and antioxidant activity to 336.34 mM TEC $100g^{-1}$. As expected, higher incorporation of the dried by-product to the pasta blend enhanced significantly ($p < 0.05$) the concentration of polyphenols and the antioxidant capability in the fettuccini pasta (Table 2). Similar results were found by Ovando-Martinez and co-workers (2009), when banana flour was added to spaghetti pasta and by Mildner-Szkudlarz and co-workers (2011) adding grape marc powder in bread formulations.

Additionally to the incorporation of the grape byproduct to the pasta formulation, it is essential to evaluate the availability of the polyphenol in the ready-to-eat product. The cooking procedure affected significantly ($p < 0.05$) the MA content and the antioxidant activity of the fettuccini pasta evaluated. RFP2.5 presented 6.70 mg C3G $100g^{-1}$ and antioxidant activity to 336.34 mM TEC $100g^{-1}$; after the cooking procedure, the samples decreased their content of both parameters to 5.25 mg C3G $100g^{-1}$ and antioxidant activity to 305.57 mM TEC $100g^{-1}$. Anthocyanins, largely presented in grape products, are soluble and heat-sensitive pigments with high antioxidant activity presented outside the cellular vacuoles of vegetable cells (Torres et al., 2010). Thus, the lower content of MA in the cooked pasta may be due to intense leaching during the cooking procedure and/or due to thermal degradation. Results suggest that capability of the pasta of scavenging the ABTS free radicals are related to the presence of anthocyanins in the final product.

The concentration of TPC and CTC in the raw and cooked pasta was not affected ($p > 0.05$) by the cooking procedure. Most glycosides of phenolics are stored inside the organelles of vegetable cells (Chism and Haard, 1996; Sakihama et al., 2002; Torres et al., 2010;), which may present a protective effect of these compounds. Moreover, Sant'Anna and co-workers (2012a) observed that CTC from grape marc are phenolics are highly bounded to the plant matrix, which suggests that these compounds are not suitable to the leaching phenomenon. The results indicate that the presence of polyphenols was little affected by the processing conditions, and incorporation of grape juice residue in fettuccini pasta may be a viable way to allocate this phenolic-rich ingredient.

12.1.3.3 Colour analysis

The results of the CIELAB analysis of the fettuccini pasta are presented in Figure 11.1. *L**-values decreased with the increase of incorporation of GMP in the pasta blend, indicating that the samples turned darker. This is because the formulation was changed by incorporating a blackish ingredient instead of a white ingredient, which is wheat flour. The increase of the *a**-values were accompanied by the increased of the incorporation of the GMP.

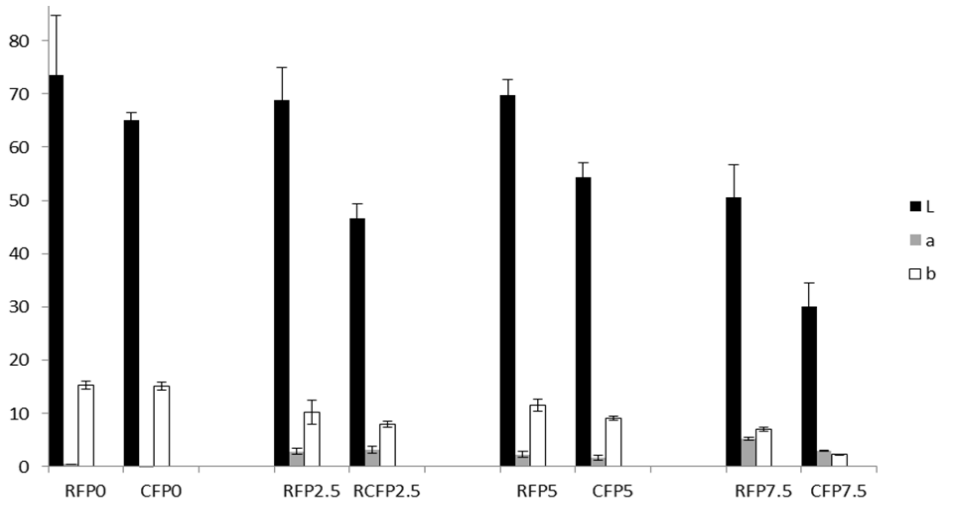

Figure 12.1 Colour analysis of the fettuccini pasta by CIELAB method. RFP0: raw control samples, without addition of grape marc powder; CFP0: cooked control samples, without addition of grape marc powder; RFP2.5: raw fettuccini pasta with 2.5% of grape marc powder incorporation; CFP2.5: cooked fettuccini pasta with 2.5% of grape marc powder incorporation; RFP5: raw fettuccini pasta with 5% of grape marc powder incorporation; CFP5: cooked fettuccini pasta with 5% of grape marc powder incorporation; RFP7.5: raw fettuccini pasta with 7.5% of grape marc powder incorporation; CFP7.5: cooked fettuccini pasta with 7.5% of grape marc powder incorporation.

This was expected, since the *a** parameter is related to the redness of the sample analyzed and the grape residue added to the pasta blend is an anthocyanin-rich product. Positive *b**-values are related to the yellowness of the samples, thus it did not present any pattern in the fettuccini preparation. The cooking procedure implied in high reduction of the *L** and *a** parameter, possibly due to the leaching process of the anthocyanins. The difference of color (ΔE) calculated by the Equation 1, showed control pasta to present slight color difference with ΔE of 8.52. Incorporation of the grape marc powder implied on high loss of changes of color of the pasta after the cooking procedure. ΔE for incorporation of FP2.5, FP5 and FP7.5 were 22.26, 15.67 and 21.23, respectively, due to leaching and thermal degradation of pigments in the fettuccini pasta.

12.3.4 Sensorial Analysis

Additionally to maintaining bioactivity of polyphenols after product processing, the quality of the product is even more critical. Figure 12.2 shows the results of the sensorial analysis of the fettuccini pasta developed with the substitution of wheat flour by GMP, where higher scores are related to better acceptance of the attributed evaluated.

Sensory evaluation of fettuccini pasta samples showed, in general, that as the level of grape marc powder increased, the product appearance, aroma, colour, texture, flavor, aftertaste and overall acceptance decreased: the substitution of wheat flour resulted in lower level of liking. Analysis of variance (ANOVA), showed that the appearance of the FP2.5, product with the lowest addition of grape marc powder, did not differ significantly ($p > 0.05$) from the control samples, while this attribute in FP5 and FP7.5 had significant change ($p < 0.05$). Since there was the incorporation of a different ingredient, panel members possibly identified an alteration of the aspect of the traditional pasta, leading to a slight rejection of the pasta in comparison to the pasta made only with wheat flour. In the same way, product colour was significantly affected ($p < 0.05$) due the substitution of the wheat flour. However, the fettuccini pasta added of grape marc powder did not differ significantly ($p > 0.05$) among them.

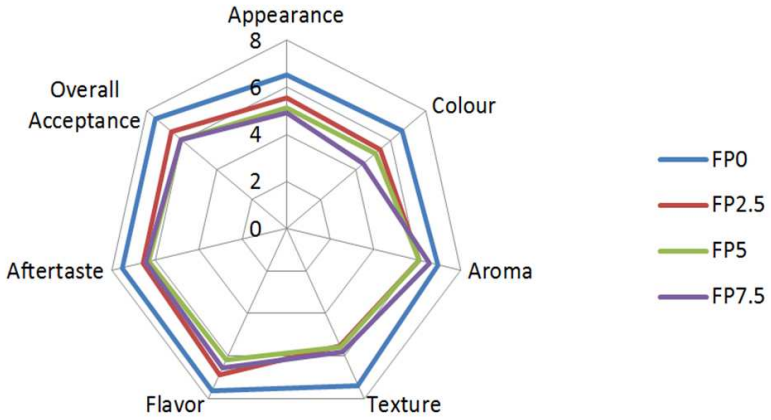

Figure 12.2 Sensorial analysis of four different fettuccini pasta: FP0 (control samples, without addition of grape marc powder), FP2.5 (substitution of 2.5% of wheat flour by grape mar powder), FP5 (substitution of 5% of wheat flour by grape mar powder), FP7.5 (substitution of 7.5% of wheat

flour by grape mar powder).

Pasta is a complex matrix and the mechanisms of interaction of incorporation of plant residues in the formulation still remain unknown. In relation to texture, fettuccini pasta added of the dried grape residue had significantly ($p < 0.05$) lower acceptance in relation to the control sample, but did not differ significantly ($p > 0.05$) among them, according to the ANOVA. Addition of fiber-rich products may increase the water absorption of the blend, due to the interaction between water and hydroxyl groups of polysaccharides through hydrogen bonding, resulting in higher hardness of the final product (Mildner-Szkudlarz et al., 2011). This fact may possibly have induced lower acceptance of the fettuccini pasta added of dried grape marc.

FP2.5 samples had similar ($p > 0.05$) acceptance of flavor and overall acceptance to the control sample, although it presented significant ($p < 0.05$) rejection in relation to aftertaste. Higher substitution resulted in lower acceptability ($p < 0.05$) in relation to flavor, aftertaste and overall acceptance. Fettuccini pasta added of GMP did not differ significantly ($p > 0.05$) among them in these attributes. This fact is possibly related to the fact that grape juice residue presents high concentration of catechins and tannins (Torres et al., 2010; Sant'Anna et al., 2012), which are responsible for astringent flavors (Scharbert and Hofmann, 2005). Then, the increase of the incorporation of grape marc powder may increase the undesirable sensorial attribute, implying in high rejection of the product.

Mildner-Szkudlarz and co-workers (2011) verified that it is possible to mix rye bread with up to 6% of grape marc with satisfactory sensorial performance. Pollard at 10% substitution had minimal impact on quality with higher antioxidant activity and fiber. Above 30%, pasta has undesirable colour, sensory properties and higher starch digestion (Aravind et al., 2012). Results suggest that FP2.5 presented the best global acceptance among the samples developed, corroborating to the idea of using plant residues as an alternative to produce foods.

12.1.4 Conclusion

The present work showed that the incorporation of grape marc powder in fettuccini pasta composition is an interesting alternative. Incorporation of 2.5% of GMP did not

interfere in the pasta cooking quality, and increased the polyphenolic concentration and the antioxidant activity in the product. Sensorial analysis suggests that this formulation had similar acceptance and colour changes, according to the CIELAB method, in comparison with the traditional fettuccini pasta. It is important to understand the bioavailability of these polyphenols in a product matrix, in order to provide real values for our health and wellbeing. Therefore, more studies are essential to allow the proper utilization of GMP as a functional ingredient.

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CAPÍTULO 13

Considerações Finais

O presente trabalho apresentou estudos de utilização de osmose direta em sistema ideal e para a concentração de suco de uva. Também foram realizados estudos para o aproveitamento do bagaço resultante do processo de fabricação do suco de uva.

Estudos em sistema ideal, com água pura como alimentação, mostraram que os aumentos da diferença de pressão osmótica, da vazão de alimentação e da temperatura do sistema acarretaram em aumento do fluxo de água através da membrana. Os resultados mostraram também que o fenômeno de polarização por concentração interna à membrana é um limitate tecnológico da técnica, reduzindo significativamente o desempenho do fluxo de permeado. Os experimentos com suco de uva mostraram que o aumento da diferença de pressão osmótica entre a alimentação e o agente osmótico e a temperatura acarretaram no incremento dos fluxos de água e sal através da membrana. O aumento da vazão de alimentação das soluções mostrou que houve aumento do fluxo de água e a diminuição do fluxo de sal durante a concentração do suco de uva. Os resultados estão de acordo com o comportamento descrito na literatura, uma vez que esses parâmetros implicam em aumento da força motriz na interface da membrana, sendo os fenômenos de polarização por concentração fatores determinantes no desempenho do processo. Ainda, os trabalhos mostraram que o suco concentrado por osmose direta não perde suas propriedades antioxidantes. Contudo, foi observado o transporte de sódio da solução osmótica para o suco, mas em concentrações pequenas, não afetando propriedades da bebida.

Os resultados de secagem do bagaço de suco de uva mostram que maiores temperaturas e velocidades de ar de secagem implicam em um processo de desidratação

mais rápido. A retenção de compostos bioativos se mostrou maior em menores temperaturas. Em relação à velocidade do ar de secagem, a retenção da concentração dos compostos fenólicos totais foi maior quando foram utilizadas maiores fluxos de ar. Os flavonóis e flan-3-óis foram sensíveis ao aumento da velocidade de ar de secagem devido a degradações oxidativas. Secagem utilizando temperaturas e velocidades do ar mais elevadas levaram a bagaço de uva secos com maiores concentrações de compostos com atividade antioxidante. Isso possivelmente se deve à formação de compostos durante o processamento que apresentam tal atividade.

Os resultados sobre extração de compostos fenólicos do bagaço de suco de uva mostraram que grande parte de compostos fenólicos, principalmente taninos condensados, estão fortemente ligados na matriz do resíduo e não são facilmente extraíveis com solventes orgânicos. Além disso, esses compostos ainda apresentam grande capacidade antioxidante, principalmente poder quelante de ferro (atividade relacionada à prevenção de doenças ligadas ao estresse oxidativo) o que mostra o grande potencial do bagaço de uva ser utilizado em aplicações alimentares, tanto na forma de extratos fenólicos, quanto na forma de farinha. O estudo cinético de extração de compostos fenólicos e de antocianinas de bagaço de uva é importante para entender os fenômenos envolvidos no seu processamento e melhor dimensionar os equipamentos em plantas industriais. Etanol em concentração de 50% em uma relação de 50 mL por grama de bagaço de uva resultou em maior extração dos compostos do resíduo da indústria produtora de suco. O modelo de pseudo-primeira ordem foi o modelo que melhor representou a extração em sistema de batelada na faixa de temperatura de 25-60ºC, com energia de ativação do processo de 23 kJ mol^{-1} para compostos fenólicos e de 29,5 kJ mol^{-1} para antocianinas monoméricas. Grande parte dos polifenóis está fortemente ligada ao bagaço e não é extraível com solvente, assim como grande parte dos bioativos com atividade antioxidante, poder redutor e atividade quelante está ligada.

No presente trabalho também foram realizados estudos de estabilidade da farinha de bagaço de uva. Os resultados mostraram que os compostos fenólicos e compostos com atividade sequestrante de radiais DPPH e de ferro se mostraram estáveis durante o armazenamento do produto a 25ºC por um período de 6 meses. Antocianinas e compostos

com a atividade de sequestrar radiais ABTS se mostraram sensíveis à degradação durante o armazenamento. Neste trabalho também foi verificado que a farinha de bagaço de uva se mostrou livre de *Salmonella* sp., *Bacillus cereus* e coliformes fecais, havendo a necessidade de cuidados com o crescimento de bolores e leveduras durante a armazenagem. Os dados mostrados nesse artigo ainda mostram que a curva de isoterma de sorção do bagaço de uva a 25ºC tem um comportamento sigmoidal, sendo o modelo de GAB o que melhor se adequou aos dados experimentais.

Finalmente, foram elaboradas massas tipo fettuccini, substituindo farinha de trigo por farinha de bagaço de uva. Os resultados mostraram que a adição do subproduto do suco de uva não interferiou na absorção de água e na perda de sólidos da massa durante o seu cozimento. Houve grande incremento de compostos fenólicos, antocianinas e de compostos com atividade antioxidante nos produtos adicionados de farinha de bagaço de uva, sendo a massa tipo fettuccini com melhor aceitação aquela em que se adicionou 2,5% do subproduto na sua preparação.

Os resultados desse trabalho mostram que a osmose direta é um processo de separação por membranas que tem grande potencial de ser utilizado como pré-concentração de suco de uva, não interferindo nas propriedades nutricionais do produto depois de ser processado pela técnica. Os estudos com o subproduto do suco de uva indicaram que a secagem por ar quente com passagem forçada de ar é uma alternativa para obter um produto microbilogica e bioquimicamente estável durante o seu armazenamento em condições usuais. Assim, farinha de bagaço de uva se motra como um componente funcional com potencial para ser utilizado tanto na indústria de alimentos quanto na agricultura familiar.

Trabalhos futuros

Os resultados do presente trabalho indicam que novos estudos devem ser realizados nas áreas abordadas a fim de promover a concentração de suco de uva por OD e utilização de farinha de bagaço de uva a nível industrial. Entre os possíveis tópicos a serem pesquisados futuramente, citam-se:

- síntese de membranas que permitam maior fluxo de água transmembrana durante a concentração de alimentos líquidos e maior retenção de sódio ao longo do processamento;

- comparação do efeito da concentração do suco por utilização de calor e por OD quanto alterações na composição de compostos fenólicos, atividade antioxidante, aroma, cor e outras características sensoriais e nutricionais importantes;
- modelar e simular os fenômenos de transporte de água transmembrana durante a concentração de alimentos líquidos por OD;
- extrair compostos fenólicos do bagaço de uva, analisando sua concentração em cromatografia líquida de alta performance;
- estudar novos parâmetros de secagem, visando a desidratação do bagaço de uva com maior eficiência;
- desenvolver produtos com farinha de bagaço de uva em nível industrial.

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