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RENATO GIOVANETTI VIEIRA

ESTABELECIMENTO DE UM PERFIL QUÍMICO PARA AMOSTRAS DE MAÇÃ A PARTIR DO SUCO

PONTA GROSSA
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Dissertação apresentada à banca examinadora como um dos requisitos à obtenção do título de Mestre em Ciência e Tecnologia de Alimentos, pelo programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Estadual de Ponta Grossa.

Orientador: Prof. Dr. Gilvan Wosiacki

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RENATO GIOVANETTI VIEIRA

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Dissertação aprovada como requisito parcial para obtenção do grau de Mestre no Curso de Pós-Graduação em Ciência e Tecnologia de Alimentos, da Universidade Estadual de Ponta Grossa, pela seguinte banca examinadora:

Orientador Prof. Dr. Gilvan Wosiacki - UEPG

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Prof. Dr.Ivo Mottin Demiate - UEPG

Ponta Grossa, 29 de julho de 2011.

Dedico este trabalho a toda minha família e amigos que me apoiaram e incentivaram durante todo o mestrado.

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Aos amigos e colaboradores do Grupo de Trabalho sobre Maçã.

RESUMO

O mercado nacional de maçã é representado por uma produção bivarietal, Fuji e Gala, correspondendo por mais de 90% do total, consequência das pesquisas para produção de frutas de alta qualidade, com baixo custo e propícias para o clima brasileiro. A avaliação quimiométrica das análises realizadas em amostras de suco durante 20 anos dessas variedades possibilitou identificar o perfil químico padrão das maçãs Gala e Fuji. Utilizou-se outras variedades comerciais brasileiras para validar este padrão, tendo limites de 0,15 a 0,35 g/100 mL (ácido málico), de 9,00 a 15,00 g/100 mL (açúcar redutor total) e de 100 a 600 mg/L (compostos fenólicos), que representam os sabores ácido, doce e amargo. Porém estes limites são insatisfatórios para a indústria de sucos e fermentados, visto que as frutas são maçãs de mesa, e aquelas não categorizadas para esta condição são então denominadas industriais. Os atuais pomares no Brasil não possuem especificamente estas variedades, mas algumas maçãs disponíveis no mercado são similares, sendo testadas para comprovar isso. Dentre as 24 variedades testadas, somente cinco apresentaram perfil semelhante à proposta industrial com teores de acidez e compostos fenólicos mais elevados, sendo as demais enquadradas como comerciais brasileiras. Porém alguns centros de pesquisas estão avaliando a produção de seleções descendentes de maçãs silvestres com maior teor de acidez e compostos fenólicos comparadas às maçãs Gala e Fuji. Com 24 amostras dessas seleções descendentes, geradas a partir de um experimento com porta enxerto da variedade comercial Baronesa e três variedades silvestres, foram comparadas com os limites do perfil das maçãs Gala e Fuji. Com a finalidade de evidenciar os resultados e explorá-los foram feitas análises de Componentes Principais (PCA) e de Agrupamentos Hierárquicos (HCA) juntamente com Gala e Fuji. Para validar estes métodos exploratórios, utilizando SIMCA (*Soft Independent Modeling of Class Analogy*) e KNN (*K-Nearest Neighbor*), tentou predizer quais eram as classes dessas novas seleções que poderiam enquadrar-se em somente Gala, somente Fuji, Gala ou Fuji ou nem Gala nem Fuji. Com os resultados obtidos, foi feita a comparação com gráficos elaborados a partir de resultados de ácido málico *versus* compostos fenólicos e da razão (ácido málico/açúcar redutor total) *versus* compostos fenólicos, encontrados na literatura. Dentre as 24 seleções, 21 apresentaram perfil industrial. As amostras industriais apresentaram níveis de compostos fenólicos acima de 600 mg/L e ácido málico acima de 0,5 g/100 mL e 50% com teores de açúcar redutor total acima de 14 g/100 mL. As restantes assemelham-se ao padrão comercial, porém com tamanhos e formatos inadequados para maçãs de mesa. Com o melhoramento e criação de novas variedades, específicas para a indústria, a produção de produtos nobres da maçã pode agregar mais valor com rendimento maior. Por meio de análises estatísticas e quimiométricas de dados das análises de amostras de suco despectinizado, é possível determinar se a matéria-prima recebida pela indústria de processamento deve ser destinada para processamento ou para consumo *in natura*, dependendo da área de atuação da indústria.

Palavras chave: Maçã, quimiometria, reconhecimento de padrões, industrial.

ABSTRACT

The domestic market is represented by an apple production bivarietal, Fuji and Gala, accounting for more than 90% of the total result of the research to produce high quality fruit with low cost and favorable climate in Brazil. Evaluation of chemometric analysis performed on samples of juice during 20 years of these varieties also identified chemical profile pattern of Gala and Fuji. Other Brazilian commercial varieties were used to validate this pattern, with limits from 0.15 to 0.35 g/100 mL (malic acid), 9.00 to 15.00 g/100 mL (total reducing sugar) and 100 to 600 mg / L (phenolic compounds), which represent the tastes sour, sweet and bitter. But these limits are unsatisfactory to the fermented juice industry and, since the fruits are apples, table and those not categorized for this condition is then called industrial. Existing orchards in Brazil specifically have these varieties, but some apples on the market are similar, being tested to prove it. Among the 24 varieties tested, only five presented a profile similar to that proposed industrial levels of acidity and higher phenolic compounds, the remainder being classified as Brazilian trade. However, some research centers are evaluating the production of selections descendants of wild apples with higher acidity and phenolic compounds compared to the Gala and Fuji apples. With 24 selections descendants of these samples, generated from an experiment with the commercial variety rootstock Baroness and three wild varieties were compared with the limits of the profile of Gala and Fuji apples. In order to highlight the results and exploit them were made of principal components analysis (PCA) and hierarchical clustering (HCA) with Gala and Fuji. To validate these exploratory methods using SIMCA (Soft Independent Modeling of Class Analogy) and KNN (K-Nearest Neighbor) tried to predict which of these new classes were the selections that could fit into only Gala, only Fuji, Gala or Fuji or or Gala or Fuji. With the results obtained, was compared with the results of malic acid *versus* phenolic compounds versus the ratio (malic acid / total reducing sugar) *versus* phenolic compounds found in the literature. Among the 24 selections, 21 showed industrial profile. The industrial samples showed levels of phenolic compounds above 600 mg / L, malic acid and above 0.5 g/100 mL and 50% with total reducing sugar content above 14 g/100 mL. The remaining resembles the commercial standard, but with sizes and shapes unsuitable for table apples. With the improvement and creation of new varieties, specific to the industry, the production of noble apple products can add more value with higher returns. Through statistical analysis and chemometric data analysis of despectinized juice sample, it is possible to determine whether the raw material received by the processing industry should be destined for processing or fresh consumption, depending on the field of industry.

Keywords: Apple, chemometric, pattern recognition, industrial

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LISTA DE ABREVIATURAS

AVG	Average
CIELAB	Sistema de Cores da Comissão Internacional de Iluminação
CP	Componentes Principais
CV	Coefficient of Variation
EPAGRI	Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina
FORTRAN	Linguagem de Programação
FRU	Frutose
GLC	Glucose
GTM	Grupo de Trabalho sobre Maçã
HCA	Hierarchical Cluster Analysis
IAPAR	Instituto Agronômico do Paraná
KNN	K Nearest Neighbors
MAL	Malic Acid
PCA	Principal Component Analysis
SD	Standard Deviation
SIMCA	Soft Independent Modeling of Class Analogy
SUC	Sucrose
TPC	Total Phenolics Compounds
TRS	Total Reducing Sugar
TSS	Total Soluble Solids
TTA	Total Titratable Acidity
UEPG	Ponta Grossa State University

SUMÁRIO

1 CAPÍTULO I

1.1 INTRODUÇÃO

O cultivo da maçã no Brasil iniciou na década de 70, na região Sul onde o clima temperado é propicio para o plantio, com menos de 1.000 ha e, em 2010, conta com uma área plantada maior que 35.000 ha. Esta expansão da cultura teve grande incentivo dos centros de pesquisas que melhoraram e selecionaram as cultivares para aumento da produção. O atual cenário da pomicultura no Brasil encontra-se baseado em duas cultivares, Gala e Fuji, que representam mais de 90% da produção total.

Há também outras variedades comerciais, menos conhecidas, desenvolvidas pelos centros de pesquisas brasileiros (EPAGRI, IAPAR) para o mercado nacional, com características interessantes para o setor agroindustrial, como resistência a doenças, menor necessidade de frio e frutos com sabor adequado para o paladar brasileiro. Essas novas variedades apresentam, em sua maioria, características semelhantes à Gala e/ou Fuji, maçãs padrão para o mercado nacional *in natura*.

Porém, não existe somente o mercado *in natura* para abastecer. Há também o setor que utiliza maçãs como matéria-prima para outros produtos como sucos, fermentados, destilados, geléias e compotas, frutas desidratadas e vinagre. No beneficiamento da maçã, são chamadas de maçãs industriais aquelas fora dos padrões de qualidade, que representam até 30% da produção total, matéria-prima para a indústria de processamento (PAGANINI et al., 2004; WOSIACKI et al., 2002).

Após um período de pesquisas para atender o comércio *in natura*, a atenção foi direcionada para variedades similares às europeias industriais, com teor de compostos fenólicos e acidez mais elevados que a maçã brasileira. Estas características são essenciais para a produção de sucos e fermentados de maçã, produtos nobres e de boa qualidade. Cada vez mais os consumidores buscam produtos diferenciados e qualitativamente superiores, o que obriga inovações e melhorias pelo mercado e indústria.

No Brasil, não existe um perfil de maçã industrial definido, pois a produção concentra-se em Gala e Fuji. Com dados obtidos durante 28 anos de pesquisa do Grupo de Trabalho sobre Maçã, juntamente com a EPAGRI, que fornece material para análise, foi realizado um estudo para definir o perfil da maçã brasileira comercial, com base nos resultados das análises de suco despectinizado das maçãs Gala e Fuji [Capítulo II]. A partir desse perfil determinado, foi feito um estudo quimiométrico de 24 variedades comerciais com dados do GTM, para classificar se apresentavam perfil comercial brasileiro (Gala e Fuji) ou industrial, segundo a literatura [Capítulo III], posteriormente ampliado com seleções de maçãs avançadas industriais e silvestres, desenvolvidas pela EPAGRI [Capítulo IV].

Estes trabalhos visam estabelecer um perfil químico, a partir do suco despectinizado de maçã, para possibilitar a classificação das maçãs destinando-as para o consumo in natura ou processamento na indústria.

1.2 OBJETIVOS

1.2.1 OBJETIVO GERAL

Estabelecer um perfil químico para classificar a maçã brasileira, a partir de dados de sucos despectinizados, como comercial ou industrial.

1.2.2 OBJETIVOS ESPECÍFICOS

Fundamentar a classificação de maçãs comerciais brasileiras;

Estudar o perfil de indicadores químicos com apelo sensorial do suco da maçã industrial;

Determinar o grau de similaridade das variedades comerciais Gala e Fuji por métodos quimiométricos;

Classificar as maçãs comerciais brasileiras, produtoras e polinizadoras, definindo seu potencial como matéria-prima;

Avaliar o potencial industrial e comercial de seleções avançadas descendentes de maçãs silvestres.

1.3 A MAÇÃ

1.3.1 A MAÇÃ COMO SÍMBOLO

Muito presente no cotidiano, a maçã é um ícone na modernidade, usada em muitos contos, lendas e histórias de muitos povos e culturas, na maioria das vezes como o gatilho de consequências ou no mundo real como um símbolo ou marca forte. Pode ser citada a lenda da mitologia grega, onde a deusa Éris, através do Pomo da Discórdia (uma maçã dourada, com a inscrição "à mais bela") provocou a Guerra de Tróia. Na Bíblia citada como o fruto da árvore do conhecimento, o fruto proibido, o passaporte de saída do Jardim do Éden, o qual Adão e Eva nunca poderiam comer. Na mitologia nórdica, a deusa Iduna, tinha um pomar de maçãs sagradas, com as quais ela alimentava os deuses, uma por dia, para que eles mantivessem a juventude e a força.

Big Apple, apelido de Nova Iorque, uma das mais influentes cidades do mundo, onde muitos dos negócios mundiais, cultura, moda e entretenimento começam e acontecem, foi criado devido a uma campanha da década de 70, quando exportava maçãs, acolhido pelos americanos

E também a empresa de tecnologia *Apple Inc.*, que se tornou a segunda maior empresa do mundo em valor de mercado em setembro de 2011, utiliza como logomarca uma maçã mordida.

1.3.2 MAÇÃ COMERCIAL E INDUSTRIAL

Até metade do século 20, no Brasil, não existiam variedades específicas para a indústria. Com a pesquisa, foram criadas e melhoradas algumas cultivares para aprimorar os produtos a partir da maçã. Dentre essas melhorias, podem ser citadas o tamanho, a forma, a resistência a doenças e a quantidade de compostos químicos, características interessantes para cada tipo de produto final (PAGANINI et al., 2004).

1.3.2.1 Produção Mundial

Segundo a FAO (2011), a atual produção anual de maçã no mundo é aproximadamente 70 milhões de toneladas. Os dois principais produtores respondem por aproximadamente 50% da produção total, China e Estados Unidos da América, com 43,5% e 6,3%, respectivamente.

A China, com um total de mais de 30 milhões de toneladas, evoluiu nos últimos cinco anos mais do que a produção total dos Estados Unidos, com média de 4,4 milhões de toneladas na última década. Essa diferença tende elevar, pois entre 1990 e 2005 a produção chinesa aumentou 450%, e nos últimos 10 anos 50%, enquanto que em outros países a produção caiu. Entretanto, mesmo com a desaceleração a tendência é de crescimento da produção mundial (FAO, 2011).

A Europa se destaca analisada enquanto União Europeia, representando 22,4% da produção mundial. Os países com maior produção são a Polônia, a Itália, a França e a Alemanha, os quais superam a marca de um milhão de toneladas cada, somando juntas cerca de oito milhões de toneladas (FAO, 2011).

1.3.2.2 Produção Nacional

A maçã no Brasil ainda é pouco consumida, visto que em países europeus o consumo *per capita* anual atinge 30 Kg, a exemplo da Bélgica, o consumo brasileiro não ultrapassa os 5 Kg. Comparado aos vizinhos Argentina e Chile, segundo a FAO (2011), ultrapassa o Chile (2,84 Kg/capita/ano), sendo quase quatro vezes menor em relação à Argentina (16,30 kg/capita/ano), valor relativamente pequeno para uma produção que deseja crescer. A produção de 2009 atingiu 1,2 milhão de toneladas, valores semelhantes com Chile e Argentina (FAO, 2011). Com consumo *per capita* pequeno e produção bivarietal (58:35), Gala e Fuji, a indústria trata a maçã de descarte como maçã industrial (ABPM, 2011a). Nos países europeus, existem pomares específicos de variedades industriais, ao passo que no

Brasil, oficialmente, existem apenas pomares experimentais de maçãs industriais (SANTOS et al., 2005).

Os dois grupos de destaque no Brasil são as variedades Gala e Fuji, sendo a primeira é originária da Nova Zelândia, do cruzamento das variedades Kidd's Orange Red e Golden Delicious. Foi trazida na década de 60, e devido ao clima temperado que garante quantidade suficiente de luz solar e amplitude térmica entre o dia e noite, a Gala brasileira é considerada a mais saborosa do mundo, mais colorida (listras vermelhas com fundo creme para amarelo claro), doce e aromática. A segunda foi desenvolvida no Japão, do cruzamento entre Ralls Janet e Red Delicious, e introduzida na década de 70, apresentando polpa doce, crocante e suco em abundância e casca com listras vermelhas sobre fundo verde claro a amarelo (ABPM, 2011b).

1.3.3 MÉTODOS DE CLASSIFICAÇÃO

Após a colheita, a maçã passa por qualificação para que cheguem à mesa do consumidor as melhores frutas. As maçãs são categorizadas e selecionadas com relação a quatro fatores: tamanho, cor, forma e presença de defeitos (LEEMANS; DESTAIN, 2004).

Segundo o Ministério da Agricultura, Pecuária e Abastecimento, a maçã só tem classificação comercial e as que não se enquadram são "industriais". A instrução normativa MAPA nº 50, de 03 de setembro de 2002, aprovou o Regulamento Técnico de Identidade e de Qualidade para a Classificação da Maçã (BRASIL, 2002), em classe ou calibre e em categorias, conforme peso (massa) médio dos frutos e a sua qualidade, respectivamente.

1.3.4 MAÇÃS COMERCIAIS

As maçãs comerciais são selecionadas por meio de sistemas manuais e automatizados. Em um primeiro momento passam por uma bancada de seleção manual, em apenas mulheres verificam se há algum defeito aparente, pois os sistemas automatizados são menos eficientes nessa detecção. Após esta etapa, as maçãs passam por um sistema automatizado, sendo classificadas por forma e tamanho e através de um fotocolorímetro, que analisa a cor da fruta, selecionada de acordo com a categoria obtida para aquela maçã de consumo *in natura* (LEEMANS; DESTAIN, 2004; PAGANINI et al., 2004).

Conforme o BRASIL (2002), o calibre ou classe da maçã é determinado de acordo com o peso médio dos frutos, conforme a Tabela 1-1, e as categorias, com os tamanhos e intensidade de defeitos da Tabela 1-2. Com relação às classes ou calibres, há no regulamento os seguintes itens: [1] Serão considerados da mesma classe ou calibre, os frutos que apresentarem 2 (duas) gramas, para mais ou para menos, em relação aos limites especificados na Tabela 1, deste Regulamento; [2] Para as Categorias 1, 2 e 3, cujas Maçãs encontram-se soltas nas caixas, serão admitidas a mistura de duas classes ou calibres contíguos; [3] Admite-se, na mesma caixa, o máximo de 10% (dez por cento) de Classes ou Calibres imediatamente superior e inferior e [4] Não se admite mistura de tamanhos a partir de duas classes imediatamente superior e/ou inferior.

Tabela 1-1 – Classes ou Calibres da Maçã, com base no número de frutos contidos numa caixa modelo Mark IV, com capacidade para conter 18 kg do produto.

Classes ou Calibres	Peso Limite Inferior (em gramas)	Peso Limite Superior (em gramas)
60	279	-
70	241	278
80	213	240
90	190	212
100	172	189
110	157	171
120	142	158
135	127	141
150	115	126
165	105	114
180	96	104
198	87	95
220	78	86
250	67	77
300	50	66

FONTE: (Brasil, 2002).

Tabela 1-2 – Natureza, causa, número e tamanho dos defeitos permitidos por Categorias.

Defeitos	Extra	Categoria 1	Categoria 2	Categoria 3
Cor = mínimo da área do fruto				
- para cultivares vermelhas	> 75	≥ 50 %	≥ 25 %	15 %
- para cultivares rajadas e mistas	> 60 %	≥ 40 %	≥ 20 %	10 %
Russeting = máximo da área, considerando a cavidade peduncular.	≤ 10 %	≤ 20 %	≤ 40 %	≤ 70 %
Bitter Pit, cortiça = área atingida	0	0	≤ 10 mm²	≤ 50 mm²
Lesão cicatrizada leve	≤ 10 mm²	≤ 30 mm²	≤ 2 cm²	≤ 10 cm²
Lesão cicatrizada grave	≤ 0 mm²	≤ 10 mm²	≤ 30 mm²	≤ 5 cm²
Dano de geada = área atingida	0	0	≤ 10% da área	≤ 30% da área
Mancha de sarna = área atingida total		≤ 5 mm²	≤ 20 mm²	≤ 150 mm²
Mancha de doenças (glomerela e Botryosphaeria)	0	≤ 3 mm²	≤ 10 mm²	≤ 50 mm²
Mancha de doenças (fuligem e sujeira de mosca), Mancha de fitotoxidez, Mancha de cochonilha e outras	0	≤ 3 mm²	≤ 10 mm²	≤ 50 mm²
Fuligem (% da área)	0	≤ 5 %	≤ 10 %	≤ 15 %
Danos mecânicos	≤ 0,5 cm²	≤ 1,0 cm²	≤ 2,0 cm²	≤ 5,0 cm²
Queimadura de sol (% da área)	0	≤ 10 %	≤ 20 %	+ de 20 %
Rachadura peduncular	0	≤ 1,0 cm	≤ 2,0 cm	≤ 3,0 cm
Lesão aberta (área ou comprimento)	0	≤ 5 mm² ou 0,5 cm	≤ 20 mm² ou 1,0 cm	≤ 70 mm² ou 2,0 cm

Fonte: (Brasil, 2002).

Com relação às categorias, há no regulamento os seguintes itens:

- Uma Maçã Extra poderá admitir somente um (1) defeito no fruto, de intensidade classificada como Extra.
- Uma Maçã de Categoria 1 poderá admitir até dois (2) defeitos por fruto, de intensidade classificada como Categoria 1.
- Uma Maçã de Categoria 2 poderá admitir até três (3) defeitos por fruto, de intensidade classificada como Categoria 2.
- Uma Maçã de Categoria 3 poderá admitir até quatro (4) defeitos por fruto, de intensidade classificada como Categoria 3.
- Uma Maçã que apresentar cinco (5) ou mais defeitos diferentes de intensidade, de Categoria 3, será considerada Industrial.

Seguindo este regulamento aqueles frutos que não foram categorizados não poderão ser comercializados *in natura*, tornando-se um fruto industrial.

1.3.5 MAÇÃS INDUSTRIAIS

Segundo BRASIL (2002), com relação à maçã, podem ser citados alguns itens do regulamento como: "4.3.1.5. Uma maçã que apresentar cinco (5) ou mais defeitos diferentes de intensidade, de Categoria 3, será considerada industrial"; "4.4.2.1. A Maçã classificada como Fora de Categoria poderá ser destinada à industrialização"; "13.2.1. Será considerada Fruta industrial a que apresentar os seguintes defeitos: Podridão, Congelamento, Desidratação, Degenerescência Interna Severa (independente da causa), Frutas Passadas (sobremaduras) e Escaldadura ". Esses itens indicam que, perante a legislação brasileira, não existe uma maçã originalmente industrial, que atenda as expectativas desse setor, sendo utilizado apenas o rejeito da classificação comercial.

Na indústria, com auxílio de modernos equipamentos, é possível obter uma elevada quantidade de informações para muitas frutas, analisadas em um breve período de tempo (BERRUETA; ALONSO-SALCES; HÉBERGER, 2007). Com os avanços tecnológicos, a classificação ficou mais rápida e eficiente. Através de métodos de classificação, é possível selecionar frutas com características adequadas para a indústria. Entretanto, o adequado para a indústria depende do produto final, que pode necessitar de frutas com acúmulo de açúcar, acidez elevada e compostos fenólicos, ou alto grau de maturação. Para selecionar estas amostras a combinação de métodos quimiométricos com resultados físico-químicos apontam as melhores formas de classificar os frutos para o seu uso específico na indústria (ALONSO-SALCES et al., 2005a; ALONSO-SALCES et al., 2005b; ALONSO-SALCES et al., 2006; BERRUETA; ALONSO-SALCES; HÉBERGER, 2007).

1.3.6 MÉTODOS QUIMIOMÉTRICOS COMO MÉTODO CLASSIFICATÓRIO

A quimiometria é uma ciência que aplica métodos estatísticos, análise multivariada, matemática aplicada e ciência da computação na mineração de dados para extração de informações relevantes e úteis de origem química. Estas técnicas tornaram-se mais comuns com a evolução da computação, que possibilitou um processamento maior de dados em menor tempo. Os primeiros usos da quimiometria foram com programadores FORTRAN que usavam mainframes para processar rotinas de bibliotecas de estatística para análises químicas. O termo quimiometria foi criado por Svante Wold na década de 70, que logo em seguida junto com Bruce Kowalski, criou a Sociedade Internacional de Quimiometria (ANDRE, 2003; BRERETON, 2007; XIE; YING; YING, 2007).

Dentro da quimiometria, podem ser citados alguns métodos utilizados para mineração dos dados, por meio de análises exploratórias, como PCA (Análise de Componentes Principais), HCA (Análise Hierárquica de Agrupamentos) e de classificação, como KNN (k-enésimo vizinho mais próximo), SIMCA (Modelagem de Classes por Componentes Principais) (FRÍAS et al., 2003; PANERO et al., 2009; SABIN; FERRÃO; FURTADO, 2004; XING; SAEYS; DE BAERDEMAEKER, 2007).

O uso da quimiometria na indústria tem uma vasta aplicação desde a discriminação geográfica de água mineral no Estado de São Paulo (SILVA et al., 2002) à identificação de fármacos anti-depressivos (BERRUETA; ALONSO-SALCES; HÉBERGER, 2007; SABIN; FERRÃO; FURTADO, 2004).

Na indústria alimentícia, mais especificamente na indústria da maçã, o uso da quimiometria pode ser observado na classificação de maçãs a partir do grau de maturação em função da composição de polifenóis (ALONSO-SALCES et al., 2005b), da detecção de defeitos na maçã por imagens (XING; SAEYS; DE BAERDEMAEKER, 2007) ou por espectrofotômetro VIS/NIR (XING et al., 2006; XING et al., 2005). Adicionalmente, também de seus produtos, como na caracterização da sidra em função do teor de proteína e características da espuma (BLANCO-GOMIS et al., 2007; 2009), classificação da maçã com relação a doçura e acidez para a produção da "Sidra de Asturias" (ABRODO et al., 2010) e classificação da Sidra Basque e Sidra Francesa por compostos fenólicos e parâmetros do CIELab (ALONSO-SALCES et al., 2005a)

1.3.7 ANÁLISE DE COMPONENTES PRINCIPAIS (PCA)

A análise de componentes principais, método quimiométrico, consiste na transformação dos dados originais em dados mais representativos, através de combinações lineares, dando origem as componentes principais (CP), as quais não são correlacionadas, que representam as variações mais importantes dos dados (BARROS NETO; SCARMINIO; BRUNS, 2006; BERRUETA; ALONSO-SALCES; HÉBERGER, 2007; PANERO et al., 2009; SABIN; FERRÃO; FURTADO, 2004).

Com esta técnica é possível analisar os dados através dos gráficos gerados, denominados de *scores* e *loadings*, que demonstram a representatividade de cada amostra (*scores*) e de cada variável (*loadings*) dentro das CPs, permitindo determinar a influência das amostras em relação às variáveis (BLANCO-GOMIS et al., 1998; BLANCO-GOMIS et al., 2009; PANERO et al., 2009; XIE; YING; YING, 2007).

Conforme Alonso-Salces et al. (2006), Barros Neto et al. (2006), Berrueta et al. (2007) esta análise reduz o número de variáveis e fornece uma visão focada dos dados evidenciando similaridades e diferenças entre as amostras.

1.3.8 ANÁLISE DE AGRUPAMENTOS HIERÁRQUICOS (HCA)

Segundo Barros Neto et al. (2006)

> A análise de agrupamentos hierárquica, por outro lado, é usada para analisar a estrutura do conjunto de dados em termos de grupos definidos de maneira hierárquica, de acordo com a similaridade entre objetos ou variáveis. Embora existam muitas variantes matemáticas, o que define a similaridade entre dois pontos é a distância entre eles no espaço multidimensional. Quanto menor essa distância,

maior a similaridade entre os objetos (ou variáveis) representados pelos dois pontos.

Panero et al. (2009) utilizaram HCA e PCA para discriminação geográfica de quiabo do Rio Grande do Norte e Pernambuco. Silva et al. (2002) avaliaram a similaridade de amostras de águas minerais do Estado de São Paulo com relação a procedência ou propriedades químicas.

1.3.9 *K-NEAREST NEIGHBOR* (KNN)

Este método utiliza a comparação entre as distâncias das amostras, calculando para cada par de amostras a distância Euclidiana, com base na proximidade às amostras do conjunto de treinamento. Para validação dos dados utiliza-se validação cruzada, onde cada amostra é retirada e testada com todas as outras, definindo sua classe. A classe é determinada a partir de quantas amostras da mesma classe estão mais perto da amostra selecionada. Aquela classe que tiver maior número de vizinhos a amostra, será a classe atribuída a amostra (ALONSO-SALCES et al., 2005b; BARROS NETO; SCARMINIO; BRUNS, 2006; SABIN; FERRÃO; FURTADO, 2004).

A utilização deste método quimiométrico pode ser vista na classificação das sidras francesas e bascas baseando-se no teor de compostos fenólicos totais e parâmetros de cor no sistema CIELAB (ALONSO-SALCES et al., 2005a) e também na determinação de adulteração de especiarias com corantes proibidos na indústria alimentícia (DI ANIBAL et al., 2009).

1.3.10 *SOFT INDEPENDENT MODELLING BY CLASS ANALOGY* (SIMCA)

Utilizado para discriminação de amostras em classes, definida através de uma análise de componentes principais para cada classe, classificando novas amostras entres as classes. Uma observação relevante neste método de classificação é que uma amostra, uma vez categorizada em uma classe, pode ser classificada em outras, pois pode haver sobreposição dos limites de cada classe (BARROS NETO; SCARMINIO; BRUNS, 2006; BLANCO-GOMIS et al., 2009; SABIN; FERRÃO; FURTADO, 2004).

SIMCA é aplicável tanto para identificar a origem das maçãs para produção da sidra basca em maçãs francesas ou galegas (ALONSO-SALCES et al., 2006) ou tomates, em transgênicos ou não transgênicos (XIE; YING; YING, 2009).

REFERÊNCIAS

ABPM. Associação Brasileira de Produtores de Maçã. **Brazilian Apple Production by variety**. Disponível em: <http://www.brazilianappleexporters.com>. Acesso em: 12 maio. 2011a.

ABPM. Maçã na escola. **Nossas principais variedades de maçã**. Disponível em: <http://www.macanaescola.org.br>. Acesso em: 15 junho. 2011b.

ABRODO, P. A.; LLORENTE, D. D.; CORUJEDO, S. J.; DE LA FUENTE, E. D.; ÁLVAREZ, M. D. G.; GOMIS, D. B. Characterisation of Asturian cider apples on the basis of their aromatic profile by high-speed gas chromatography and solid-phase microextraction. **Food Chemistry**, v. 121, n. 4, p. 1312-1318, 2010.

ALONSO-SALCES, R. M.; GUYOT, S.; HERRERO, C.; BERRUETA, L. A.; DRILLEAU, J.-F.; GALLO, B.; VICENTE, F. Chemometric classification of Basque and French ciders based on their total polyphenol contents and CIELab parameters. **Food Chemistry**, v. 91, n. 1, p. 91-98, 2005a.

ALONSO-SALCES, R. M.; HERRERO, C.; BARRANCO, A.; BERRUETA, L. A.; GALLO, B.; VICENTE, F. Classification of apple fruits according to their maturity state by the pattern recognition analysis of their polyphenolic compositions. **Food Chemistry**, v. 93, n. 1, p. 113-123, 2005b.

ALONSO-SALCES, R. M.; HERRERO, C.; BARRANCO, A.; LÓPEZ-MÁRQUEZ, D. M.; BERRUETA, L. A.; GALLO, B.; VICENTE, F. Polyphenolic compositions of Basque natural ciders: A chemometric study. **Food Chemistry**, v. 97, n. 3, p. 438-446, 2006.

ANDRE, M. Multivariate Analysis and Classification of the Chemical Quality of 7-Aminocephalosporanic Acid Using Near-Infrared Reflectance Spectroscopy. **Analytical Chemistry**, v. 75, n. 14, p. 3460-3467, 2003.

BARROS NETO, B. D.; SCARMINIO, I. S.; BRUNS, R. E. 25 anos de quimiometria no Brasil. **Química Nova**, v. 29, n., p. 1401-1406, 2006.

BERRUETA, L. A.; ALONSO-SALCES, R. M.; HÉBERGER, K. Supervised pattern recognition in food analysis. **Journal of Chromatography A**, v. 1158, n. 1-2, p. 196-214, 2007.

BLANCO-GOMIS, D.; FERNANDEZ-RUBIO, P.; GUTIÉRREZ-ALVAREZ, M. D.; FERNANDEZ-RUBIO, P.; MANGAS-ALONSO, J. J. Use of High performance liquid chromatographic-chemometric techniques to differentiate apple juices clarified by microfiltration and ultrafiltration. **Analyst**, v. 123, n., p. 125-129, 1998.

BLANCO-GOMIS, D.; MANGAS-ALONSO, J. J.; JUNCO-CORUJEDO, S.; GUTIÉRREZ-ÁLVAREZ, M. D. Cider Proteins and Foam Characteristics: A Contribution to Their Characterization. **Journal of Agricultural and Food Chemistry**, v. 55, n. 7, p. 2526-2531, 2007.

BLANCO-GOMIS, D.; MANGAS-ALONSO, J. J.; JUNCO-CORUJEDO, S.; GUTIÉRREZ-ÁLVAREZ, M. D. Characterisation of sparkling cider by the yeast type used in taking foam on the basis of polypeptide content and foam characteristics. **Food Chemistry**, v. 115, n. 1, p. 375-379, 2009.

BRASIL, Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa Nº 50**. Brasília, 2002.

BRERETON, R. G. **Applied Chemometrics for Scientists**. Chichester: John Wiley & Sons, Ltd, 2007.

DI ANIBAL, C. V.; ODENA, M.; RUISÁNCHEZ, I.; CALLAO, M. P. Determining the adulteration of spices with Sudan I-II-II-IV dyes by UV-visible spectroscopy and multivariate classification techniques. **Talanta**, v. 79, n. 3, p. 887-892, 2009.

FRÍAS, S.; CONDE, J. E.; RODRÍGUEZ-BENCOMO, J. J.; GARCÍA-MONTELONGO, F.; PÉREZ-TRUJILLO, J. P. Classification of commercial wines from the Canary Islands (Spain) by chemometric techniques using metallic contents. **Talanta**, v. 59, n. 2, p. 335-344, 2003.

LEEMANS, V.; DESTAIN, M. F. A real-time grading method of apples based on features extracted from defects. **Journal of Food Engineering**, v. 61, n. 1, p. 83-89, 2004.

FAO. Organização das Nações Unidas para a Agricultura e Alimentação. **Food and Agricultural commodities production**. Disponível em: <http://faostat.fao.org/site/567/default.aspx>. Acesso em: 05 jul. 2011.

PAGANINI, C.; NOGUEIRA, A.; DENARDI, F.; WOSIACKI, G. Análise da aptidão industrial de seis cultivares de maçãs, considerando suas avaliações físico-químicas (dados da safra 2001/2002). **Ciência e Agrotecnologia**, v. 28, n. 6, p. 1336-1343, 2004.

PANERO, F. S.; VIEIRA, M. D. F. P.; CRUZ, A. M. F. D.; MOURA, M. D. F. V. Application of the exploratory analysis of data in the geographical discrimination of okra of Rio Grande do Norte and Pernambuco. **Orbital: The Electronic Journal of Chemistry**, v. 34, n. 3, p. 33-40, 2009.

SABIN, J. G.; FERRÃO, M. F.; FURTADO, J. C. Análise multivariada aplicada na identificação de fármacos antidepressivos. Parte II: Análise por componentes principais (PCA) e o método de classificação SIMCA. **Revista Brasileira de Ciências Farmacêuticas**, v. 40, n., p. 387-396, 2004.

SANTOS, L. C. D.; PAGANINI, C. C.; NOGUEIRA, A.; WOSIACKI, G. Composição Química de Sucos Provenientes de Maçãs de Dez Diferentes Genótipos. Safra 2002/2003. **Brazilian Journal of Food Technology**, v. 8, n. 2, p. 5, 2005.

SILVA, F. V.; KAMOGAWA, M. Y.; FERREIRA, M. M. C.; NÓBREGA, J. A.; NOGUEIRA, A. R. A. Discriminação geográfica de águas minerais do Estado de São Paulo através da análise exploratória. **Eclética Química**, v. 27, n., p. 91-102, 2002.

WOSIACKI, G.; NOGUEIRA, A.; SILVA, N. C. C.; DENARDI, F.; CAMILO, A. P. Apple varieties growing in subtropical áreas. The situation in Santa Catarina - Brazil. **Fruit Processing**, v. 12, n. 1, p. 19-28, 2002.

XIE, L.; YING, Y.; YING, T. Combination and comparison of chemometrics methods for identification of transgenic tomatoes using visible and near-infrared diffuse transmittance technique. **Journal of Food Engineering**, v. 82, n. 3, p. 395-401, 2007.

XIE, L.; YING, Y.; YING, T. Classification of tomatoes with different genotypes by visible and short-wave near-infrared spectroscopy with least-squares support vector machines and other chemometrics. **Journal of Food Engineering**, v. 94, n. 1, p. 34-39, 2009.

XING, J.; BRAVO, C.; MOSHOU, D.; RAMON, H.; DE BAERDEMAEKER, J. Bruise detection on Golden Delicious apples by vis/NIR spectroscopy. **Computers and Electronics in Agriculture**, v. 52, n. 1-2, p. 11-20, 2006.

XING, J.; SAEYS, W.; DE BAERDEMAEKER, J. Combination of chemometric tools and image processing for bruise detection on apples. **Computers and Electronics in Agriculture**, v. 56, n. 1, p. 1-13, 2007.

XING, J.; VAN LINDEN, V.; VANZEEBROECK, M.; DE BAERDEMAEKER, J. Bruise detection on Jonagold apples by visible and near-infrared spectroscopy. **Food Control**, v. 16, n. 4, p. 357-361, 2005.

2 CAPÍTULO II: CHEMICAL PATTERN OF BRAZILIAN APPLES. A CHEMOMETRIC APPROACH BASED ON THE FUJI AND GALA VARIETIES

CHEMICAL PATTERN OF BRAZILIAN APPLES. A CHEMOMETRIC APPROACH BASED ON THE FUJI AND GALA VARIETIES

ABSTRACT

The chemical composition of apple juices may be used to discriminate varieties for consumption and for raw material. Fuji and Gala have a chemical pattern that can be used for this classification. Multivariate methods correlate independent continuous chemical descriptors with the categorical apple variety. Three main descriptors of apple juice were selected: malic acid content, total reducing sugar and total phenolic compounds. A chemometric approach, employing PCA and SIMCA, was used to classify apple juice samples. PCA was performed with 24 juices from Fuji and Gala, and SIMCA, with 15 juices. The exploratory and predictive models recognized 88% and 64%, respectively, as belonging to a mixed domain. The apple juice from commercial fruits shows a pattern related to cv. Fuji and Gala with limits from 0.18 to 0.39 g/100 mL (malic acid), from 8.65 to 15.18 g/100 mL (total reducing sugar) and from 100 to 400 mg/L (total phenolic compounds) Such boundaries were slightly shorter in the remaining set of commercial apple juices, specifically from 0.16 to 0.36 g/100 mL, from 9.25 to 15.5 g/100 mL and from 180 to 606 mg/L for acidity, reducing sugar and phenolic compounds, respectively, representing the acid, sweet and bitter tastes.

Keywords: Chemometry, chemical descriptor, pattern recognition

PADRÃO QUÍMICO DA MAÇÃ BRASILEIRA. UMA ABORDAGEM QUIMIOMÉTRICA BASEADA NAS VARIEDADES FUJI E GALA

RESUMO

A composição química de sucos de maçã pode ser usada para diferenciar as variedades de mesa das industriais. As maçãs Fuji e Gala apresentam características químicas que permitem essa classificação. Métodos multivariados relacionam descritores químicos com a categoria da variedade de maçã. Três principais descritores do suco de maçã foram selecionados: ácido málico, açúcar redutor total e compostos fenólicos. Uma abordagem quimiométrica, empregando PCA e SIMCA foi usada para classificar amostras de sucos de maçã. A PCA foi realizada com 24 amostras de suco de Fuji e Gala, e a SIMCA, com 15 amostras. Os modelos exploratórios e preditivos reconheceram 88% e 64%, respectivamente, como pertencentes a um domínio misto. Os sucos de maçã de frutas comerciais mostraram um padrão similar as cultivares Fuji e Gala com limites de 0,18 a 0,39 g/100 mL (ácido málico), de 8,65 a 15,18 g/100 mL (açúcar redutor total) e de 100 a 400 mg/L (compostos fenólicos), mas tais limites foram levemente menores no conjunto restante de sucos de maçãs comerciais, especificamente de 0,16 a 0,36 g/100 mL, 9,25 a 15,5 g/mL e 180 a 606 mg/L para acidez, açúcar redutor total e compostos fenólicos, respectivamente, representando os sabores ácido, doce e amargo.

Palavras chave: Quimiometria, descritores químicos, reconhecimento de padrões

2.1 INTRODUCTION

The production of apples actually began in the early 1970s with cultivars adapted to the highland locations in the Southern States of Brazil. The economic viability had been explained to personnel involved with investments in the apple business, focusing the technological intensification on the Fuji and Gala varieties, which represent 93% of the apple production (KREUZ, 2002). The remaining 7% of the apple production comprises many precocious varieties, most of them with a very low chilling requirement and harvesting time much earlier than cv. Gala, which currently represents good options to small fruit growers and attractive prices. In a world-wide perspective, Gala and Fuji have been ranked as the 3rd and 4th apples produced, in metric ton, just after Golden Delicious (1st) and Delicious (2nd) (WORLD APPLE REVIEW, 2008).

Fuji and Gala apples were brought to Brazil in the 1960s, respectively from Japan and New Zealand (WOSIACKI et al., 2002). Even in the colder regions of South Brazil, the chilling requirement for these apples is not satisfied in areas below an altitude of 1.300 m, where chemical treatments are needed to break bud dormancy. For cv. Fuji, even with good flowering, cropping and a non-biannual bearing, the fruit quality is better above an altitude of 1.300 m. In contrast, Gala has good performance even at lower elevations. According to Henschke (1994) more studies must be conducted to improve the quality of main varieties concerning appearance, juice yield, size, shape, storage capacity and to reach 25 ton/ha of high quality apples aiming to export them to other places, within Brazilian borders or overseas. Harvested commercial apples are driven through a complex selection and physical classification system where 70% are classified as table fruit and, 10% as waste (negative costs) and 20% is damaged but healthy fruit, which constitutes the actual Brazilian industrial raw material. The classification of apples for commercialization was made according to sensorial aspects such as acid, sweet and bitter tastes. The amounts of malic acid and total phenolic compounds have been used for the commercial classification of apples, usually as bitter sweet, but such praxis does not include the total reducing sugar content.

The use of multivariate methods to establish the relationship between the independent continuous variable and the categorical variable is becoming widespread (BARROS NETO et al., 2006; BLANCO-GOMIS et al., 1998).

Exploratory techniques such as Principal Component Analysis (PCA) may be used to select the main continuous descriptors of a categorical variable and, with the auto-scaled data, may provide an exploratory model. Classificatory techniques such as Soft Independent Modeling of Class Analogy (SIMCA) may be used to classify similar categorical variables (BARROS NETO et al., 2006). In 2005, Alonso-Salces et al. used the pattern recognition analysis of the apple phenolics compounds to classify them by their maturity state. In 1998, Blanco-Gomis et al. discriminated apple juices clarified by microfiltration or by ultra filtration by an exploratory HCA to select the best descriptor, using PCA to quantify the components, and LDA (Linear Discriminant Analysis) and KNN to classify the juices with 100% correctness. In 2000, González and Peña-Méndez were able to differentiate must and wine by chemometric analysis such as Discriminant Analysis (DA), PCA, Biplot (BA) and Factor Analysis (FA). Molfetta et al. (2005) studied the structure – activity relationship (SAR) of quinone compounds with biological activity by employing PCA, HCA, SIMCA and KNN.

A multivariate analyses approach may be useful to classify apple varieties according to a few independent chemical descriptors of apple juices. The main purpose of this article was to establish the chemical pattern of Brazilian commercial apples according to the selected chemical descriptors of the Fuji and Gala varieties by employing multivariate methods.

2.2 MATERIALS AND METHODS

2.2.1 MATERIALS

Samples of commercial and experimental varieties of apple have being collected during many seasons, since 1986 to the 2007, from many places in the Southern States of Brazil.

2.2.2 METHODS

In these juices was carried out different analyses and the results stored in the GTM database. From this database, the chemical descriptors of many samples from Fuji and Gala juices were recovered for an initial screening to select the main descriptors for juice classification. Then, the chemical descriptors of 12 samples from Gala and Fuji varieties, as well from 15 authentic apple juices obtained from commercial varieties, were recovered in order to test the capacity of the predicting model. Usually 5-10 kg of sample was processed to obtain depectinized juice. Stabilized apple juices were used for further analyses after a qualitative paper filtration, when necessary. Pectinolytic enzymes samples (Pectinex 3 XL - NOVO Nordisk do Brazil) used for apple juice clarification were given by LNF Ltd., from Bento Gonçalves RS. Chemical products used in the analyses or in the experiments were also from quality pro analysis.

Processing. Apple juice was obtained by mechanical pressure according to the protocol described by Wosiacki et al. (1989). This juice was depectinized in a hot step in a water bath (2 h, 45 ºC), filtered through paper, bottled, closed and stabilized by freezing.

Analysis. Total Soluble Solids (TSS) were determined in a refractometer adjusted to 20 ºC, expressed in ºBrix (IAL, 2008). The content of glucose (GLC) was determined with a glucose oxidase kit assay and the amount of fructose (FRU) was then calculated as the remainder from reducing sugar. The amount of sucrose (SUC) was determined as the difference from total reducing sugar and reducing sugar (NELSON, 1944; SOMOGYI, 1945). Total titrable acidity (TTA) was determined with 0.1 M NaOH and expressed as malic acid (MAL) in g/100 mL. Total phenolic compounds (TPC) were determined by the Folin-Ciocalteau reagent (TANNER; BRUNNER, 1985) and expressed as (+) catechin, in mg/L. The ratio was calculated by total reducing sugar / total titrable acidity as an adimensional figure. The Descriptive Statistic Analysis of the chemical descriptors was made by employing the Statistic® 7.0 software. The Principal Component (PCA) was employed in the exploratory analysis and the pattern recognition method Soft Independent Modeling of Class Analogy (SIMCA) was used to classify the apple varieties (categorical) according to their relationship with selected chemical descriptors (continuous variable). The auto scaled processing allowed the results to be compared to each other on the same scale (BARROS NETO et al., 2006). PCA and SIMCA were performed with Pirouette® software, version 4.1 (Infometrix).

2.3 RESULTS AND DISCUSSION

Table 2-1 shows the main chemical attributes of Fuji and Gala apple juice samples, recovered from the database maintained by the GTM (Apple Working Team) at Ponta Grossa State University. All the independent chemical attributes were tested aiming to select those more interesting to be used as a discriminative attribute, or chemical descriptor.

Table 2-1 – Main physical-chemical attributes of Gala and Fuji apple juices

Analysis	Fuji						Gala					
	N	**Min**	**Avg**	**Max**	**Std. Dev.**	**Var**	**N**	**Min**	**Avg**	**Max**	**Std. Dev.**	**Var**
Malic acid, g/100 mL	40	0.136	0.249	0.434	0.082	0.01	55	0.123	0.285	0.401	0.058	0.003
Reducing Sugar, g/100 mL	40	6.46	10.36	15.31	2.09	4.37	55	6.30	8.70	14.65	1.90	3.600
Total reducing sugar, g/100 mL	39	10.17	12.75	16.29	1.56	2.44	54	9.00	11.06	15.04	1.51	2.280
Ratio	24	24.82	67.10	113.92	28.62	819.22	33	25.52	40.52	56.54	8.60	73.89
Total phenolic compounds, mg/L	37	130	254	404	69	4.72	52	124	297	544	98	9.580
Fructose, g/100 mL	33	3.33	7.36	9.84	1.63	2.64	48	4.87	6.99	9.99	1.54	2.360
Glucose, g/100 mL	34	0.99	2.98	5.20	1.11	1.23	49	0.82	1.46	2.29	0.29	0.080
Sucrose, g/100 mL	38	0.52	2.62	5.62	1.22	1.50	53	0.55	2.52	5.95	0.96	0.920

Note: Standard deviation - STD; Minimum – Min; Average – Avg; Maximum – Max; Variance - Var

All attributes were submitted for exploratory analyses and those with more capability of discriminate, for further pattern recognition methods, were selected as chemical descriptors. The main independent descriptors were total malic acid (MAL), total reducing sugar (TRS) and total phenolic compound (TPC) contents. These descriptors represent the acid taste, the sweetness or sweet taste and bitterness or bitter mouth feeling. A relative descriptor, namely ratio, a function of the total reducing sugar and total malic acid contents, ratio [r= f (TRS, MAL)], was considered also in the first step of apple characterization.

A group of 24 authentic Fuji and Gala apple juices, whose descriptors are shown in Table 2-2, was used in the exploratory experiments with multivariate methods. The values found for both fruit cultivars were close, but they suggest that Fuji (MAL=0.275 ± 0.045 g/100 mL; TRS=12.52 ± 1.44 g/100 mL; TPC=255 ± 77 mg/L) is sweeter than Gala (MAL=0.278 ± 0.056 g/100 mL; TRS=11.30 ± 1.64 g/100 mL; TPC=317 ± 116 mg/L), which has a higher acidity and bitterness.

Table 2-2 – Chemical descriptors of 24 samples of apple juices from Fuji and Gala, analyzed 1986-2007

Code	Variety	Crop	MAL (g/100 mL)	TRS (g/100 mL)	TPC (mg/L)	Ratio
1	Fuji	1986-1987	0.287	15.80	404	55.05
2	Fuji	1990-1991	0.250	12.04	200	48.14
3	Fuji	1991-1992	0.330	13.37	200	40.52
4	Fuji	1993-1994	0.220	12.08	130	54.91
5	Fuji	1994-1995	0.265	12.29	195	46.38
6	Fuji	1995-1996	0.310	12.50	261	40.32
7	Fuji	2000-2001	0.290	12.04	260	41.52
8	Fuji	2001-2002	0.310	11.46	259	36.97
9	Fuji	2002-2003	0.346	10.17	374	29.39
10	Fuji	2004-2005	0.196	13.59	309	69.60
11	Fuji	2005-2006	0.238	13.65	228	80.05
12	Fuji	2006-2007	0.259	11.33	240	43.92
13	Gala	1986-1987	0.317	15.04	544	47.45
14	Gala	1990-1991	0.315	12.70	200	40.32
15	Gala	1991-1992	0.330	12.00	200	36.36
16	Gala	1993-1994	0.200	10.62	222	53.10
17	Gala	1994-1995	0.162	10.62	222	65.76
18	Gala	1999-2000	0.320	11.11	403	34.72
19	Gala	2000-2001	0.312	10.33	309	33.15
20	Gala	2001-2002	0.313	10.56	293	33.78
21	Gala	2002-2003	0.250	9.32	493	37.28
22	Gala	2004-2005	0.249	12.86	392	51.60
23	Gala	2005-2006	0.329	11.21	238	35.19
24	Gala	2006-2007	0.240	9.20	291	38.33

Note: MAL: Malic acid, TRS: Total reducing sugar, TPC: Total phenolic compounds

Although the results of total phenolic compounds from samples Gala 13 and Gala 21 seem to be quite different in the Gala set, these samples were not excluded by the Q-test (SHUGAR; BALLINGER, 1996).

The chemical descriptors of a set with 15 authentic commercial varieties of apple juices, selected to verify the effectiveness of the model, are shown in Table 2-3. Average results of the selected descriptors (MAL = 0.295 ± 0.087 g/100 mL [29.7%], TRS = 12.12 ± 1.52 g/100 mL [12.57%], TPC = 349 ± 131 mg/L [37.59%]) reflect a more acid and bitter set, although intermediate in sweetness.

Table 2-3 – Chemical descriptors of 15 apple juices from commercial varieties from different crops

Code	Variety	Malic acid (g/100 mL)	Total reducing sugar (g/100 mL)	Ratio	Total phenolic compounds (mg/L)
25	Catarina	0.265	14.00	52.82	250.42
26	Condessa	0.177	11.26	63.75	447.49
27	Daiane	0.286	13.79	48.15	397.93
28	Eva	0.403	10.22	26.21	244.94
29	Golden Delicious	0.312	10.53	33.76	535.08
30	Imperatriz	0.250	14.71	58.77	460.57
31	Joaquina	0.194	13.04	67.78	250.54
32	Kiko	0.227	12.94	57.00	257.80
33	Mollies	0.260	11.27	43.80	523.31
34	NF Golden	0.324	10.24	31.61	381.28
35	Red Delicious	0.206	13.52	66.07	518.69
36	Royal Gala	0.270	10.36	38.37	318.03
37	Sansa	0.337	12.89	38.21	364.20
38	Eva	0.454	11.98	26.90	183.50
39	Princesa	0.456	11.06	24.25	114.00

According to Schobinger et al. (1995) and Lea and Drilleau (2003), the scatter plot of the amount of malic acid and total phenolic content (Figure 2-1) may define four groups as the first separate the sweet (< 0.45 g/100 mL) from the sharp (>0.45 g/100 mL) and the last, the bitter (> 200 mg/L) from the sweet (< 200 mg/L). These four apple groups are named sweet, sharp, bitter sweet and bitter sharp by these criteria. Schobinger et al. (1995) used the central criteria but stretched the boundaries, creating domains with expressions such as “very low acid – flat", "very high acid – sour", for instance, and proposed a circular area inside the diagram where the combination was harmonious. It must be considered that the enzymatic treatment on apple juices do have lower amounts of phenolic compounds and so the process should be kept the same (NOGUEIRA et al., 2003; SCHOBINGER et al., 1995).

Figure 2-1*A* shows the position of each of the 24 samples of apple juice from the set of Fuji and Gala varieties concerning the malic acid and phenolic compound coordinates. The chemical domain of these commercial apples is defined within the limits from 0.15 to 0.35 g/100 mL of malic acid and from 100 to 600 mg/L of total phenolic compounds, classified as sweet and bittersweet apples. However, neither the Fuji nor the Gala domains can be discriminated in such a classification procedure. Likewise, Figure 2-1*B* shows the position of each of the 15 authentic samples of commercial apple juices, and it was confirmed that they are located in the same area, with the exception of Eva [28; 39] and Princesa [38], which are slightly more acid than 0.35 g/100 mL.

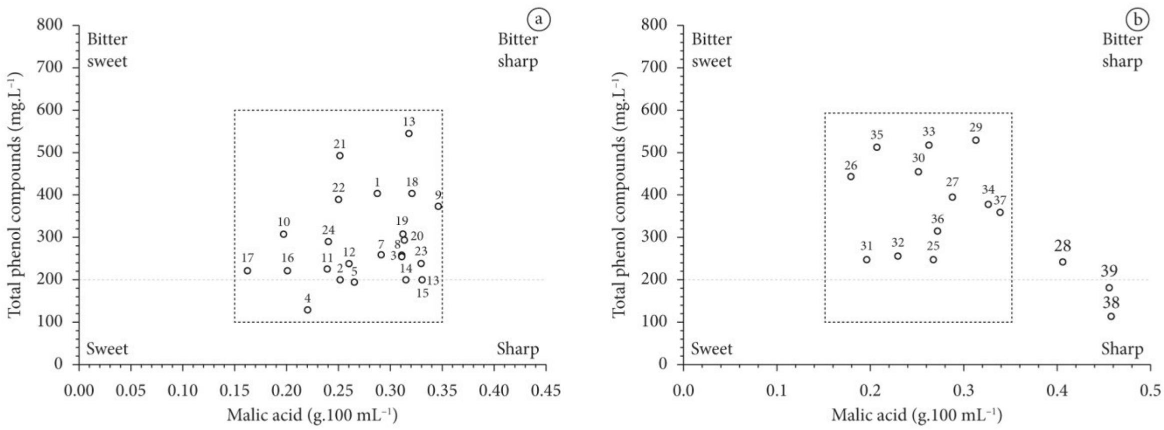


Figure 2-1 – Scatter plot (malic acid vs. total phenolic compounds) of standard commercial Fuji and Gala samples [A] and the group of authentic selected commercial samples [B] of Brazilian apples.

Czelusniak et al. (2003) analyzed fruits harvested in a commercial orchard including fruits from producer and pollinator trees during the 2001/2002 season. Among 27 apple juices, one was classified as sweet, 14 as bittersweet within the boundaries of this experiment, three were found to contain more phenolic compounds, and ten were categorized as bitter sharp. The first 15 samples were commercial apples. Usually, table apples are qualified as bittersweet (SCHOBINGER et al., 1995). According to these criteria, Paganini et al. (2004), qualified five apples as bittersweet and two as sweet from seven commercial apple varieties harvested in the 2003/2004 season. Fertonani et al. (2006) reported that the samples of Gala, Fuji and Joaquina used as raw material to pectin extraction from pomace were indeed qualified as bitter. Nogueira et al. (2006) reported that in a total of 14 samples of apple juices, comprising four commercial and ten advanced selections, five were qualified as bitter sharp and could be used as industrial raw material and the others as bittersweet, obviously table apples.

The ratio between total reducing sugar and malic acid provides a descriptor for the quality and the value 20 (adimensional) separates the industrial from the commercial apples (SCHOBINGER et al., 1995). Figure 2-2 *A* and *B* show that all apples listed in Table 2-2 and Table 2-3 are table fruits and do not fit any technological utilization. This experiment states that none of the samples tested falls in the domain of industrial set of apples considering their own profile of quality, completely clustered in the domain of commercial apples. This can be extended to the conclusion that the apples used for juice processing in Brazil are indeed of commercial quality and they do not fit any industrial pattern. But, while working with 27 samples of apple varieties (including three producers and pollinizers), Czelusniak et al. (2003) proved that, although many components of this set belong to the commercial category, they have been classified as industrial. The highest is Einshemer, with high malic acid content around 1.0 g/100 mL, and Rainha, Primicia, and Princesa, with malic acid contents of 0.64, 0.46 and 0.42 g/100 mL, respectively.

These approaches to classify apples do not give any direct information concerning the total reducing sugar and both plots in Figure 2-2 are not quite correct since a high ratio (TRS/MAL) may be achieved either by acid-poor or by sugar-poor apples.

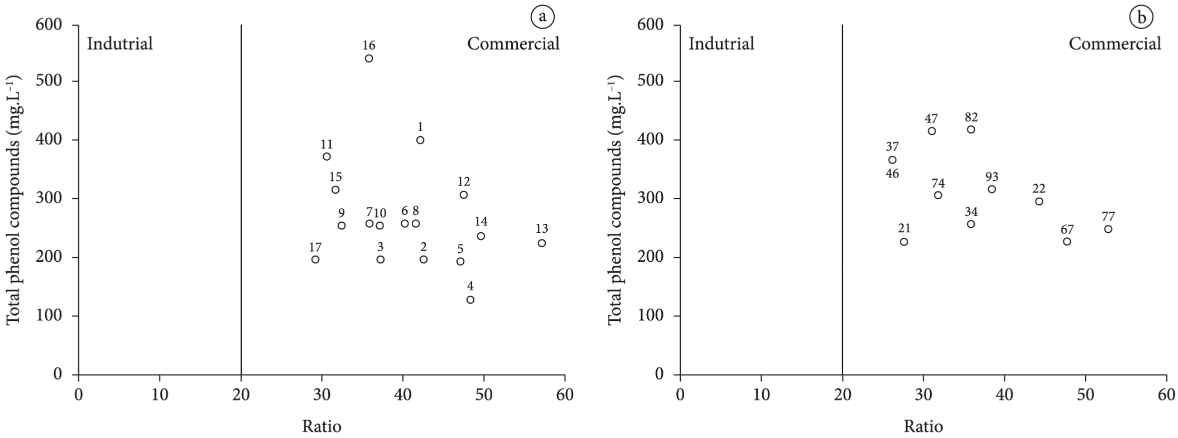


Figure 2-2 – Scatter plot (ratio vs. total phenolic compounds) of the Fuji and Gala apples samples [A] and of the commercial apple varieties [B].

In this experiment, it was shown that the main commercial Brazilian apples have an intrinsic profile of quality and so chemometric methods may be used to recognize this pattern in their juices. It seems reasonable to look for techniques of pattern recognition to classify apples for definitive commercial and industrial purposes.

The Principal Component Analysis (PCA) aims to group the chemical descriptors generating a new set of variables called Principal Components (PCs), which are linear combinations of the original continuous variables, completely independent. After several attempts to obtain a good classification for the categorical variables (apple varieties), the best results were found with three independent and continuous variables (Table 2-2 These three PC explain of total variance as follows: PC1=42.50%, PC2=31.00%, PC3=26.50%. The plots of scores were examined and the most informative one is presented in Figure 2-3, which shows the PC1 against PC2, corresponding to a total of 73.50%. Such a plot revealed that sample discrimination is related to the first principal component (PC1).

Figure 2-3*A* shows the separation of the sweeter Fuji juices from the more acidic and bitter Gala juices, according to the loadings of the continuous variables, shown in Figure 2-3*B*. The samples Gala 14 and Fuji 10 were in a mixed domain because their descriptors are so close that the model could not discriminate them. The samples of Fuji juice have a descriptor with less dispersion, more homogeneous once they occupy a smaller space than that of Gala's juices. The test apple juices can be qualified either as Fuji or/and as Gala due to they are spatial domains where Fuji and Gala may fit due to their similarity.

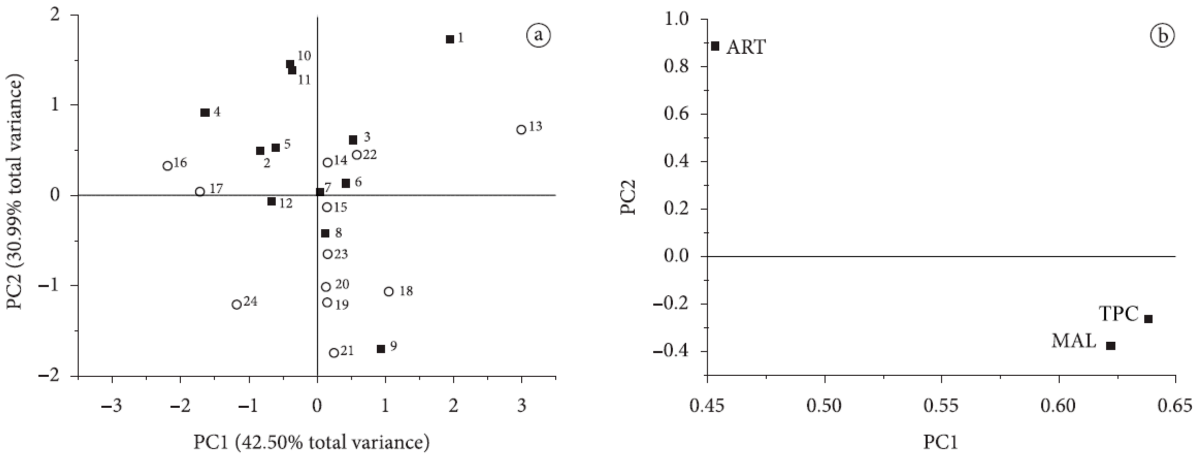


Figure 2-3 – Score [A] and loading [B] plots of data concerning samples of Fuji (■) and Gala (○) apple juice.

SIMCA, related to PCA because both are based on the generation of new variables that are a linear combination of the original, may also be used for pattern recognition and classification of apple varieties. The 3D plot of PC1, PC2 and PC3 factor will be shown in Figure 2-4.

Table 2-4 shows the pattern recognition of the standard and of some authentic samples of apple cultivars stating its similarity for Fuji, for Gala, for both, or for none of them. In the set of authentic Fuji and Gala samples, none of the samples were recognized as Fuji, but three samples belong to Gala's domain and 21 (88%) belong to the mixed domain. As expected, none of the samples become ungrouped. In the set of 15 authentic commercial samples to be modeled, none of them were predicted to be Fuji, and four were found in the Gala domain, nine (60%) were found in the mixed domain and three were excluded by the model. These results confirm the pattern recognition of the model, and the possibility to qualify any commercial apple as Fuji and/or Gala.

Table 2-4 – Results of SIMCA concerning similarity to Fuji or to Gala apples

Domain	Exploratory model	Predictive model
Only Fuji	None	None
Only Gala	1, 13, 21	26, 30, 33, 35
Either Fuji or Gala	2, 3, 4, 5,6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24	25, 27, 28, 29, 31, 32, 34, 36, 37
Neither Fuji nor Gala	None	38, 39

Figure 2-4*A* shows the SIMCA model for authentic Fuji and Gala samples and Figure 2-4*B* shows the qualification of authentic commercial apple samples. The chemical pattern established by the chemical descriptors of Fuji and Gala was recognized until the 3rd level by multivariate statistic methods (SIMCA). Curiously, none of the commercial authentic samples was qualified as Fuji, but four (Condessa, Imperatriz, Mollie's Delicious and Red Delicious) were categorized as Gala alone. All other test samples belong to the mixed domain, except for Princesa and Eva, which were ungrouped. It seems reasonable to quantify the selected descriptors in order to restrict the chemical pattern as that based in the Fuji and Gala domain, and to respect the borders defining the model. Table 2-5 shows such values, including the borders of the model.

Table 2-5 – Descriptor values of the Fuji and Gala domain.

Descriptors	Average	Borders
Malic acid (g/100 mL)	0.259±0,051	0.156-0.361
Total Reducing Sugars (g/100 mL)	11.38±1.574	9.24-15.51
Total Phenolic Compounds (mg/L)	392±107	178-606

Those values were not comparable to those reported by Czelusniak et al. (2003) on the chemical classification of samples from apples of 27 varieties, because there are not any internal reference varieties. However, if one takes into account only the fruits fitting the commercial frontier in this article, based on Fuji and Gala apples, their results are similar because MAL = 0.274 ± 0.068 g/100 mL with a minimum of 0.150 and a maximum 0.340, TRS = 10.65 ± 1.4 g/100 mL, from 8.25 to 12.70, and TPC = 286 ± 97 mg/L, from 222 to 403.

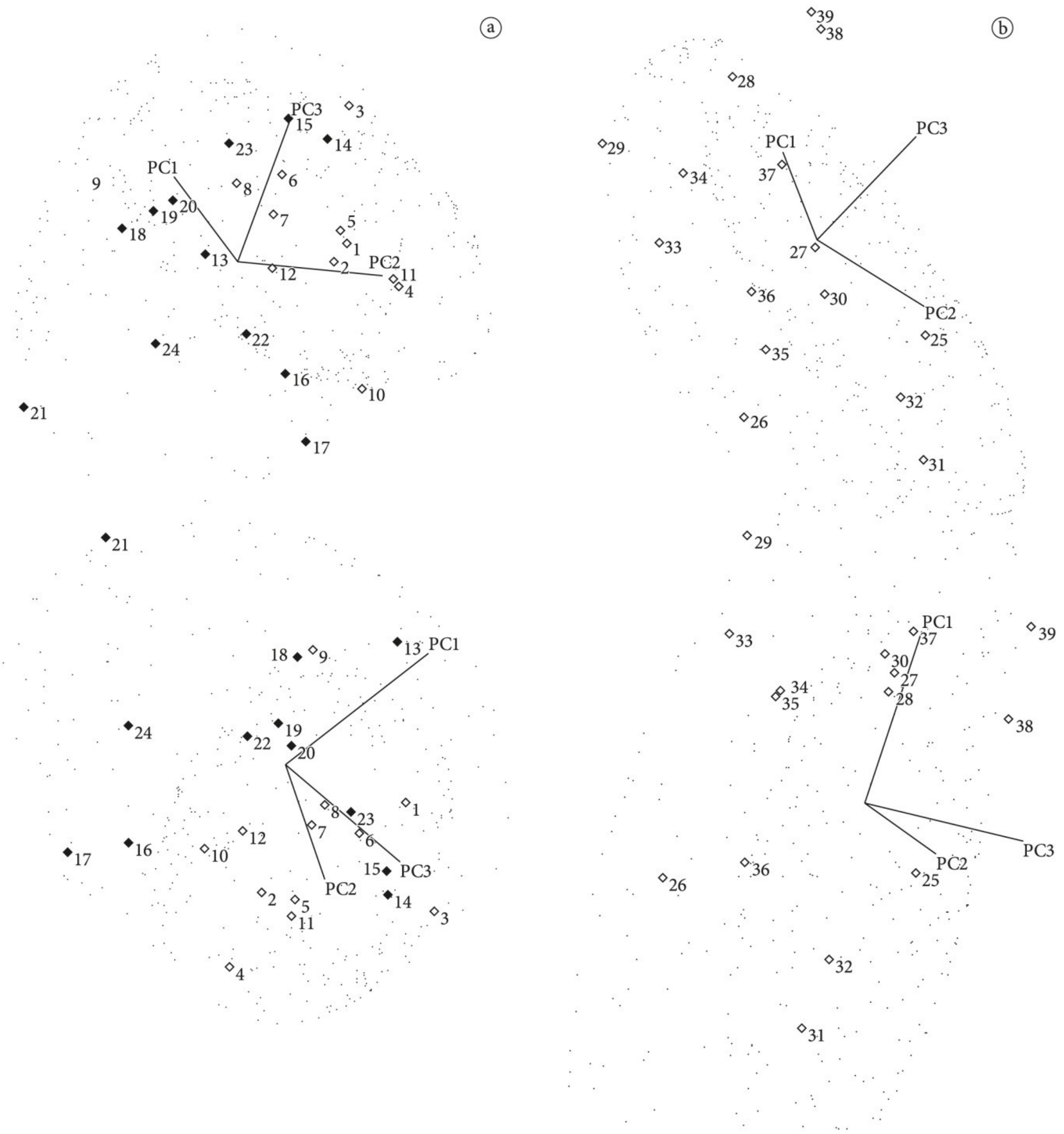


Figure 2-4 – SIMCA model obtained with a) descriptors of Fuji (◊) and Gala (♦) and the b) predictive classification of authentic commercial apples, from two different point of view.

To illustrate such a chemical pattern, Figure 2-5 shows a box and whisker plot with a broader amplitude of the Fuji and Gala descriptor than that of the commercial apple juice samples and a slight exception in what concerns malic acid – the apple juices used to test the model are slightly more acidic than the standard juices.

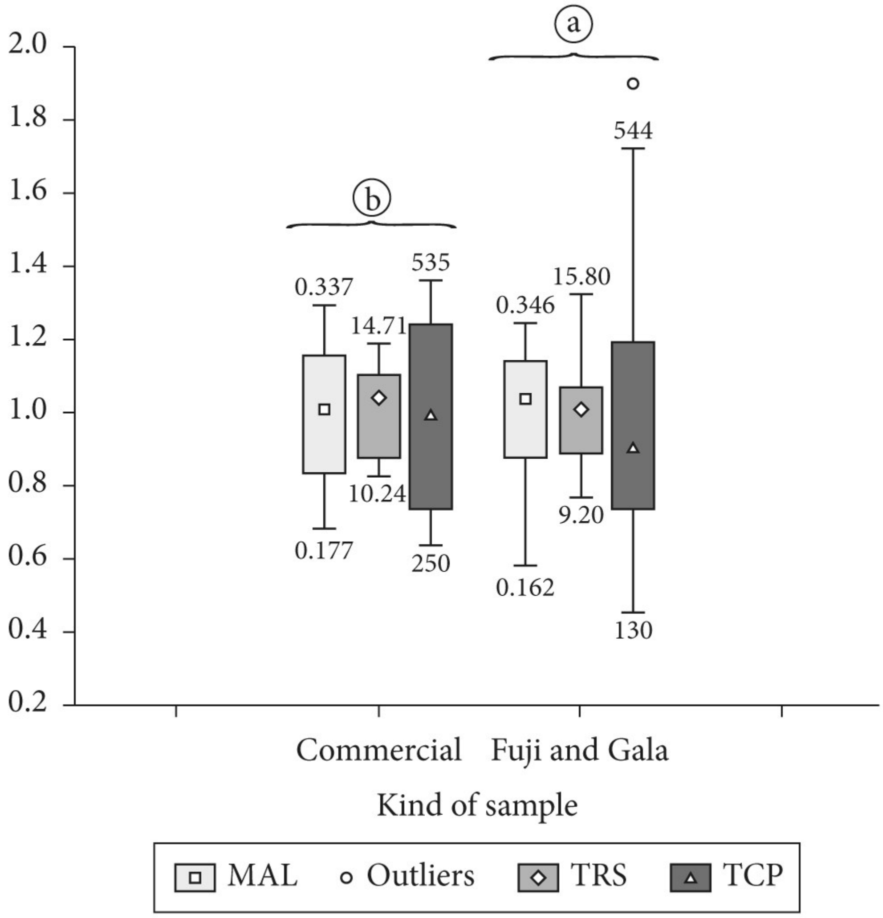


Figure 2-5 – a) Representation of the inferior and superior normalized values of the chemical descriptors from 24 authentic commercial Fuji and Gala apples. b) Representation of the inferior and superior borders of 15 authentic commercial apples.

2.4 CONCLUSIONS

The chemical descriptors malic acid and total phenolic compounds state the chemical pattern based on the main commercial variety in Brazil, namely Fuji and Gala, in the domain 0.15-0.35 g/100 mL (malic acid) and 100-600 mg/L (total phenolic compounds), qualified as sweet and bittersweet apples, respectively. None of the authentic samples of apple studied presented the condition to be considered as industrial, with a ratio TRS/MAL below 20.

The exploratory PCA screened and auto-scaled the best malic acid, total reducing sugar and total phenolic compound chemical descriptors to be used in the classificatory SIMCA, whose model discriminates all commercial varieties as Fuji, as Gala or as both Fuji and Gala, with 100% correctness.

The chemical pattern of commercial apple varieties could be conventionally established and be included within the borders of malic acid (MAL = 0.16-0.33 g/100 mL), total reducing sugar (TRS = 9.32-13.33 g/100 mL) and total phenolic compounds (TPC = 130-418 mg/L) based on Fuji and Gala apple juice.

REFERENCES

ALONSO-SALCES, R. M.; HERRERO, C.; BARRANCO, A.; BERRUETA, L. A.; GALLO, B.; VICENTE, F. Classification of apple fruits according to their maturity state by the pattern recognition analysis of their polyphenolic compositions. **Food Chemistry**, v.93, n.1, p.113-123, 2005.

BARROS NETO, B. D.; SCARMINIO, I. S.; BRUNS, R. E. 25 anos de quimiometria no Brasil. **Química Nova**, v.29, p.1401-1406, 2006.

BLANCO-GOMIS, D.; FERNANDEZ-RUBIO, P.; GUTIÉRREZ-ALVAREZ, M. D.; FERNANDEZ-RUBIO, P.; MANGAS-ALONSO, J. J. Use of High performance liquid chromatographic-chemometric techniques to differentiate apple juices clarified by microfiltration and ultrafiltration. **Analyst**, v.123, p.125-129, 1998.

CZELUSNIAK, C.; OLIVEIRA, M. C. S. D.; NOGUEIRA, A.; SILVA, N. C. C.; WOSIACKI, G. Qualidade de maçãs comerciais produzidas no Brasil: Aspectos físico-químicos. **Brazilian Journal of Food Technology**, v.6, n.1, p.25-31, 2003.

FERTONANI, H. C. R.; SIMÕES, D. R. S.; NOGUEIRA, A.; WOSIACKI, G. Potencial da variedade Joaquina para o processamento de suco clarificado e vinho seco de maçã**. Ciência e Tecnologia de Alimentos**, v.26, n.2, p.434-440, 2006.

GONZÁLEZ, G.; PEÑA-MÉNDEZ, E. M. Multivariate data analysis in classification of must and wine from chemical measurements. **European Food Research and Technology**, v.212, n.1, p.100-107, 2000.

HENSCHKE, R. A maçã e o Mercosul. **Revista Agropecuária Catarinense**, v.7, n.2, p.51, 1994.

IAL. **Métodos físicos e químicos para análise de alimentos**. In: IAL. Normas analíticas. São Paulo: Instituto Adolfo Lutz, 2008.

KREUZ, C. L. Rentabilidade da cultura da macieira cultivar Gala em duas densidades de plantio. **Pesquisa Agropecuária Brasileira**, v.37, p.229-235, 2002.

LEA, A. G. H.; DRILLEAU, J.-F. Cidermaking. In: A. G. H. Lea e J. R. Piggott (Ed.). **Fermented Beverage Production**. London: Springer, 2003, p.59-87.

MOLFETTA, F. A.; BRUNI, A. T.; HONÓRIO, K. M.; DA SILVA, A. B. F. A structure-activity relationship study of quinone compounds with trypanocidal activity. **European Journal of Medicinal Chemistry**, v.40, n.4, p.329-338, 2005.

NELSON, N. A photometric adaptation of the Somogyi method for determination of glucose. **Journal Biology Chemistry**, v.153, p.375-380, 1944.

NOGUEIRA, A.; SANTOS, L. D.; WIECHETECK, F. V. B.; GUYOT, S.; WOSIACKI, G. Efeito do processamento no teor de compostos fenólicos em suco de maçã. **Publicatio UEPG - Ciências Exatas e da Terra, Ciências Agrárias e Engenharias**, v.9, n.3, p.7-14, 2003.

NOGUEIRA, A.; SWIECH, B. D. P.; DENARDI, F.; WOSIACKI, G. Características físico-químicas e sensoriais de suco de maçã clarificado e fermentado. **Publicatio UEPG - Ciências Exatas e da Terra, Ciências Agrárias e Engenharias**, v.12, n.3, p.15-23, 2006.

PAGANINI, C.; NOGUEIRA, A.; DENARDI, F.; WOSIACKI, G. Análise da aptidão industrial de seis cultivares de maçãs, considerando suas avaliações físico-químicas (dados da safra 2001/2002). **Ciência e Agrotecnologia**, v.28, n.6, p.1336-1343, 2004.

SCHOBINGER, U.; BARBIC, I.; DÜRR, P.; WALDVOGEL, R. Phenolic compounds in apple juice - positive and negative effects. **Fruit Processing**, v.6, p.171-178, 1995.

SHUGAR, G.; BALLINGER, J. **Chemical Technicians' Ready Reference Handbook**: McGraw-Hill Professional, v. 1996. 979 p.

SOMOGYI, M. A new reagent for the determination of sugar. **Journal Biology Chemistry**, p.61-68, 1945.

TANNER, H.; BRUNNER, H. R. **Getränke Analytik-Untersuchungsmethode für die labor -und Betriebspraxis**. Wädenswill: Helles, v. 1985.

WORLD APPLE REVIEW. Pullman: Belrose, Inc., v. 2008. 138 p.

WOSIACKI, G.; NAMIUCHI, N. N.; CERIBELLI, M. I. P. F.; SATAQUE, E. Y.; SICHIERI, V. L. F. S.; OLIVEIRA, T. C. R. M.; CESAR, E. O. Estabilidade do suco clarificado de maçã. Parte I - Processo de obtenção do suco de maçã. **Arquivos de Biologia e Tecnologia**, v.32, n.4, p.775-786, 1989.

WOSIACKI, G.; NOGUEIRA, A.; SILVA, N. C. C.; DENARDI, F.; CAMILO, A. P. Apple varieties growing in subtropical áreas. The situation in Santa Catarina - Brazil. **Fruit Processing**, v.12, n.1, p.19-28, 2002.

3 CAPÍTULO III: CHEMICAL AND SENSORY PROFILE OF APPLE. A NEW APPROACH ABOUT QUALITY

CHEMICAL AND SENSORY PROFILE OF APPLE. A NEW APPROACH ABOUT QUALITY

ABSTRACT

In 2009, apple production in Brazil reached 1.222.890 metric tons and this confirmed the success of the installation of this culture in Southern States, as result of the cooperation project comprehending researchers, producer and governmental assistance. The main purpose was to supply the domestic market with high quality fruits at small production cost, leading to produce only Fuji and Gala varieties. This bivarietal production does not supply the sector of juice and fermented beverage processing, because these fruits are not properly for industrial purposes. But these apples are the only raw material available and a really industrial orchard is necessary at the moment, installed with industrial cultivars, both producer and pollinator or obtained by a gradual conversion of apples commercial in industrial varieties. In this article descriptors of quality of 12 samples of Fuji and 12 of Gala were collected in the database of GTM (Apple Research Group) were recovered as standard and another set of 24 commercial apples were used as test. Since there were many apple varieties with some features useful for processing, the classification by different procedures was done aiming to describe them and pointing to that varieties that show the features of industrial apples.

Keywords: chemometric, acid, sugar, phenolic compounds

PERFIL QUÍMICO E SENSORIAL DA MAÇÃ. UMA NOVA ABORDAGEM SOBRE QUALIDADE

RESUMO

Em 2009, a produção de maçã no Brasil alcançou 1.222.890 toneladas e isto confirmou o sucesso da instalação dessa cultura nos Estados do Sul, como resultado do projeto de cooperação abrangendo pesquisadores, produtores e assistência governamental. O principal objetivo foi abastecer o mercado interno de frutas de alta qualidade a baixo custo de produção, priorizando apenas das variedades Fuji e Gala. Esta produção bivarietal não supre o setor processamento de sucos e bebidas fermentadas, visto que esses frutos não são apropriados para fins industriais. Entretanto, essas maçãs são a única matéria-prima disponível. Um pomar industrial realmente é necessário no momento, instalado com cultivares industriais, tanto produtoras ou polinizadoras ou obtidas por conversão gradual de maçãs comerciais em variedades industriais. Neste artigo, descritores de qualidade de 12 amostras de Fuji e 12 de Gala foram coletados no banco de dados do GTM (Apple Research Group) e recuperados como padrão e outro conjunto de 24 maçãs comerciais foram utilizados como teste. Uma vez que havia muitas variedades de maçãs com algumas características úteis para o processamento, a classificação por diferentes procedimentos foi realizada com o objetivo de descrevê-los, indicando as variedades que se adaptam às características de maçãs industriais.

Palavras chave: quimiometria, acidez, açúcar, compostos fenólicos

3.1 INTRODUCTION

The apple production in Brazil, among the 15 top producing country (FAO, 2011), is based only on two varieties namely Gala and Fuji, which are indeed the 3rd and 4th in the international ranking as the most sold fruits in its category (WORLD APPLE REVIEW, 2008). As this production reach the 1.222.890 ton, the domestic market is supplied with almost 850.000 metric ton of these varieties in a 58:35 proportion. It is supposed that the unclassified apple (30%) may contain at least 20% of sound fruits although with some defects in appearance (ABPM, 2011, FAO. 2011). They can perfectly be processed into juice or fermented beverage. In such case, the demand includes special varieties with regular production in a specific period of time to optimize the correct utilization either technical or economical of the available resources. The large amounts of apples, Fuji and Gala, have been processed and the products do not necessarily fit the international legislation (BIRUS, 2001).

The installation of pomiculture in the Southern States of Brazil was successful also in sensorial acceptation because there was a previous, simultaneous and continuous Research Program to actualize varieties and to create new cultivars but it is required that the production cost must be equal or even cheaper than the current (WOSIACKI et al., 2002).

There are lots of varieties in commercial orchards that were recommended by Research Institutes and many of them may have quality profile of an industrial variety that could be used to improve the quality of a product obtained with Fuji or Gala because there are not any perceptible movement in the direction to look for an apple truly industrial (WOSIACKI et al., 2000).

In the middle of the 1980's, as results of interaction from some remarkable german scientists, one book was published with the name of RSK (NEUHÄUSER, 1998). The content of such book includes the quality profile of many fruits and the first example was that of apple. Since then, all descriptors of apple are perfectly qualified, with the average, standard deviation as well as minimum and maximum scores ever found. The analytical approach was in agreement with official methodology at that time, nowadays slightly modified to obey actual legislation (IFU ,2011).

Among the noble products of the apple industry, there is cider, widely consumed in Europe. For this product has an excellent quality should be a proper balance of sugar, acidity and phenolic compounds (LEA, 1995; CAMPO, 2005). According to Campo (2005 apud ACRRE; MCLELLAN, 1989) apple juice is composed of approximately 11% sugar, responsible for sweet taste, 0.4% of acidity that causes the perception of sour and bitter is mainly attributed to phenolic compounds.

In this article, at the same time several varieties are classified through empiric and classical methods and following a multivariate approach, the *leit motiv* is to indicate a practical and efficient way to find out if apple varieties in commercial orchards have features that promote its use as raw material for processing.

3.2 MATERIAL AND METHODS

3.2.1 MATERIAL

Samples of commercial varieties were collected during 2001 to 2007 from many places of southern Brazil, mainly in the region of Fraiburgo. The selected samples, 24 in total, were Anna (AN), Baronesa (BA), Belgolden (BG), Carícia (CR), Catarina (CT), Condessa (CD), Daiane (DA), Einshemer (ES), Eva (EV), Fred Hough (FH), Fuji Suprema (SU), Golden Delicious (GD), Granny Smith (GS), Imperatriz (IZ), Imperial Gala (IG), Joaquina (JO), Melrose (MR), Mollies Delicious (MD), Princesa (PR), Rainha (RA), Red Delicious (RD), Royal Gala (RG), Sansa (SA), Starkrimson (ST), available to commerce *in natura*. Despectinized juice was obtained from these samples, using samples of pectinolytic enzymes (Pectinex 3XL – Novo Nordisk do Brazil). All chemicals used in the analysis and experiments were quality *pro analisys*.

3.2.2 METHODS

Apple Juice processing. The apples were handled carefully by trained personal, driven to the industry unit for washing, selected and stored at low temperature (+0.5^{o}C). There was a definite protocol to be followed at a laboratory level regardless the amount of apple under investigation. *Circa* 5 kg are washed, selected and cut with knife in four pieces, then are put into a knife mill arranged with the largest knife to obtain discrete pieces but minimum of free juice. Then the milled apples were arranged in four layers of sieves, pressed twice at 3 kgf/cm^2 during 10 min. The brut extract of the press is the natural apple juice and part of it can be stored at -18^{o}C for further trials.

Apple juice depectinization. The press extract of milled apple contains all soluble chemical compounds and the processing follows the protocol. The brown apple juice receives a pectinolytic treatment (3 mL/hL) at hot conditions (45^{o}C/60 min.) or preferentially at cold conditions (8^{o}C/15 hours), and the upper cleaner part is removed by siphoning and then is filtered in qualitative paper without pressing. The transparent and pectin-free apple juice is stored in plastic bottle at -18^{o}C.

Analysis. Total Soluble Solids (TSS) were determined in a refractometer adjusted to 20^{o}C (ITAL, 2008). The content of glucose (GLC) was determined with glucose oxidase kit assay (Gold Analisa Diagnóstica Ltda.) and the amount of fructose (FRU) was then calculated as the remainder from reducing sugar, and that of sucrose (SUC), as the difference from total reducing sugar and reducing sugar (NELSON, 1944; SOMOGYI, 1945). Total titrable acidity (TTA) was determined with NaOH 0.1M and expressed as malic acid (MAL) in g/100mL. Total phenolic compound level (TPC) was determined by the Folin-Ciocalteau reagent (TANNER; BRUNNER, 1985) and expressed as (+) catechin, in mg/L. The Descriptive Statistic Analysis of the chemical descriptors was made by employing the Statistic® 7.0 software.

Qualifying procedures. The samples of apple used in this article belong to authentic fruit, with descriptors within the range recognized by the official current legislation. With a referential set of 24 samples of the commercial apple varieties Fuji and Gala and another set of samples of 24 varieties was classified according four different procedures and the results were considered for practical utilization. These varieties are considered categorical variables and the descriptors malic acid, total reducing sugar and total phenolic compounds, the independent and continuous variables, among glucose, fructose, and sucrose, considered unimportant during the data processing. [1] A similar procedure uses as descriptor the refractive index named degree brix (TANNER; BRUNNER, 1985) but both methods determine the total soluble solids (BEECH, 1972; BEECH; CHALLINOR, 1951; DRILLEAU, 1991). [2] Descriptors malic acid (MAL) and total phenolic compounds (TPC). The scatterplot according to these axes creates four regions in a Cartesian 2D and the apple distribution can be used to qualify them within conventional figures or varieties (SCHOBINGER et al., 1995). [3] Descriptors ratio (total reducing sugar/malic acid) and total phenolic compounds. The apple relative position enables it to be qualified as commercial or industrial, using the cutoff 20 to ratio (DRILLEAU, 1991; NOGUEIRA et al., 2003). [4] Multivariate analysis is now a tool to qualify apples since that a discriminatory power exists, what is evaluated by exploratory PCA and HCA and later, the pattern recognition of SIMCA and KNN can be used to correlates the categorical apple with the independent or continuous variables (PADIN et al., 2001).

Chemical descriptors. It can be state that the magnitude order of figures are 10^{-1} for malic acid, 10^{1} for total reducing sugar and 10^{-3} for total phenolic compounds and to compare them it was utilized a process to normalize the data, specifically using a factor (x^{-1}).

3.3 RESULTS AND DISCUSSION

3.3.1 STANDARD AND TEST COMMERCIAL APPLES.

3.3.1.1 Quality profile of the Brazilian apple Gala and Fuji.

The chemical descriptors of 24 despectinized apple juice of samples from cultivars of commercial apples were recovered from the data bank of GTM (Apple Research Group) at UEPG (Ponta Grossa State University) and were considered as test fruit to be classified by a set of 24 apple samples of the varieties Fuji (1-12) and Gala (13-24). Three continuous and independent descriptors of the data bank it were selected, precisely corresponding to a sensorial taste response as acidity for malic acid, sweetness for sugar and bitterness for total phenolic compounds (SCHOBINGER et al., 1995). It must to emphasize that the lowest and highest values for malic acid should correspond to unbalanced-sweet or flat and unbalanced-sour or flat, respectively. The same occurs with total phenolic compound, they correspond to adstringent-sweet and adstringent-sour, respectively. This set of descriptors is characterized quantitatively in

Table 3-1 and it corresponds to the Brazilian commercial apple quality profile, focusing at the mentioned varieties (VIEIRA et al., 2011). This set of Fuji and Gala shows a similar average of malic acid, with the Gala a little bit more spread than Fuji. The data for total reducing sugar are not so similar but are at least 5% higher for Fuji. Total phenolic compounds shows more spread and highest values for Gala.

In Table 3-1 the results of the simple descriptive statistic of 24 authentic samples of the standard varieties are shown.

Table 3-1 – Simple descriptive statistic of 24 Brazilian samples of Gala and Fuji

Quantitative Chemical Descriptors	FUJI			GALA			Qualitative Sensorial correspondence
	AVG	SD	CV(%)	AVG	SD	CV(%)	
MAL	0.275	0.045	16.36	0.278	0.056	20.14	Acidity
TRS	12.52	1.43	11.47	11.30	1.64	14.55	Sweetness
TPC	255	77	30.17	317	116.19	36.45	Bitterness

AVG = average; SD = standard deviation; CV = coefficient of variation; MAL = malic acid; TRS = total reducing sugar; TPC = total phenolic compounds

Source:(VIEIRA et al., 2011)

3.3.1.2 Quality profile of 24 Brazilian Commercial apple.

In Table 3-2, the descriptive statistic simple of the three descriptors from 24 commercial apples are shown. The set of varieties shows an average value for malic acid 0.388 ± 0.148 g/100 mL (39.67%), with values minimum of 0.198 g/100 mL and maximum, 0.821 g/100mL, what represent a difference from the standard of 33% in average and tree times the maximum value. Certainly, there are varieties completely different from the standard set according to this descriptor. In what concerns total reducing sugar, the average was 11.68 ± 1.103 g/100 mL (9.47%) and the data was spread in a large distribution with values ranging from minimum of 9.49 g/100 mL to a maximum of 13.52 g/100 mL. Total phenolic compound shows average figure 379 ± 126 mg/L (33.39%), ranging from 173 up to 689 mg/L. In sensorial terms these set can be compared as more acidic and less sweet than the standard. Perhaps some actual industrial variety can be included in the test group.

In Table 3-2 the characteristic of the sample of 24 commercial samples varieties are shown.

Table 3-2 – Simple descriptive statistic of 24 Brazilian commercial samples of apple

Quantitative chemical descriptors	**Apple varieties in test (24)**					**Qualitative sensorial correspondence**
	AVG	**SD**	**Min**	**Max**	**CV (%)**	
MAL g/100 mL	0.388	0.148	0.198	0.821	39.67	Acidity
TRS g/100 mL	11.68	1.103	9.49	13.52	9.45	Sweetness
TPC mg/L	379	126	173	689	33.29	Bitterness

AVG = average; SD = standard deviation; CV = coefficient of variation; MAL = malic acid; TRS = total reducing sugar; TPC = total phenolic compounds

3.3.2 METHODS FOR APPLE QUALIFICATION

3.3.2.1 Acid taste and bitterness

In Figure 3-1*A* and *B*, it is shown the distribution of the descriptors acidity and bitterness, according to the results of malic acid and total phenolic compounds as seen by the English point of view (NOGUEIRA; WOSIACKI, 2010). Such scatter plot describes the Brazilian apple varieties according to Gala(G) and Fuji(F) can be characterized as bitter sweet (15/24), bitter (7/24), sweet (2/24) and bitter acid (1/24), if were followed the English standard (BEECH, 1972). The standard apple may be described with the average value for malic acid 0.264±0.033 g/100 mL, with values from 0.246 to 0.303 g/100 mL at 95% confidence, minimum of 0.200 g/100 mL and maximum, 0.346 g/100mL. Concerning to total reducing sugar, the average was 12.50±4.66 g/100 mL and the data was spread in a large distribution with values ranging from 11.61 to 13.43 g/100 mL at 95% confidence, minimum of 10.17 g/100 mL and maximum, 15.80 g/100mL. At last, the total phenolic compound 347±80 mg/L show values from 205 to 304 mg/L at 95.5 confidence, minimum 130 mg/L and maximum, 404 mg/L. In sensorial terms they are related to a set of apple with extremes as a low acid taste and bitterness – which is considered unbalanced composition or flat. The total reducing sugar is considered high and so the fruits or their juices present a considerable sweetness. With such attributes, it is possible to state that this kind of apple has more characteristics of commercial fruits and do not satisfy nor contains characteristics to the industrial sector (VIEIRA et al., 2011).

The test set of apples showed similar values with Gala and Fuji, bitter (13/24), bitter-sweet (2 / 24), bitter-acid (5 / 24). However, four samples showed characteristic bitter-sour, because its phenolic compounds values were superior to 300 mg/L and acidity above 90 mEq/L.

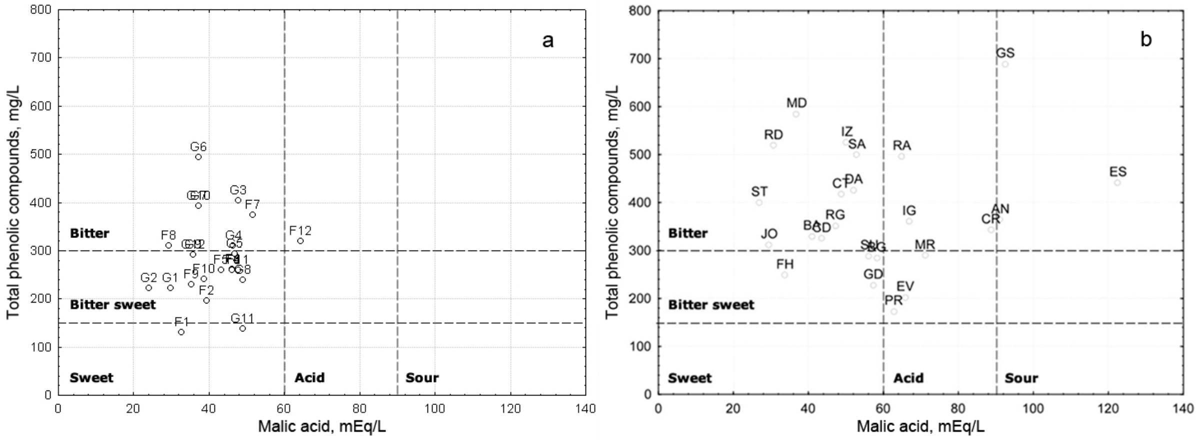


Figure 3-1 – a) Distribution of the standard apples according to mEq/L of malic acid and total phenolic compouns contents. Gala(G), Fuji(F). b) Distribution of test set of commercial apples

In Figure 3-2 is shown the same distribution calculated as malic acid but with larger axis to amplify the space aiming to include and compare the standard and test apples.

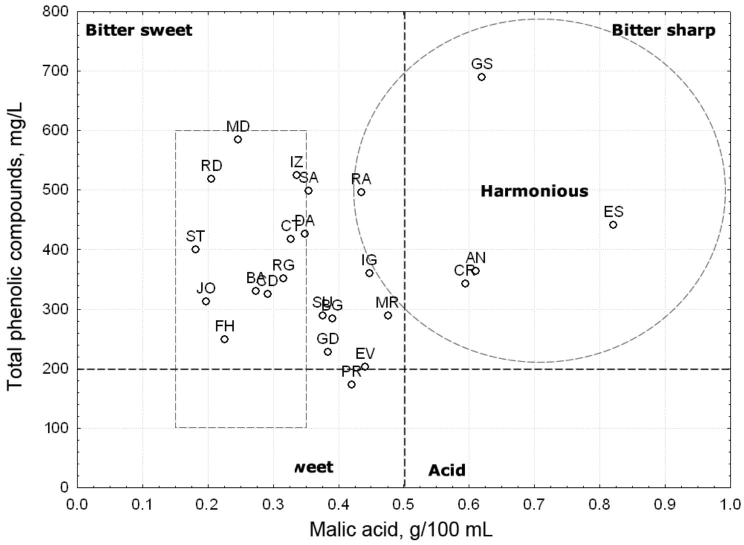


Figure 3-2– Classes of apple spread in a Cartesian 2D space

In fact, with the Cartesian space determined by the axes (X:Y) of malic acid and phenolic compounds, respectively, it is possible to have four patterns as follows: bitter sharp (+ ; +), bitter sweet (- ; +), sweet (- ; -) and sharp (- ; +) (BEECH, 1972; SCHOBINGER et al., 1995). At least five varieties belong to the group more similar to the harmonious industrial set: 'Granny Smith', 'Carícia', 'Anna', 'Einshemer' and 'Rainha'. There is not any one in the sharp set but at least one (Princesa) in the sweet set. Large fraction of the entire set is spread in the bitter sweet, most of them (12) within space determined elsewhere (VIEIRA et al., 2011). In fact the spread standard apples are confined in a small part of the 2nd class, from 0.15 up to 0.35 g/100 mL of malic acid as can be seen in Figure 3-2, with interrupted line and the pattern does not include apples with malic acid from 0.35 to 0.5 g/100 mL. The apples of this sub group are too acid for table fruits and not acid enough to be used as raw material besides are much proper for processing then the Fuji or Gala. With such criteria in mind, four apples belong to a group of industrial varieties here named bitter sharp, twenty varieties as bitter sweet (commercial) and eight should have a further study because are actually considered neither Fuji nor Gala. In the 3rd group, sweet, there are only one variety with a bad score in this set. All 24 samples were than classified in four groups. If the group related to the standard would considered as the real bitter sweet, these apples showing acidity from 0.35 up to 0.5 g/100 mL, there would be eight varieties fitting this space, an intermediary set showing slightly more acid than the bitter sweet and less than the bitter sharp, namely, ‘Melrose’, ‘Rainha’, ‘Fuji Suprema’, ‘Golden delicious’, ‘Belgolden’, ‘Princesa’, ‘Eva’ and ‘Imperial Gala’.

3.3.2.2 Table apples and raw material

The factors did not consider amounts of total reducing sugars (TRS), an important attribute of juice, a right criteria to establish a precise cut off to separate the categories into commercial and industrial varieties must be defined. In this sense, it was create a new continuous variable as the ratio which represents the relation between total reducing sugar and malic acid, an adimensional value. This ratio increases when the sugar increase and when the malic acid decrease. The value of 20 is empirically separating both categories. This kind of discrimination is dependent to environmental conditions like the season, weather and cultural tracts. This procedure also includes the bitterness. In Figure 3-3 is possible to visualize two categories, the industrial (left of value 20) and the commercial, at right. In this set of apples in test, four of them were in the class industrial and 20 remained in the commercial class. The cut off 20 is questionable: why is it not 21 or 19? If it was 25 the cultivar Melrose would be industrial, and 30, at least five others but it is necessary to go back to the experimental number to see why the ratio is low and there are two possibilities: rather the sugar or the acid are high values once increase. To take decisions with such results, it is curious and critical because Melrose may be a better raw material than the Granny Smith and Carícia; Anna and Einshemer. These latter, producer and pollinator, respectively, although good enough for industrial processes are practically eradicated in Brazil.

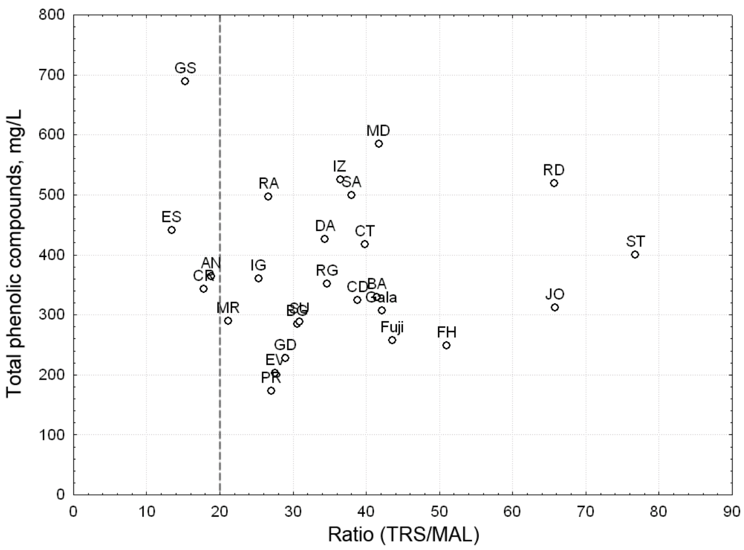


Figure 3-3 – Separation of industrial (ratio < 20) from commercial (ratio > 20) apples

3.3.2.3 Modeling standard and pattern recognition methods to predict class

The principal components analysis (PCA), an method design to project n-dimensional figures in a lower dimension space, usually two or three (FERREIRA et al., 2002) actually delineates the component by linear combinations of the original independent variables. The analysis has an exploratory sense once it provides some hypothesis to be extract from the original data and also redundant and randomized information are discharged. The clustering of the categorical and continuous in the 2D array defines the structure of the data of the scores and loads. The scores describe the composition of the components relative to the categorical sample and the loads, the continuos descriptors.

In this exploratory study seven chemical descriptors were tested against 24 categorical samples of two apple varieties, Gala and Fuji, (matrix 24 x 7) and the results of analyses were that only three selected descriptors gave the same answer with 100% of the variance. With the new matrix (24 x 3) the dimensions were reduced but the information was preserved.

From these three descriptors were tested 24 samples of commercial apples, Gala and Fuji, along with the test set of commercial samples of apples, totaling 48 in order to relate them to other classification methods.

Principal Component Analysis

An exploratory analysis with the standard categorical and the independent and continuous information sets (48 apples x 3 attributes) was done. The data were auto scaled and Principal Component Analysis (PCA) was performed with Pirouette®. Three descriptors were able to explain 100% of all variance as the PC1 explains 46.36% and PC2, more 31.74%.

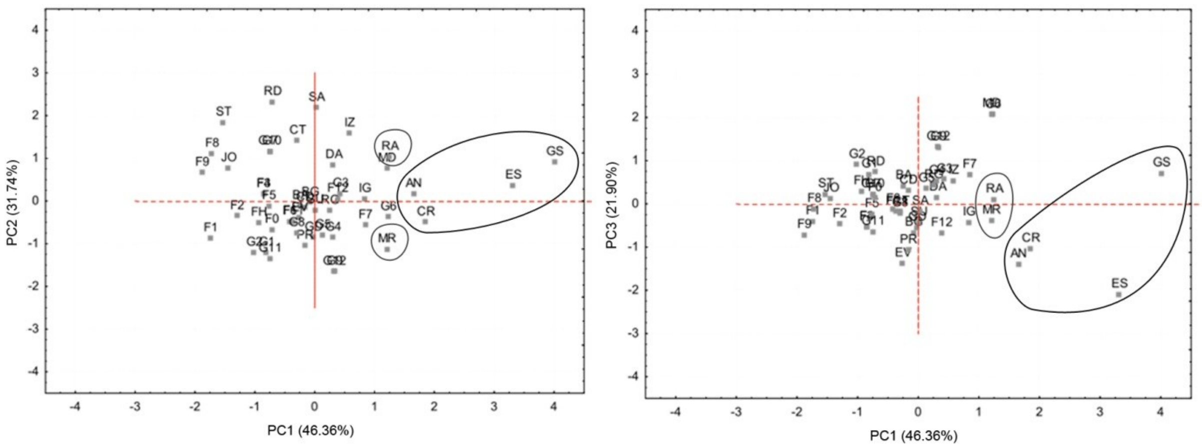


Figure 3-4 – Projection of the scores of 24 Gala and Fuji and 24 commercial samples of apple.

In Figure 3-4, the PCA shows the clustering of table apples, which together with the Gala and Fuji apples are considered commercial. The samples of apple, Granny Smith (GS), Einshemer (ES), Carícia (CR) and Anna (AN), are more scattered and out of range of the grouping of Gala and Fuji, with a different profile, similar industrial apples. It can be noticed that variety Melrose is on the edge of the cluster, as well as other classification methods, a profile showing both commercial and industrial.

Hierarchical Cluster Analysis

To complement the PCA, hierarchical cluster analysis (HCA) groups (Figure 3-5), according to the similarity of these samples was considered. The last group (Green), are apples (AN, CR, EN, GS, MR) that have lower similarity (10%) with the rest. Comparing this result data with PCA, these apples are those located outside the boundaries of the commercial group of apples, including Melrose, and which have an industrial profile, with high levels of acid malic and total compounds phenolics.

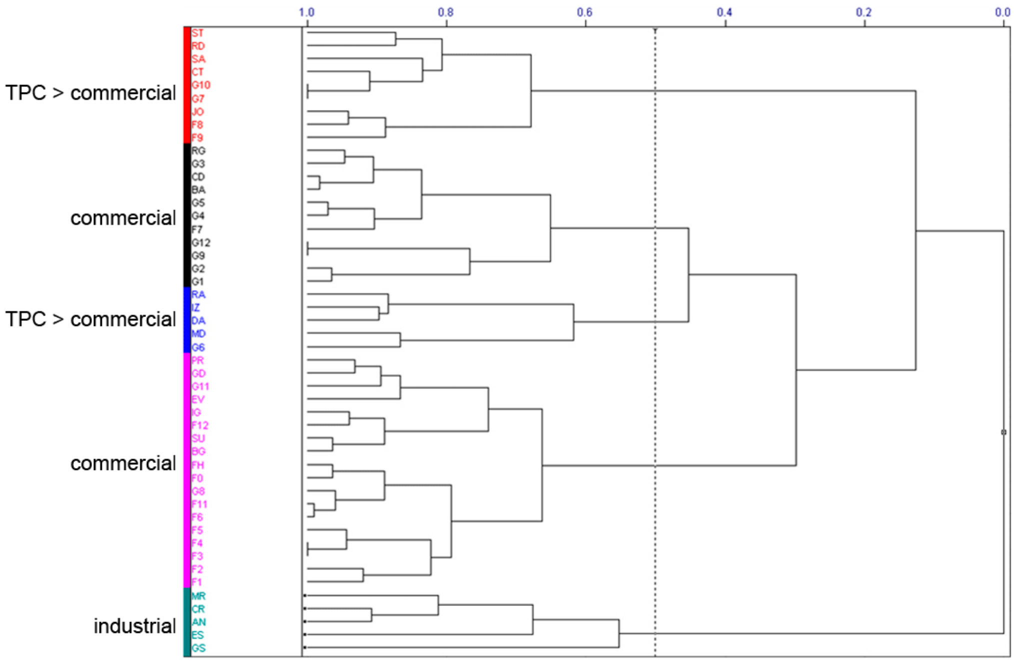


Figure 3-5 – Hierarchical clustering analysis of 48 samples of different varieties apples.

The other groups consist of apples that have a commercial profile table. Groups 1 and 3 (Red and Blue) are apples that present values higher than the average total phenolic compounds of Fuji and Gala apples, and groups 2 and 4 (Black and Pink) are the apples that have the same levels of TPC , TRS and MAL that Fuji and Gala.

K-nearest neighbours

In order to generate the model with the qualifying classes, 24 samples of Fuji and Gala apples were used, and then these same samples were classified to determine k. Analyzing percentages of correct answers for each k, the chosen value was 2. After determining the value of k, set of commercial apples were classified according to Table 3-3.

Table 3-3 – Pattern recognition Gala and Fuji with KNN

Recognized pattern	KNN k=2
Fuji	13
Gala	11
Total	24

Soft independent modelling of class analogy (SIMCA)

SIMCA evaluates the derivative of the main components for each class separately. Create a template functions for each class separately, and the samples tested are classified according to the distance from the class template.

With 24 commercial samples of apples (Gala and Fuji), the model was made to allow the classification of the other 24 samples.

In Figure 3-6 and Figure 3-7 the model prediction and the model with the other 24 samples of apples are shown, respectively. According to this method the apples can be classified into four groups, as (1)Fuji (2) Gala, (3) Fuji or Gala and (4) neither Fuji nor Gala. By figures it can be verified that those varieties previously classified as industrial and that have bitter-acid profile (AN, CR, EN, GS, MR), are far from the model, and those that were between the classification made by Vieira et al. (2011) and group harmonious are not within the cloud (standard model), but are very close to it.

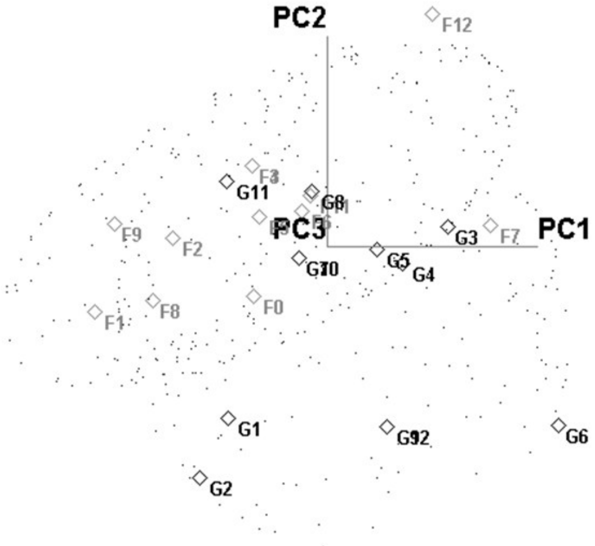


Figure 3-6 – Model made from the 24 samples of Gala and Fuji apples

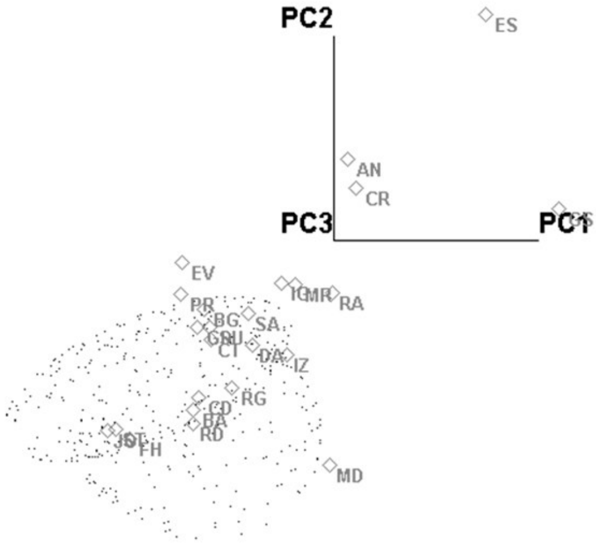


Figure 3-7 – Testing of 24 samples commercial of apples in the developed model

The classification given by the SIMCA method has this fit as shown in Table 3-4. Apples were classified in group 4 (neither Fuji nor Gala) are Anna, Carícia, Granny Smith, Einshemer, Melrose, Red Delicious and Starkrimson.

Table 3-4 – Pattern recognition Gala and Fuji with SIMCA

Recognized pattern	SIMCA
Only Fuji	0
Only Gala	7
Either Fuji or Gala	10
Neither Fuji nor Gala	7
Total	24

Comparison of methods

All results in the four methods of qualification are summarized in Figure 3-8. The few varieties that had an industrial profile, with satisfactory amounts of malic acid, reducing sugars and total phenolics, according to the classification methods were Anna, Carícia, Einshemer, Granny Smith and Melrose. This last variety can be used as commercial or industrial because it was classified at the boundaries between commercial and industrial apples.

Varieties that are classified in Cluster 1 presented similar levels of MAL and TRS of Fuji and Gala, but have higher levels of TPC. Two samples (RD, ST) showed the classification of neither Gala nor Fuji, even belonging to Cluster 1, because they have values of MAL below the averages of Fuji and Gala, but in all other classifications, fit as commercial. Varieties that are part of Cluster 2 are those that are within the limits of Fuji and Gala.

Figure 3-8 – Apple juice class according to several analytical procedures

Variety	Acidity and Bitterness*	Ratio and bitterness**	SIMCA/KNN	PCA/HCA
Anna	**Bitter sharp**	**Industrial**	**NOR**	**Cluster 3**
Baronesa	Bitter sweet	Commercial	EOR2	Cluster 2
Belgolden	Bitter sweet	Commercial	EOR1	Cluster 2
Carícia	**Bitter sharp**	**Industrial**	**NOR**	**Cluster 3**
Catarina	Bitter sweet	Commercial	OG	Cluster 1
Condessa	Bitter sweet	Commercial	EOR2	Cluster 2
Daiane	Bitter sweet	Commercial	EOR2	Cluster 1
Einshemer	**Bitter sour**	**Industrial**	**NOR**	**Cluster 3**
Eva	Bitter sweet	Commercial	OG	Cluster 2
Fred Hough	Bitter sweet	Commercial	EOR2	Cluster 2
Fuji Suprema	Bitter sweet	Commercial	EOR1	Cluster 2
Golden Delicious	Bitter sweet	Commercial	EOR1	Cluster 2
Granny Smith	**Bitter sharp**	**Industrial**	**NOR**	**Cluster 3**
Imperatriz	Bitter sweet	Commercial	OG	Cluster 1
Imperial Gala	Bitter sweet	Commercial	EOR1	Cluster 2
Joaquina	Bitter sweet	Commercial	EOR1	Cluster 1
Melrose	**Bitter sweet**	**Industrial**	**NOR**	**Cluster 3**
Mollies Delicious	Bitter sweet	Commercial	OG	Cluster 1
Princesa	Sweet	Commercial	OG	Cluster 2
Rainha	Bitter sweet	Commercial	OG	Cluster 1
Red Delicious	Bitter sweet	Commercial	NOR	Cluster 1
Royal Gala	Bitter sweet	Commercial	EOR2	Cluster 2
Sansa	Bitter sweet	Commercial	OG	Cluster 1
Starkrimson	Bitter sweet	Commercial	NOR	Cluster 1
Note: OF: Only Fuji; OG: Only Gala; EOR1: Either Fuji or Gala; EOR2: Either Gala or Fuji; NOR: Neither Fuji nor Gala. * (BEECH, 1972). ** (DRILLEAU, 2005)				

3.4 CONCLUSIONS

The commercial varieties destined to industrial processing are those that present levels of malic acid and phenolic compounds higher than standard Gala and Fuji.

With these methods, the apple should have the following classification to be considered industrial: [1]; acidity and bitterness – bitter sharp; [2] Ratio (TRS/MAL) and bitterness – industrial; [3] SIMCA/KNN - neither Fuji nor Gala; and [4] PCA and HCA – cluster 3.

REFERENCES

ABPM. Associação Brasileira de Produtores de Maçã. **Brazilian Apple Production by variety**. Disponível em: <http://www.brazilianappleexporters.com>. Acesso em: 12 maio. 2011.

ACREE, T. E.; MCLELLAN, M. R. Flavor Components and Quality Attributes In: DOWNING, D. L. (Ed.). **Processed Apple Products**. New York: Springer, 1989. p. 323-341.

BEECH, F. W. English Cidermaking - Technology, Microbiology and Biochemistry. In: HOCKENHULL, D. J. D. (Ed.). **Progress in Industrial Microbiology**. London: Churchill Livingstone, v.11, 1972. p.123-213.

BEECH, F. W.; CHALLINOR, S. W. **Maceration and defecation in cider-making I. Changes occurring in the pectin and nitrogen contents of apple juices**. In: (Ed.), 1951.

BIRUS, T. **Moderne Apfelsaft-Technologie: Das Fruchtsaft-Fachbuch für den Pratiker aus der Fachbuchserie des Fachverlages flüssiges obst**. Oberhonnerfeld: Flüssiges Obst GmbH. 2001. 192 p.

BOULLANGER, E. **Le matieres premieress de la fabrication du cidre**. Industries Agricoles de Fermentation, v.2, n.29. 1903.

DRILLEAU, J.-F. Consolider les connaissances et maîtriser la qualité du produit fini. Revue **Pomme à Cidre**, v.23, p.23-25. 1991.

FERREIRA, E. C.; RODRIGUES, S. H. B. G.; FERREIRA, M. R. M. C.; NÛBREGA, J. A. J.; NOGUEIRA, A. R. A. Análise exploratória dos teores de constituintes inorgânicos em sucos e refrigerantes de uva. **Eclética Química**, v.27, n.special, p.77-90. 2002.

IFU. International Federation of Fruit Juice Producers. **IFU Methods**. Disponível em: <http://www.ifu-fruitjuice.com/ifu-methods>. Acesso em: 15 jul. 2011.

NELSON, N. A photometric adaptation of the Somogyi method for determination of glucose. **Journal Biology Chemistry**, v.153, p.375-380. 1944.

NEUHÄUSER, K. The way from RSK to CoP (AIJN). **Fruit Processing**, v.8, n.2, p.59-63. 1998.

NOGUEIRA, A.; PRESTES, R. A.; SIMÕES, D. R. S.; DRILLEAU, J.-F.; WOSIACKI, G. Análise dos indicadores físico-químicos de qualidade da sidra brasileira. **Semina: Ciências Agrárias**, Londrina, v. 24, n. 2, p.289-298. 2003.

NOGUEIRA, A.; WOSIACKI, G. Sidra. In: VENTURINI FILHO, W. G. (Ed.). **Tecnologia de Bebidas**: Ed. Blucher, 2005. p.383-422.

PADIN, P. M.; PENA, R. M.; GARCIA, S.; IGLESIAS, R.; BARRO, S.; HERRERO, C. Characterization of Galician (N.W. Spain) quality brand potatoes : a comparison study of several pattern recognition techniques. **Analyst**, v. 126, n. 1, p. 97-103, 2001.

RSK. **RSK- Wert. Die Gesamtdarstellung**. Bonn: Flüssiges Obst GmbH. 1987. 204 p.

SCHOBINGER, U.; BARBIC, I.; DÜRR, P.; WALDVOGEL, R. Phenolic compounds in apple juice - positive and negative effects. **Fruit Processing**, v. 6, p. 171-178, 1995.

SOMOGYI, M. A new reagent for the determination of sugar. **Journal Biology Chemistry**, v. 160, p. 61-68, 1945.

TANNER, H.; BRUNNER, H. R. **Getränke Analytik-Untersuchungsmethode für die labor -und Betriebspraxis**. Wädenswill: Helles, 1985.

VIEIRA, R. G.; ROSILENE APARECIDA, P.; DENARDI, F.; NOGUEIRA, A.; WOSIACKI, G. Chemical Pattern of Brazilian Apples. A chemometric approach based on the Fuji and Gala varieties. **Ciência e Tecnologia de Alimentos**, v. 31, n. 2, p. 1-9. 2011.

WORLD APPLE REVIEW. Pullman: Belrose, Inc. 2008. 138 p.

WOSIACKI, G.; NOGUEIRA, A.; SILVA, N.C.C. Brazilian apple production – a few years later. **Fruit Processing**, Schönborn, v. 10, n. 12, p. 472-475, dez., 2000.

WOSIACKI,G.; NOGUEIRA,A.; SILVA,N.C.C.; DENARDI,F.; CAMILO,A.P Apple varieties growing in subtropical áreas. The situation in Santa Catarina – Brazil. **Fruit Processing,** Schönborn, v. 12, n. 1, p. 19-28, 2002.

WOSIACKI, G.; SILVA, N. C. C.; NOGUEIRA, A.; DENARDI, F. The apple and its fructose content. Cultivar Sansa - A case study. **Publicatio UEPG. Ciências Exatas e da Terra, Ciências Agrárias e Engenharias**, v.11, n.2, p.27-39. 2005.

4 CAPÍTULO IV: PATTERN RECOGNITION OF DEPECTINIZED JUICES FROM ADVANCED SELECTIONS OF CRAB APPLES DESCENDENTS

PATTERN RECOGNITION OF DEPECTINIZED JUICES FROM ADVANCED SELECTIONS OF CRAB APPLES DESCENDENTS

ABSTRACT

The processing of apples in Brazil includes high level procedures programmed for the withdrawal of fruits that do not have the profile of quality necessary to be competitive in the market. The disqualified fruits for marketing are considered as raw material the production of juices and fermented beverages. The industry small or large sized demand truly apples industrial with characteristics of acidity and higher phenolic compounds, those presented by Fuji and Gala. These varieties will remain important in the quantitative point of view since they explain 90% of Brazilian production, and are shown in 3rd and 4th place respectively in the world. It should be taken into account that the main explanation for this production bivarietal rests in first place, the cost of production. Another apples grown in commercial orchards, has been researched in order to encourage options more acidic or bitter. Thus appear on the scene of the pomiculture the figure of crab apples, not yet genetically eroded and has been used as a pollinator because of its flowering period wider and more constant. In an experiment with rootstock the commercial variety Baronesa and three wild varieties were evaluated to determine its potential as an adjunct processing or ingredient in the final product. These fruits, harvested in 2008 and 2009 were presented for morphological characterization, and they are unacceptable from the viewpoint of consumers in relation to its size, similar to cherries. According to current procedures in the industry by Premium juice, these apples were characterized as commercial or industrial, and as being acidic or bitter. With the same chemical descriptors related to taste sour, sweet and bitter these selections were compared with wild Fuji and Gala apples through a Principal Component Analysis (PCA) and Hierarchical Cluster (HCA) in an experiment still exploratory. With a pattern recognition approach, now with the intention to predict which group each fruit would be more compatible, the techniques were used KNN (K-Nearest Neighbor) and SIMCA (Soft Independent Modeling of Class Analogy). It was concluded that one selection has the chemical patterns of Fuji, four of Gala and 19 selections were outside the standards commercial varieties used by the chemical descriptors.

Keywords: Multivariate Classification, pollinators of apples, chemometric

RECONHECIMENTO DE PADRÕES DE SUCOS DESPECTINIZADOS DE SELEÇÕES AVANÇADAS DESCENDENTES DE MAÇÃS SILVESTRES

RESUMO

O beneficiamento das maçãs no Brasil compreende procedimentos de alto nível, programados para a retirada das frutas sem perfil de qualidade necessário para o mercado. As desqualificadas para a comercialização são consideradas como matéria prima para a produção de sucos e de fermentados. A indústria de pequeno ou de grande porte demanda maçãs verdadeiramente industriais apresentando características de acidez e de compostos fenólicos mais elevados dos apresentados pela Fuji e Gala. Estas variedades continuarão importantes do ponto de vista quantitativo uma vez que explicam 90% da produção brasileira e figuram em 3º e 4º lugar, respectivamente, em termos mundiais. A principal explicação para esta produção bivarietal repousa, em primeiro lugar, no custo de produção. As demais maçãs produzidas em pomares comerciais têm sido pesquisadas com a finalidade de oferecer opções mais ácidas ou amargas. Assim, surgem no cenário da pomicultura as maçãs silvestres, ainda não erodidas geneticamente e utilizadas como polinizadoras, dado o seu período de florescência, mais amplo e mais constante. De um experimento com porta enxerto da variedade comercial Baronesa e três variedades silvestres foram avaliadas visando determinar o seu potencial como adjunto no processamento ou ingrediente no produto final. Estas frutas, colhidas nas safras de 2008 e 2009 foram apresentadas para caracterização morfológica, e de pronto se deduz que são inaceitáveis do ponto de vista dos consumidores face ao seu tamanho, com dois cm de diâmetro. De acordo com os procedimentos atuais na indústria, estas maçãs foram caracterizadas como comerciais ou industriais, e como sendo ácidas ou amargas. Com os mesmos descritores químicos relacionados aos sabores ácido, doces e amargos estas seleções selvagens foram comparadas às maçãs Fuji e Gala, mediante Análise dos Componentes Principais (PCA) e do Agrupamento Hierárquico (HCA) num experimento ainda exploratório. Com abordagem de reconhecimento de padrões, com a intenção de predizer o grupo compatível para cada fruta, foram usadas as técnicas SIMCA (Soft Independent Modeling of Class Analogy) e KNN (K-Nearest Neighbor). Foi concluído que uma seleção apresenta os padrões químicos da Fuji, quatro da Gala e 19 seleções ficaram fora dos padrões das variedades comerciais pelos descritores químicos usados.

Palavras chave: Análise multivariada, Classificação de maçãs Polinizadoras, Quimiometria

4.1 INTRODUCTION

The successful implantation of commercial orchard of apple in Brazil changed significantly the utilization of the unqualified fruit, initially commercialized at low rate and nowadays, used as industrial. However, the main varieties produced are Fuji and Gala, (*circa* 93%) with features indeed undesirable either for apple juice or for fermentation processing (FAO, 2011; ABPM, 2011). The products from these varieties are too sweet, taste enhanced by the low acidity and also with low bitter taste (VIEIRA et al., 2011; MADRERA et al. 2010).

The juice and cider processing sector demands as raw material apples with a specific profile (VIEIRA et at., 2011; CAMPO et al., 2005) in order to offer high quality product with two main objectives to stimulate the domestic market with new attractive beverages and to compete in the European market with traditional ones (LEA; DRILLEAU, 2003; CAMPO et al., 2008). Due to the industries are interested on the apple quality (Videira, SC), the research agency maintaining an amazing apple trees collection (Caçador - SC) and the UEPG (Ponta Grossa State University) with an experience of more than 30 years in apple science and technology, the main actors in such scenery, still have much to do to indeed give a definite solution and to make clear the new direction to set up for Brazilian apple.

The selection of apple varieties trees to be used for the composition of commercial orchard (DENARDI; CAMILO, 1997) was ever one of the objectives of the research agencies and now Fuji and Gala, with their low costs of production represent a pattern to be respected and followed. The classification of these apples determined by a multivariate procedure points out to a well-defined composition in terms of acidity, sugar and phenolic compound (VIEIRA et al., 2011; CAMPO et al., 2008). To evaluate the experimental apples it is clear that all chemical descriptors for sweet, acidity and bitter tastes must be higher than standard but the production cost should be still lower.

Crab apple trees are currently used in Brazilian commercial orchard due to some features as a large flowering period, regular flower production each season, and rusticity (what means low costs) although still demand temperate climate. As a matter of fact in the Estação Experimental de Caçador – Epagri a current research investigates since 2003 two sets of descendants of the variety Baronesa (*Malus domestica*) producing commercial-size fruits, growing in open pollination with samples of crab apples (Malus silvestris) smaller, with 1cm to 2cm diameter as showed in Figure 4-1 (WOSIACKI et al., 2009).

In a commitment with the former chapter, here is described how is the dispersion of the small-size crab apple descendant according to the sweet, acidity and bitter tastes throughout the descriptors sugar, malic acid and total phenolic compounds.

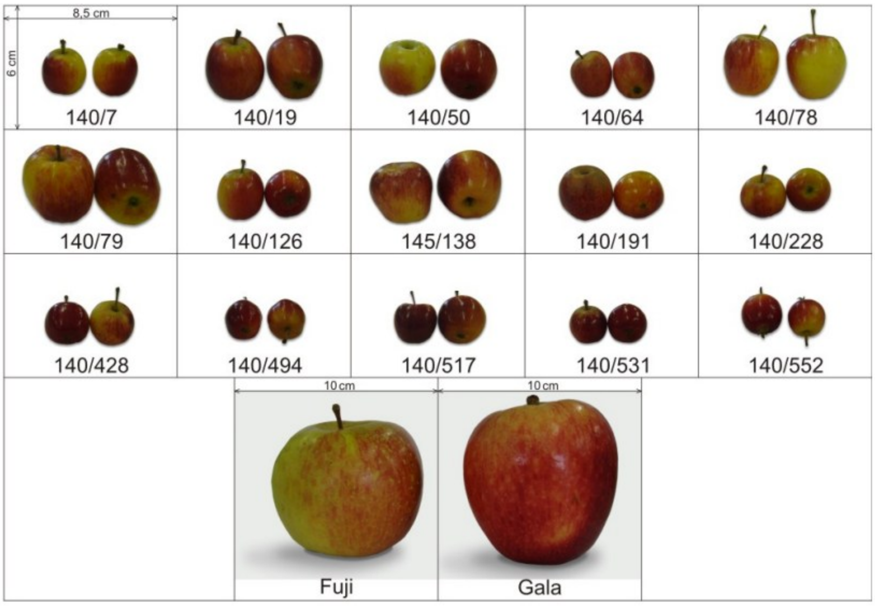


Figure 4-1 - Comparison between the size of the commercial apples and crab apples

4.2 MATERIAL AND METHODS

4.2.1 MATERIAL

Samples of crab apple descendant fruits, a total of 26 (140/228-1, 140/228-2, 140/64, 140/79, 140/98, 140/191-1, 140/191-2, 140/7, 140/19-1, 140/19-2, 140/50, 140/78-1, 140/78-2, 140/126-1, 140/126-2, 140/428, 140/494, 140/517, 140/531, 140/552, 140/105, 140/107, 140/118, 140/250, 140/281, 140/314), manually harvested at *Estação Experimental de Caçador* (EPAGRI) by specialized people in two seasons, were kept at -0.5ºC and left at 18-25ºC overnight before processing. All enzyme preparations were from NOVO and donated by their representative in Brazil, LNF (Bento Gonçalves RS). All chemical products were quality *pro analysis.*

4.2.2 METHODS

4.2.2.1 Apple juice extracted by mechanical press

Samples of crab apple (5 Kg) were selected, sanitized, weighted and fragmented in a knife mill (METVISOR) and the juice from the milled apple was extracted in a hydraulic press (SENGER), under 3 Kgf/cm. The integral apple juice was treated with 3g/hL of pectinolytic enzyme (Pectinex 100) during 60 min at 45 ºC. After paper filtering, the limpid apple juice was stabilized by thermal treatment (80ºC/20 min) and stored in a room temperature. Alternatively, the stabilization was achieved by storing the apple beverage immediately (-18ºC), until further use.

4.2.2.2 Physico-chemical analysis.

Total titrable acidity was determined with NaOH 0,1N and expressed as malic acid in g/100mL (IAL, 2008). Reducing sugar (RS) and total reducing sugar (TRS) were determined directly in the sample and after HCl 0.1 N hydrolysis of sucrose, respectively, by the traditional gravimetric technique proposed by Somogyi (1945) as adapted to colorimetry by Nelson(1944). D-glucose was determined by the enzymatic technique. Sucrose and fructose were estimated by difference. Total phenolic compounds were determined according to Folin Ciocalteau reagent (TANNER; BRUNNER, 1985).

4.2.2.3 Experimental design

The results of the trials were analyzed by simple descriptive statistics (Statistica®) (BARROS NETO et al., 2007). PCA and HCA. Principal Component Analysis (PCA) and the Hierarchical Clustering Analysis (HCA) were used to explore the physico-chemical results and to establish the relationship between the categorical and the continuous and independent variables using the software Pirouette, 4.11 (Infometrix) (FERREIRA et al., 2002). Predictable capacity was evaluated by crossed validation (MARTENS; NAES, 1992). SIMCA e KNN. Pattern recognization models KNN and SIMCA were used to the establishing sets of samples by similarity among physico-chemical properties, determining models to confirm the identification and clustering of unknown samples, for further classification (BEEBE et al., 1998; VIEIRA et al., 2011).

4.3 RESULTS AND DISCUSSION

4.3.1 CHEMICAL DESCRIPTORS

The results of physico-chemical analysis made with the despectinized pressing extract are shown in Table 4-1 in the form of simple descriptive statistics related to a 26 samples set. The most independent continuous variables were malic acid (MAL), total phenolic compounds (TPC) and total reducing sugar (TRS).

Table 4-1 – Simple Descriptive Statistics Analysis

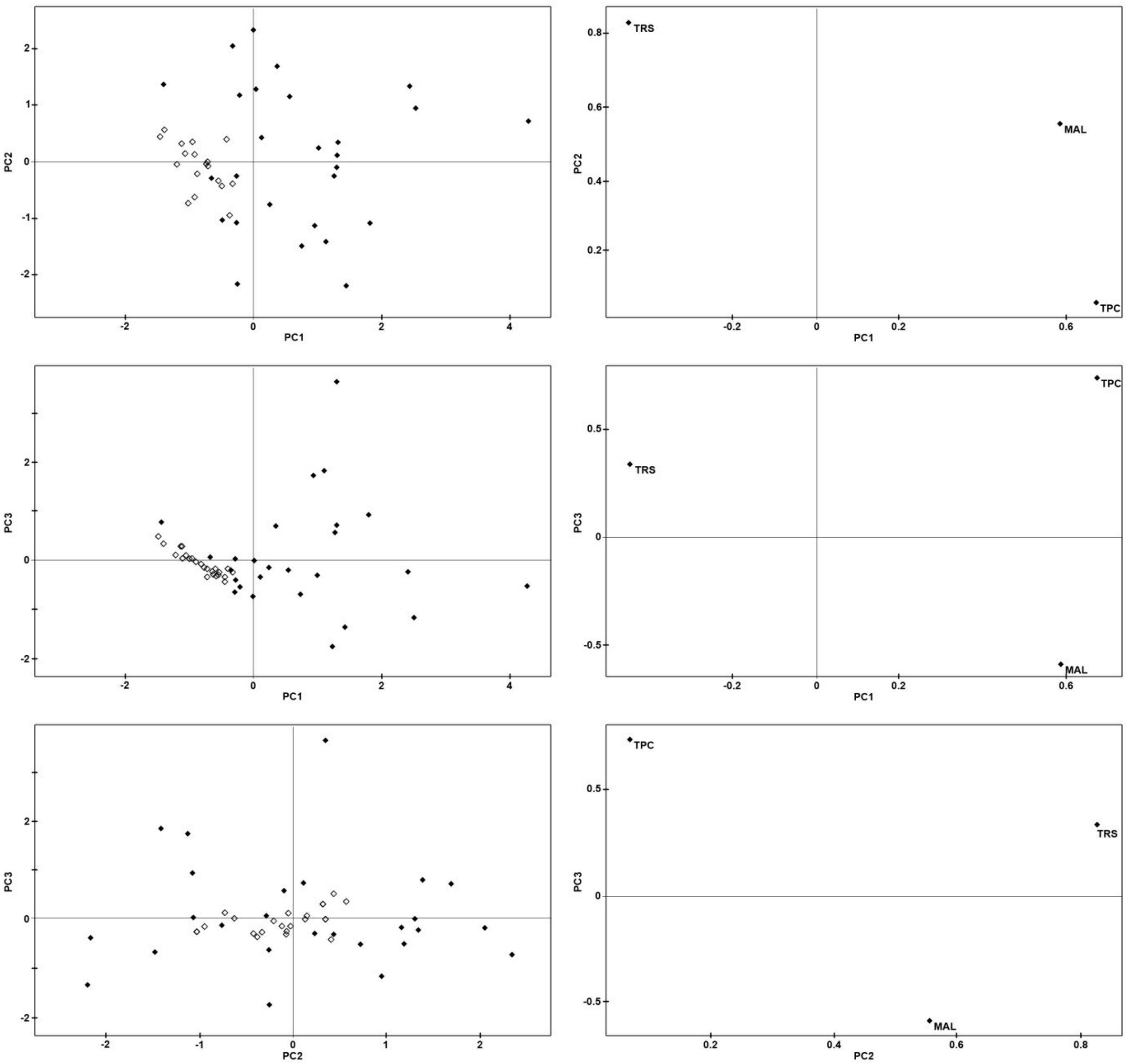
	N	Avg	Min	Max	CV	SD
MAL	26	0.480	0.11	1.05	0	0.25
TRS	26	10.37	4.14	15.54	8	2.88
RS	26	7.21	3.93	10.35	3	1.68
RATIO	26	29.15	7.07	94.14	401	20.01
TPC	26	1764	200	5594	402	1.97
FRU	26	4.32	1.23	7.68	4	1.97
GLC	26	2.89	1.23	4.74	1	1.05
SUC	26	3.15	0.24	8.38	4	1.95
%FRU	26	41.42	17.23	62.24	199	14.12

Avg = Average, Min = Minimum, Max = Maximum, CV = Coefficient of variation, SD = Standard deviation, MAL = acid malic (g/100 mL), TRS = total reducing sugar (g/100 mL), RS = reducing sugar (g/100 mL), RATIO = TRS/MAL, TPC = total phenolic compounds (mg/L), FRU = fructose (g/100 mL), GLC = glucose (g/100 mL), SUC = sucrose (g/100 mL), %FRU = FRU/SUC

4.3.2 EXPLORATORY STEP

The descriptors malic acid (MAL), total phenolic compounds (TPC) and total reducing sugars (TRS), correspond intrinsically the acid taste, bitterness and sweetness, respectively. The Principal Component Analysis (PCA) modifies these chemical descriptors in order to create a new informational set that are linear continuous combination from the former what means, completely independent variables. After testing all chemical descriptors available these three gave the best response in recognizing the categorical variables, what means, the different genotypes still considered advanced selections of crab apples. The 24 different samples of commercial apples from Fuji and Gala varieties were used to an exploratory analysis to define a model that defines a space domain pointing to a quality profile and such results are expressed in the graphic form concerning scores and loadings as shown in the Figure 4-2. It is possible to observe that some wild apples were very close to that of commercial and others do not have the same pattern. The Hierarchical Cluster Analysis (HCA) gave as result the dendrogram shown in Figure 4-3 which defines five cluster at 50% similarity, pointing to four groups, two of them similar and other two, some different features, from the commercial apple.

In Figure 4-2 the scores show that there are a set of categorical variables at the same space of Fuji and Gala are. The loads in Figure 4-2 show that such set features experimental apples like 140/7, 140/64, 140/78, 140/79, 140/517, 140/98, 140/191. Together, this figure is showing that there are a set of crab apples featuring some similarity to the Brazilian commercial standard but also others with higher levels of sugar, or phenolic compounds or even more acidic fruits.



a b

Figure 4-2 – Scores (a) and loadings (b) of PCA with model of Gala and Fuji (◇) and 26 samples of crab apples (◆)

The Hierarchical Cluster Analysis confirm such genotypic discrimination and the results are shown in Figure 4-3 in the format of a dendrogram, poiting to 5 different sets at 50% of similarity (0.5).

Grouping (black) with similarity to the Gala and Fuji, are those with values near or within the limits presented at Vieira et al. (2011), except for 140/78 selection that represents a value of phenolic compounds above the average Brazilian commercial apples. The first group (red) of the dendrogram indicates the selections 140/78, 140/494, 140/98, 140/105, 140/250, 140/281, 140/314, these with values of acidity and phenolic compounds higher than the group of commercial. The third group (blue), made up of selections 140/531, 140/228, 140/118, 140/19 and 140/126, the latter two in two different seasons, phenolic compounds show significantly above the average, but with values near the average of acidity and sugar in average. The fourth group (pink), 140/191, 140/552 and 140/107, are those that stand out of most of the cultivars, since they have much higher values of acidity and phenolic compounds than others. The fifth group (green), 140/50, 140/228, 140/428, are selections with values of acidity and phenolic compounds above average, but sugars with lower values than average.

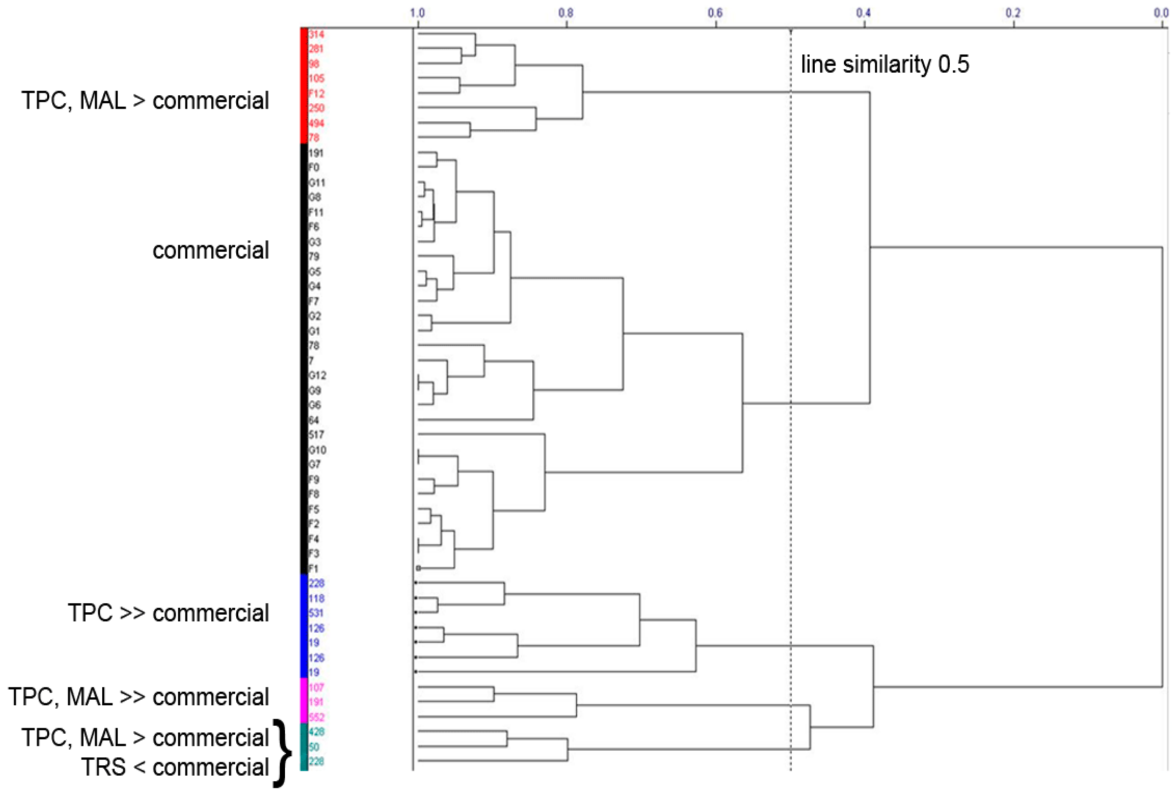


Figure 4-3 – Hierarquical Cluster Analysis of 24 samples of Gala (G) and Fuji (F), and 26 samples of crab apples

The utilization of these both statistical tools reveals clearly which are the samples of advanced selections that can be considered similar or not at 50% of similarity.

4.3.3 VALIDATION OF MODEL EXPLORATION

The validation was performed using the same samples of apple varieties references (Fuji and Gala) and Table 4-2 provides the validity of the final exploratory model without any sample being found outside the limits. The principal components analysis, with a level of statistical analysis of 99.995%, reverts special attention to the analysis of Fuji and Gala, which participate in this process as a point of comparison. The exploratory model was validated using the same samples used for their establishment, and all recognized within the standard Fuji or Gala and no errors were found (Table 4-2), therefore wild selections can be submitted as categorical variables.

Table 4-2 – Validation of exploratory model of Fuji and Gala Apples.

Real	Predicted Fuji	Predicted Gala	Without predicted
Fuji	11	1	0
Gala	1	11	0

Note.: (VIEIRA et al., 2011)

4.3.4 CLASSIFICATION OF CATEGORICAL VARIETIES

SIMCA, related to the PCA because both are based in the establishing of new, linear combination of the originals, can be used to pattern recognition of apples supporting the classification. The Figure 4-4 show the predictive model containing the Cartesian space the varieties of reference and the same amplified space, it is possible to compare the relative positions of output varieties: Fuji and Gala (model) are marked with the black color and the wild with gray.

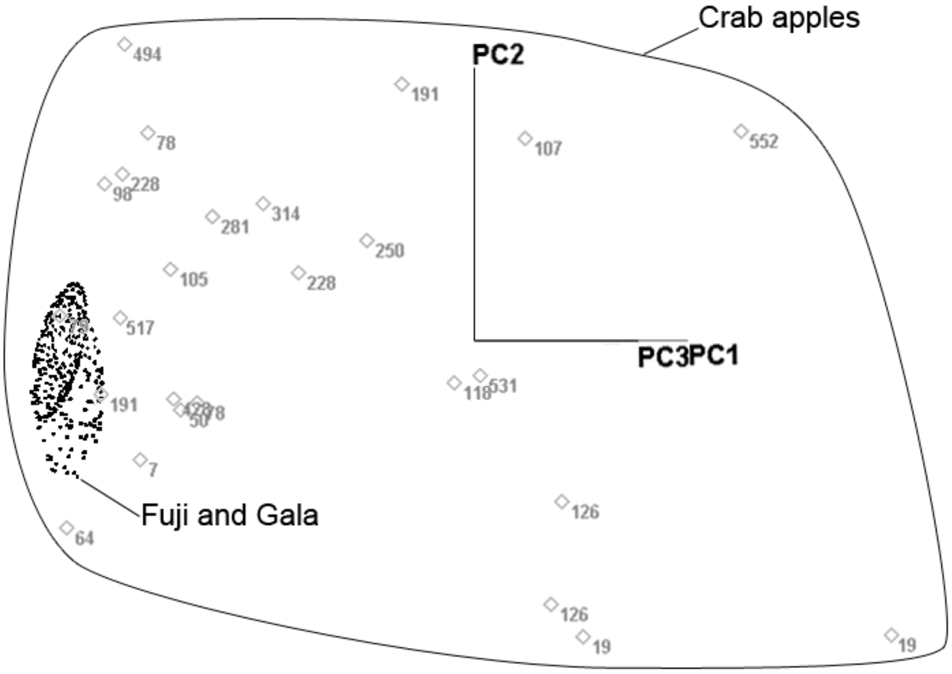


Figure 4-4 – Model of classification of standard apples (black cloud) and (gray) as compared to the wild apple

The tools SIMCA and KNN utilized to the model predict in the exploration phase were effective with 100% correctness in the referential varieties Fuji and Gala, indicated the existence of 5 samples of wild apple with the same patterns of Fuji (1) and Gala (4), and that other samples are out of the referential areas (21), indicated in the Table 4-3.

Table 4-3 – Validation of predicted model with SIMCA

Samples	Predicted as Fuji	Predicted as Gala	Incompatible
Fuji	0	0	0
Gala	0	0	0
Crab apple	1	4	21

Table 4-4 discriminates crab apples, one by one, about the four possible classes, only Fuji, only Gala, Fuji or Gala and neither Fuji nor Gala. Among the classes, Gala or Fuji showed no correspondence. Crab apples showed values quite different of chemical descriptors in relation to commercial apples, but some were classified as only Fuji or only Gala, because these showed some punctual similarity.

Table 4-4 – Discrimination of categorical variables

Class	Selections
Fuji	140/228-1
Gala	140/64, 140/79, 140/98, 140/191-2
Fuji or Gala	None
Neither Fuji nor Gala	140/7, 140/19-1, 140/50, 140/78-1, 140/126-1, 140/191-1, 140/428, 140/494, 140/517, 140/531, 140/552, 140/19-2, 140/78-2, 140/105, 140/107, 140/118, 140/126-2, 140/228-2, 140/250, 140/281, 140/314

Through the tool KNN was made prediction using the model Fuji and Gala and also a test with all crab apples and commercials, the prediction discriminate between the two varieties and in the test among five different classes as shown in Table 4-5. According to the model, the class Gala had more matches (22) than the Fuji (4), because the area of Gala is much more dispersed, and in the test set of 50 samples (26 wild, 12 Fuji and 12 Gala) discriminated five classes (A, B, C, D, E), being three composed only with wild and another two mixed.

The class A consists of crab apples that had high values for all descriptors. In class B, there are selections with very high values for TPC, but with lower MAL. In class C the values of MAL and TPC are slightly above the commercial, but the TRS is higher. And in classes D and E, there are selections that most closely with the commercial.

Table 4-5 – Grouping categorical validaded for KNN

Group	Categorical Variable	Chemical tastes descriptors		
		MAL	TPC	TRS
A	140/552,140/191, 140/107	High	High	Medium
B	140/126,140/126,140/19,140/19	Low	High	Medium
C	140/78, 140/98, 140/105,140/118, 140/228, 140/250,140/281, 140/314, 140/494,140/531,	Medium	Medium	High
D	140/517, 10 Fuji e 4 Gala,	Low	Medium	Medium
E	140/7, 140/50, 140/64, 140/78, 140/79, 140/191, 140/228 , 140/428, (2 Fuji + 8Gala).	Low	Medium	Medium
Gala	140/7, 140/19, 140/50, 140/64, 140/79, 140/126-1, 140/191-1, 140/428, 140/517, 140/531, 140/552, 140/19-2, 140/78-2, 140/105, 140/107, 140/118, 140/126-2, 140/191-2, 140/228-2, 140/250, 140/281, 140/314			
Fuji	140/78-1, 140/228-1, 140/494, 140/98			

MAL = malic acid; TPC = total phenolic compound; TRS = total reducing sugar

4.3.5 COMPARISON OF THE TRADITIONAL PROCEDURE

Compared with the chemometrical methods, the scatter plot of malic acid and total phenolic compounds and ratio (TRS/MAL) and phenolic compounds also show information relevant to the classification of new selections. In Figure 4-4 these two graphs show that the most were classified as bitter or bitter-sharp and also as industrial according to Vieira et al. (2011).

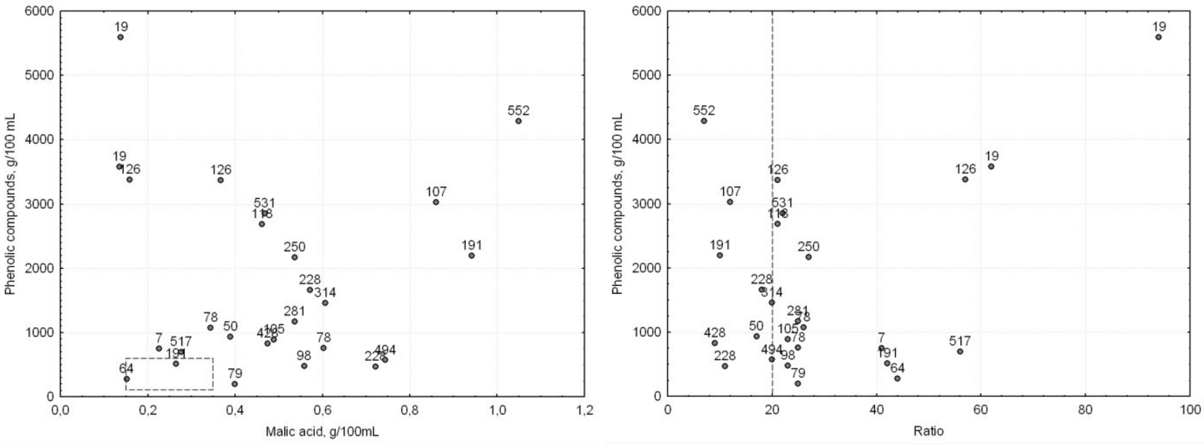


Figure 4-5 – Classification of varieties of apples according with the graphics of dispersion used for process factory.

4.4 CONCLUSIONS

It is possible fix a predicted model of quality of wild apples according to characteristics of commercials quality apples of varieties Fuji and Gala. The profile of commercial quality apples is defined in descriptors chemicals malic acid, total compounds phenolics and total reduced sugar. The crab apples have values higher this chemical descriptors except 4 samples, in similar level that commercials, what don`t become commercials because your format and bad size.

The wild selections 140/107, 140/191 e 140/552 have high level of acid and 140/78, 140/98, 140/105,140/118, 140/228, 140/250,140/281, 140/314, 140/494,140/531, that have high levels of sugar can be used like ingredient in process of juice.

REFERENCES

ABPM. Associação Brasileira de Produtores de Maçã. **Brazilian Apple Production by variety**. Disponível em: <http://www.brazilianappleexporters.com>. Acesso em: 12 maio. 2011.

BARROS NETO, B. D.; SCARMINIO, I. S.; BRUNS, R. E. **Como Fazer Experimentos - Pesquisa e Desenvolvimento na Ciência e na Indústria**. Campinas: UNICAMP. 2007

BEEBE, K. R.; PELL, R. J.; SEASHOLTZ, M. B**. Chemometrics: A Practical Guide**. Wiley-Interscience, v.1. 1998. 360 p.

CAMPO, G. D.; SANTOS, J. I.; BERREGI, I.; MUNDUATE, A. Differentiation of Basque cider apple juices from different cultivars by means of chemometric techniques. **Food Control**, v. 16, n. 6, p. 549-555, 2005.

CAMPO, G. D.; BERREGI, I.; SANTOS, J. I.; DUEÑAS, M.; IRASTORZA, A. Development of alcoholic and malolactic fermentations in highly acidic and phenolic apple musts. **Bioresource Technology**, v. 99, n. 8, p. 2857-2863, 2008.

DENARDI, F.; CAMILO, A. P. Novas culivares de macieira: proposta de nova composição de pomares com polinizadoras / produtoras. **Revista Agropecuária Catarinense**, v.10, n.2, p.25-30. 1997.

FAO. Organização das Nações Unidas para a Agricultura e Alimentação. **Food and Agricultural commodities production**. Disponível em: <http://faostat.fao.org/site/567/default.aspx>. Acesso em: 05 jul. 2011.

FERREIRA, E. C.; RODRIGUES, S. H. B. G.; FERREIRA, M. M. C.; NÔBREGA, J. A.; NOGUEIRA, A. R. A. Análise exploratória dos teores de constituintes inorgânicos em sucos e refrigerantes de uva. **Eclética Química**, v.27, n.especial, p.77-90. 2002.

IAL. Métodos físicos e químicos para análise de alimentos. In: IAL (Ed.). **Normas analíticas**. Sâo Paulo: Instituto Adolfo Lutz, 2008.

LEA, A. G. H.; DRILLEAU, J.-F. Cidermaking. In: LEA, A. G. H.; PIGGOTT, J. R. (Ed.). **Fermented Beverage Production**. London: Springer, 2003. Cap.4. p. 59-87.

MADRERA, R. R.; LOBO, A. P.; ALONSO, J. J. M. Effect of cider maturation on the chemical and sensory characteristics of fresh cider spirits. **Food Research International**, v. 43, n. 1, p. 70-78, 2010.

MARTENS, H.; NAES, T. **Multivariate Calibration**: John Wiley Professional. 1992. 438 p.

NELSON, N. A photometric adaptation of the Somogyi method for determination of glucose. **Journal Biology Chemistry**, v.153, p.375-380. 1944.

SOMOGYI, M. A new reagent for the determination of sugar. **Journal Biology Chemistry**, p.61-68. 1945.

TANNER, H.; BRUNNER, H. R. **Getränke Analytik-Untersuchungsmethode für die labor -und Betriebspraxis**. Wädenswill: Helles, 1985.

VIEIRA, R. G.; ROSILENE APARECIDA, P.; DENARDI, F.; NOGUEIRA, A.; WOSIACKI, G. Chemical Pattern of Brazilian Apples. A chemometric approach based on the Fuji and Gala varieties. **Ciência e Tecnologia de Alimentos**, v. 31, n. 2, p. 1-9, 2011.

WOSIACKI, G.; NOGUEIRA, A.; PRESTES, R. A.; DENARDI, F.; ZARDO, D. M.; VIEIRA, R. G. New wild apple selectfions. Some features of 15 samples obtained under free pollination. **Fruit Processing**, v. 19, n. 4, p. 188-193, 2009.

5 CAPÍTULO V

5.1 CONSIDERAÇÕES FINAIS

No primeiro trabalho [**Capítulo II**], com dados obtidos do banco de dados do GTM relativo a 20 anos de produção (1986-2007), das variedades Fuji e Gala, foi estabelecido o perfil químico do suco, com referência aos teores de ácido málico, açúcar redutor total e compostos fenólicos. Neste trabalho os valores encontrados e definidos foram: acidez entre 0,16 e 0,33 g/100 mL; açúcar entre 9 e 16 g/mL; compostos fenólicos entre 130 e 540 mg/L. Foram testadas algumas variedades consideradas comerciais, presentes no banco de dados para verificar se podiam ser enquadradas dentro dos limites estabelecidos pela Fuji e Gala. Dentre as quinze variedades testadas, treze apresentaram-se dentro dos valores da escala comercial. Desta forma, foi possível estabelecer os valores que as maçãs comerciais brasileiras devem apresentar, dentro dos limites de 0,15 a 0,35 g/100 mL de acidez, 100 e 600 mg/L de compostos fenólicos e razão açúcar redutor total e ácido málico superior a 20.

No segundo trabalho [**Capítulo III**], com os limites definidos da maçã comercial brasileira, foi feito um estudo quimiométrico para classificar algumas variedades comerciais segundo esta escala e este modelo proposto. Utilizando 24 amostras de Fuji e Gala e 24 outras variedades comerciais, foi feita sua avaliação utilizando métodos exploratórios (HCA e PCA) e métodos classificatórios (KNN e SIMCA). Dentre as 24 variedades testadas, apenas cinco apresentaram valores não coincidentes com as maçãs Gala e Fuji, com teores elevados de acidez e compostos fenólicos, o que as tornam mais atraentes para a indústria.

No terceiro e último trabalho [**Capítulo IV**], com as escalas já definidas e testadas, foi realizada a avaliação de novas variedades de maçã, apresentadas pelo centro de pesquisa EPAGRI (Caçador – SC), sendo novamente utilizados os mesmos métodos (PCA, HCA, KNN e SIMCA). Dentre as 26 amostras apresentadas, 21 foram classificadas como maçãs industriais, pois apresentaram valores de acidez e compostos fenólicos superiores os da Gala e de Fuji e a razão entre açúcar redutor total e acidez inferior a 20. Essas seleções apresentam características interessantes para a indústria na fabricação de produtos nobres. Através destas informações, o centro de pesquisa pode continuar ou encerrar a melhoria destas seleções, dependendo do interesse. Conforme exposto anteriormente, o foco agora adotado dentro do centro de pesquisa é desenvolver novas variedades que tenham a relação custo/benefício viáveis à indústria, podendo servir de matéria-prima para produtos nobres.

REFERÊNCIAS

ABPM. Associação Brasileira de Produtores de Maçã. **Brazilian Apple Production by variety**. Disponível em: <http://www.brazilianappleexporters.com>. Acesso em: 12 maio. 2011a.

ABPM. Maçã na escola. **Nossas principais variedades de maçã**. Disponível em: <http://www.macanaescola.org.br>. Acesso em: 15 junho. 2011b.

ABRODO, P. A.; LLORENTE, D. D.; CORUJEDO, S. J.; DE LA FUENTE, E. D.; ÁLVAREZ, M. D. G.; GOMIS, D. B. Characterisation of Asturian cider apples on the basis of their aromatic profile by high-speed gas chromatography and solid-phase microextraction. **Food Chemistry**, v. 121, n. 4, p. 1312-1318, 2010.

ACREE, T. E.; MCLELLAN, M. R. Flavor Components and Quality Attributes In: DOWNING, D. L. (Ed.). **Processed Apple Products**. New York: Springer, 1989. p. 323-341.

ALONSO-SALCES, R. M.; GUYOT, S.; HERRERO, C.; BERRUETA, L. A.; DRILLEAU, J.-F.; GALLO, B.; VICENTE, F. Chemometric classification of Basque and French ciders based on their total polyphenol contents and CIELab parameters. **Food Chemistry**, v. 91, n. 1, p. 91-98, 2005a.

ALONSO-SALCES, R. M.; HERRERO, C.; BARRANCO, A.; BERRUETA, L. A.; GALLO, B.; VICENTE, F. Classification of apple fruits according to their maturity state by the pattern recognition analysis of their polyphenolic compositions. **Food Chemistry**, v. 93, n. 1, p. 113-123, 2005b.

ALONSO-SALCES, R. M.; HERRERO, C.; BARRANCO, A.; LÓPEZ-MÁRQUEZ, D. M.; BERRUETA, L. A.; GALLO, B.; VICENTE, F. Polyphenolic compositions of Basque natural ciders: A chemometric study. **Food Chemistry**, v. 97, n. 3, p. 438-446, 2006.

ANDRE, M. Multivariate Analysis and Classification of the Chemical Quality of 7-Aminocephalosporanic Acid Using Near-Infrared Reflectance Spectroscopy. **Analytical Chemistry**, v. 75, n. 14, p. 3460-3467, 2003.

BARROS NETO, B. D.; SCARMINIO, I. S.; BRUNS, R. E. 25 anos de quimiometria no Brasil. **Química Nova**, v. 29, p. 1401-1406, 2006.

BARROS NETO, B. D.; SCARMINIO, I. S.; BRUNS, R. E. **Como Fazer Experimentos - Pesquisa e Desenvolvimento na Ciência e na Indústria**. Campinas: UNICAMP. 2007

BEEBE, K. R.; PELL, R. J.; SEASHOLTZ, M. B**. Chemometrics: A Practical Guide**. Wiley-Interscience, v.1. 1998. 360 p.

BEECH, F. W. English Cidermaking - Technology, Microbiology and Biochemistry. In: HOCKENHULL, D. J. D. (Ed.). **Progress in Industrial Microbiology**. London: Churchill Livingstone, v.11, 1972. p.123-213.

BEECH, F. W.; CHALLINOR, S. W. **Maceration and defecation in cider-making I. Changes occurring in the pectin and nitrogen contents of apple juices**. In: (Ed.), 1951.

BERRUETA, L. A.; ALONSO-SALCES, R. M.; HÉBERGER, K. Supervised pattern recognition in food analysis. **Journal of Chromatography A**, v. 1158, n. 1-2, p. 196-214, 2007.

BIRUS, T. **Moderne Apfelsaft-Technologie: Das Fruchtsaft-Fachbuch für den Pratiker aus der Fachbuchserie des Fachverlages flüssiges obst**. Oberhonnerfeld: Flüssiges Obst GmbH. 2001. 192 p.

BLANCO-GOMIS, D.; FERNANDEZ-RUBIO, P.; GUTIÉRREZ-ALVAREZ, M. D.; FERNANDEZ-RUBIO, P.; MANGAS-ALONSO, J. J. Use of High performance liquid chromatographic-chemometric techniques to differentiate apple juices clarified by microfiltration and ultrafiltration. **Analyst**, v. 123, n., p. 125-129, 1998.

BLANCO-GOMIS, D.; MANGAS-ALONSO, J. J.; JUNCO-CORUJEDO, S.; GUTIÉRREZ-ÁLVAREZ, M. D. Cider Proteins and Foam Characteristics: A Contribution to Their Characterization. **Journal of Agricultural and Food Chemistry**, v. 55, n. 7, p. 2526-2531, 2007.

BLANCO-GOMIS, D.; MANGAS-ALONSO, J. J.; JUNCO-CORUJEDO, S.; GUTIÉRREZ-ÁLVAREZ, M. D. Characterisation of sparkling cider by the yeast type used in taking foam on the basis of polypeptide content and foam characteristics. **Food Chemistry**, v. 115, n. 1, p. 375-379, 2009.

BOULLANGER, E. **Le matieres premieress de la fabrication du cidre**. Industries Agricoles de Fermentation, v.2, n.29. 1903.

BRASIL, Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa Nº 50**. Brasília, 2002.

BRERETON, R. G. **Applied Chemometrics for Scientists**. Chichester: John Wiley & Sons, Ltd, 2007.

CAMPO, G. D.; BERREGI, I.; SANTOS, J. I.; DUEÑAS, M.; IRASTORZA, A. Development of alcoholic and malolactic fermentations in highly acidic and phenolic apple musts. **Bioresource Technology**, v. 99, n. 8, p. 2857-2863, 2008.

CAMPO, G. D.; SANTOS, J. I.; BERREGI, I.; MUNDUATE, A. Differentiation of Basque cider apple juices from different cultivars by means of chemometric techniques. **Food Control**, v. 16, n. 6, p. 549-555, 2005.

CZELUSNIAK, C.; OLIVEIRA, M. C. S. D.; NOGUEIRA, A.; SILVA, N. C. C.; WOSIACKI, G. Qualidade de maçãs comerciais produzidas no Brasil: Aspectos físico-químicos. **Brazilian Journal of Food Technology**, v.6, n.1, p.25-31, 2003.

DENARDI, F.; CAMILO, A. P. Novas culivares de macieira: proposta de nova composição de pomares com polinizadoras / produtoras. **Revista Agropecuária Catarinense**, v.10, n.2, p.25-30. 1997.

DI ANIBAL, C. V.; ODENA, M.; RUISÁNCHEZ, I.; CALLAO, M. P. Determining the adulteration of spices with Sudan I-II-II-IV dyes by UV-visible spectroscopy and multivariate classification techniques. **Talanta**, v. 79, n. 3, p. 887-892, 2009.

DRILLEAU, J.-F. Consolider les connaissances et maîtriser la qualité du produit fini. Revue **Pomme à Cidre**, v.23, p.23-25. 1991.

FAO. Organização das Nações Unidas para a Agricultura e Alimentação. **Food and Agricultural commodities production**. Disponível em: <http://faostat.fao.org/site/567/default.aspx>. Acesso em: 05 jul. 2011.

FERREIRA, E. C.; RODRIGUES, S. H. B. G.; FERREIRA, M. M. C.; NÔBREGA, J. A.; NOGUEIRA, A. R. A. Análise exploratória dos teores de constituintes inorgânicos em sucos e refrigerantes de uva. **Eclética Química**, v.27, n.especial, p.77-90. 2002.

FERTONANI, H. C. R.; SIMÕES, D. R. S.; NOGUEIRA, A.; WOSIACKI, G. Potencial da variedade Joaquina para o processamento de suco clarificado e vinho seco de maçã**. Ciência e Tecnologia de Alimentos**, v.26, n.2, p.434-440, 2006.

FRÍAS, S.; CONDE, J. E.; RODRÍGUEZ-BENCOMO, J. J.; GARCÍA-MONTELONGO, F.; PÉREZ-TRUJILLO, J. P. Classification of commercial wines from the Canary Islands (Spain) by chemometric techniques using metallic contents. **Talanta**, v. 59, n. 2, p. 335-344, 2003.

GONZÁLEZ, G.; PEÑA-MÉNDEZ, E. M. Multivariate data analysis in classification of must and wine from chemical measurements. **European Food Research and Technology**, v.212, n.1, p.100-107, 2000.

HENSCHKE, R. A maçã e o Mercosul. **Revista Agropecuária Catarinense**, v.7, n.2, p.51, 1994.

IAL. Métodos físicos e químicos para análise de alimentos. In: IAL (Ed.). **Normas analíticas**. Sâo Paulo: Instituto Adolfo Lutz, 2008.

IFU. International Federation of Fruit Juice Producers. **IFU Methods**. Disponível em: <http://www.ifu-fruitjuice.com/ifu-methods>. Acesso em: 15 jul. 2011.

KREUZ, C. L. Rentabilidade da cultura da macieira cultivar Gala em duas densidades de plantio. **Pesquisa Agropecuária Brasileira**, v.37, p.229-235, 2002.

LEA, A. G. H.; DRILLEAU, J.-F. Cidermaking. In: LEA, A. G. H.; PIGGOTT, J. R. (Ed.). **Fermented Beverage Production**. London: Springer, 2003. Cap.4. p. 59-87.

LEEMANS, V.; DESTAIN, M. F. A real-time grading method of apples based on features extracted from defects. **Journal of Food Engineering**, v. 61, n. 1, p. 83-89, 2004.

MADRERA, R. R.; LOBO, A. P.; ALONSO, J. J. M. Effect of cider maturation on the chemical and sensory characteristics of fresh cider spirits. **Food Research International**, v. 43, n. 1, p. 70-78, 2010.

MARTENS, H.; NAES, T. **Multivariate Calibration**: John Wiley Professional. 1992. 438 p.

MOLFETTA, F. A.; BRUNI, A. T.; HONÓRIO, K. M.; DA SILVA, A. B. F. A structure-activity relationship study of quinone compounds with trypanocidal activity. **European Journal of Medicinal Chemistry**, v.40, n.4, p.329-338, 2005.

NELSON, N. A photometric adaptation of the Somogyi method for determination of glucose. **Journal Biology Chemistry**, v. 153, p. 375-380, 1944.

NEUHÄUSER, K. The way from RSK to CoP (AIJN). **Fruit Processing**, v. 8, n. 2, p. 59-63, 1998.

NOGUEIRA, A.; PRESTES, R. A.; SIMÕES, D. R. S.; DRILLEAU, J.-F.; WOSIACKI, G. Análise dos indicadores físico-químicos de qualidade da sidra brasileira. **Semina: Ciências Agrárias**, Londrina, v. 24, n. 2, p. 289-298, 2003.

NOGUEIRA, A.; SANTOS, L. D.; WIECHETECK, F. V. B.; GUYOT, S.; WOSIACKI, G. Efeito do processamento no teor de compostos fenólicos em suco de maçã. **Publicatio UEPG. Ciências Exatas e da Terra, Ciências Agrárias e Engenharias**, v. 9, n. 3, p. 7-14, 2003.

NOGUEIRA, A.; SWIECH, B. D. P.; DENARDI, F.; WOSIACKI, G. Características físico-químicas e sensoriais de suco de maçã clarificado e fermentado. **Publicatio UEPG. Ciências Exatas e da Terra, Ciências Agrárias e Engenharias**, v. 12, n. 3, p. 15-23, 2006.

NOGUEIRA, A.; WOSIACKI, G. Sidra. In: VENTURINI FILHO, W. G. (Ed.). **Tecnologia de Bebidas**: Ed. Blucher, 2005. p.383-422.

PADIN, P. M.; PENA, R. M.; GARCIA, S.; IGLESIAS, R.; BARRO, S.; HERRERO, C. Characterization of Galician (N.W. Spain) quality brand potatoes : a comparison study of several pattern recognition techniques. **Analyst**, v. 126, n. 1, p. 97-103, 2001.

PAGANINI, C.; NOGUEIRA, A.; DENARDI, F.; WOSIACKI, G. Análise da aptidão industrial de seis cultivares de maçãs, considerando suas avaliações físico-químicas (dados da safra 2001/2002). **Ciência e Agrotecnologia**, v. 28, n. 6, p. 1336-1343, 2004.

PANERO, F. S.; VIEIRA, M. D. F. P.; CRUZ, A. M. F. D.; MOURA, M. D. F. V. Application of the exploratory analysis of data in the geographical discrimination of okra of Rio Grande do Norte and Pernambuco. **Orbital: The Electronic Journal of Chemistry**, v. 34, n. 3, p. 33-40, 2009.

RSK. **RSK- Wert. Die Gesamtdarstellung**. Bonn: Flüssiges Obst GmbH. 1987. 204 p.

SABIN, J. G.; FERRÃO, M. F.; FURTADO, J. C. Análise multivariada aplicada na identificação de fármacos antidepressivos. Parte II: Análise por componentes principais (PCA) e o método de classificação SIMCA. **Revista Brasileira de Ciências Farmacêuticas**, v. 40, n., p. 387-396, 2004.

SANTOS, L. C. D.; PAGANINI, C. C.; NOGUEIRA, A.; WOSIACKI, G. Composição Química de Sucos Provenientes de Maçãs de Dez Diferentes Genótipos. Safra 2002/2003. **Brazilian Journal of Food Technology**, v. 8, n. 2, p. 5, 2005.

SCHOBINGER, U.; BARBIC, I.; DÜRR, P.; WALDVOGEL, R. Phenolic compounds in apple juice - positive and negative effects. **Fruit Processing**, v. 6, p. 171-178, 1995.

SHUGAR, G.; BALLINGER, J. **Chemical Technicians' Ready Reference Handbook**: McGraw-Hill Professional, v. 1996. 979 p.

SILVA, F. V.; KAMOGAWA, M. Y.; FERREIRA, M. M. C.; NÓBREGA, J. A.; NOGUEIRA, A. R. A. Discriminação geográfica de águas minerais do Estado de São Paulo através da análise exploratória. **Eclética Química**, v. 27, n., p. 91-102, 2002.

SOMOGYI, M. A new reagent for the determination of sugar. **Journal Biology Chemistry**, v. 160, p. 61-68, 1945.

TANNER, H.; BRUNNER, H. R. **Getränke Analytik-Untersuchungsmethode für die labor -und Betriebspraxis**. Wädenswill: Helles, 1985.

VIEIRA, R. G.; ROSILENE APARECIDA, P.; DENARDI, F.; NOGUEIRA, A.; WOSIACKI, G. Chemical Pattern of Brazilian Apples. A chemometric approach based on the Fuji and Gala varieties. **Ciência e Tecnologia de Alimentos**, v. 31, n. 2, p. 1-9. 2011.

WORLD APPLE REVIEW. Pullman: Belrose, Inc. 2008. 138 p.

WOSIACKI, G.; NAMIUCHI, N. N.; CERIBELLI, M. I. P. F.; SATAQUE, E. Y.; SICHIERI, V. L. F. S.; OLIVEIRA, T. C. R. M.; CESAR, E. O. Estabilidade do suco clarificado de maçã. Parte I - Processo de obtenção do suco de maçã. **Arquivos de Biologia e Tecnologia**, v. 32, n. 4, p. 775-786, 1989.

WOSIACKI, G.; NOGUEIRA, A.; SILVA, N.C.C. Brazilian apple production – a few years later. **Fruit Processing**, Schönborn, v. 10, n. 12, p. 472-475, 2000.

WOSIACKI, G.; NOGUEIRA, A.; SILVA, N. C. C.; DENARDI, F.; CAMILO, A. P. Apple varieties growing in subtropical áreas. The situation in Santa Catarina - Brazil. **Fruit Processing**, v. 12, n. 1, p. 19-28, 2002.

WOSIACKI, G.; SILVA, N. C. C.; NOGUEIRA, A.; DENARDI, F. The apple and its fructose content. Cultivar Sansa - A case study. **Publicatio UEPG. Ciências Exatas e da Terra, Ciências Agrárias e Engenharias**, v.11, n.2, p.27-39. 2005.

WOSIACKI, G.; NOGUEIRA, A.; PRESTES, R. A.; DENARDI, F.; ZARDO, D. M.; VIEIRA, R. G. New wild apple selectfions. Some features of 15 samples obtained under free pollination. **Fruit Processing**, v. 19, n. 4, p. 188-193, 2009.

XIE, L.; YING, Y.; YING, T. Combination and comparison of chemometrics methods for identification of transgenic tomatoes using visible and near-infrared diffuse transmittance technique. **Journal of Food Engineering**, v. 82, n. 3, p. 395-401, 2007.

XIE, L.; YING, Y.; YING, T. Classification of tomatoes with different genotypes by visible and short-wave near-infrared spectroscopy with least-squares support vector machines and other chemometrics. **Journal of Food Engineering**, v. 94, n. 1, p. 34-39, 2009.

XING, J.; VAN LINDEN, V.; VANZEEBROECK, M.; DE BAERDEMAEKER, J. Bruise detection on Jonagold apples by visible and near-infrared spectroscopy. **Food Control**, v. 16, n. 4, p. 357-361, 2005.

XING, J.; BRAVO, C.; MOSHOU, D.; RAMON, H.; DE BAERDEMAEKER, J. Bruise detection on Golden Delicious apples by vis/NIR spectroscopy. **Computers and Electronics in Agriculture**, v. 52, n. 1-2, p. 11-20, 2006.

XING, J.; SAEYS, W.; DE BAERDEMAEKER, J. Combination of chemometric tools and image processing for bruise detection on apples. **Computers and Electronics in Agriculture**, v. 56, n. 1, p. 1-13, 2007.