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FACULDADE DE FARMÁCIA
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LEGUMES MINIMAMENTE PROCESSADOS E PRÉ-COZIDOS MANTIDOS EM ATMOSFERA MODIFICADA

Avaliação Sensorial, Físico-Química/Compostos Bioativos e Microbiológica

Evolução da Qualidade ao Longo do Armazenamento com Apoio da Quimiometria

Carla Dulcinea Andrade Cerqueira de Borlido Barbosa

Porto, 2014

Tese de Doutoramento

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Dissertação de candidatura ao grau de Doutor em Ciências Farmacêuticas - Nutrição e Química dos Alimentos, apresentada à Faculdade de Farmácia da Universidade do Porto

Orientação

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Porto

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Resumo

Os legumes prontos-a-consumir, minimamente processados (MP), apenas cortados e lavados, ou pré-cozidos (PC), são habitualmente usados como acompanhamento das dietas atlântica e mediterrânica. São considerados saudáveis pelo importante aporte de micronutrientes e compostos bioativos possuidores de atividade antioxidante.

Portugal é reconhecido como um país produtor de vegetais de elevada qualidade, sendo os procedimentos de manipulação dos vegetais e da embalagem essenciais para garantir a extensão da vida útil, mantendo a frescura e o valor nutricional. Geralmente, estes produtos estão disponíveis em embalagens com atmosfera modificada (AM). O uso de elevadas concentrações de dióxido de carbono (CO_2) conjugado com uma percentagem reduzida ou ausência de oxigénio (O_2) poderá favorecer a interação dos micronutrientes com a atmosfera envolvente. Como consequência, alguns parâmetros físico-químicos (pH, humidade, cinzas, minerais, acidez total, textura e análise da microestrutura, cor, atividade antioxidante, fenóis totais, flavonoides, carotenoides e antocianinas) bem como as propriedades organoléticas, podem ser afetados.

O trabalho desenvolvido teve como objetivo principal melhorar as condições de conservação de diferentes legumes (couve, cenoura, feijão-verde, pimentos verde, vermelho e amarelo) com vista à extensão dos habituais prazos de validade. Para tal, foram simuladas práticas industriais de processamento mínimo e de pré-cozinhado, embalamento em AM e armazenamento refrigerado. Estudou-se ainda a influência de alguns materiais de embalagem e diferentes misturas de gases para definição das atmosferas modificadas a implementar. A qualidade e segurança dos produtos foram monitorizadas, recorrendo a parâmetros sensoriais, físico-químicos, nutricionais e microbiológicos. Na avaliação sensorial recorreu-se a um painel de provadores, selecionado previamente para este trabalho, e treinado para avaliação de atributos específicos para este tipo de produtos. Foram, também, realizadas análises com consumidores para avaliação da aceitabilidade dos produtos.

Relativamente aos legumes MP foram testadas diferentes atmosferas (10, 15% O_2 e 40, 45% CO_2) e dois tipos de embalagem (filmes com diferentes características de permeabilidades) ao longo de 10 e 17 dias de armazenamento. A melhor combinação de gases foi 10/45, com perdas negligenciáveis na atividade antioxidante, sem alteração nas propriedades organoléticas e desenvolvimento microbiano aceitável. Os resultados obtidos permitiram o alargamento do prazo de validade em cerca de 4 dias, sem colocar em risco a qualidade e segurança destes produtos.

No que respeita aos PC, e à semelhança dos MP, foram ensaiadas inicialmente três atmosferas (%O_2/%CO_2: 0/40; 2,5/40; 2,5/60) ao longo de 20 dias de armazenamento. A

atmosfera com melhor comportamento (0/40) foi ensaiada durante um período mais longo (28 dias). Apenas pequenas alterações, sem tendência consistente, foram observadas nos parâmetros físico-químicos e microbiológicos. Tal foi confirmado pela avaliação do painel de provadores.

No tratamento e interpretação dos dados experimentais foram usadas técnicas quimiométricas (análise de componentes principais (ACP) clássica, ACP com auto*biplots*, análise de variáveis canónicas, análise de correlações canónicas, entre outras). Estas metodologias mostraram-se facilitadoras da redução do volume de informação permitindo chegar a conclusões assertivas.

Os resultados obtidos permitem concluir pela viabilidade de extensão do tempo de vida útil dos diferentes produtos estudados pelo recurso às técnicas de preservação implementadas.

Com esta informação, a indústria e os produtores poderão usufruir de mais alguns dias de armazenamento, que ajuda a racionalizar operações de colheita e pós-colheita e facilitará as operações de logística. No final da cadeia de distribuição, o consumidor beneficiará de mais variedade de vegetais, de elevada qualidade organolética e nutricional e, ainda, permitirá a redução do desperdício alimentar.

Palavras-chave: Legumes prontos-a-consumir; legumes minimamente processados; pré-cozidos; embalagem em atmosfera modificada; quimiometria; análise sensorial; atividade antioxidante; compostos bioativos; textura e microestrutura

Abstract

Ready-to-use (RTU) vegetables are considered a need of modern lifestyle and a requirement of many catering activities. RTU vegetables may be presented do the consumers as minimally processed (MP) or precooked (PC) both packed ready-to-eat.
MP (fresh-cut and washed) or precooked (PC), are commonly used as side dishes in the Atlantic and Mediterranean diets being considered healthy due to the fact that they are important sources of micronutrients and of a large variety of bioactive compounds which possess antioxidant activity. Portugal is recognized as country producing high quality vegetables, and it is therefore essential to implement procedures for product manipulation and packaging to ensure extended shelf life, keeping freshness and nutritional value.

Generally, RTU products are available in packs with modified atmosphere (MAP). The use of high concentrations of carbon dioxide (CO_2) combined with a reduced percentage or absence of oxygen (O_2), could favour the interaction of micronutrients, metabolic pathways and natural microflora with the surrounding atmosphere. As a result, some physicochemical parameters (pH, moisture, ash, minerals, acidity, texture microstructure, colour, antioxidant activity, total phenolic, flavonoids, carotenoids and anthocyanins) and the organoleptic properties may be affected.

The work aimed at improving preservation conditions of different vegetables (cabbage, carrots, green beans, green, red and yellow peppers) in an attempt to extend the usual "best before" dates. For that, the work was carried out simulating industry procedures of minimal processing, pre-cooking, packaging with modified atmosphere and refrigerated storage. the influence of the packaging materials and different gas mixtures was studied in order to defining the best modified atmosphere to be implemented. The quality and safety of products were monitored, using sensory, physical, chemical, nutritional and microbiological parameters. For sensory evaluation a panel of judges, previously trained for this work was used with a special training in this type of products. Some consumer studies to evaluate product acceptability were also performed.

Concerning MP vegetables, different atmospheres were tested (10, 15 and 40% O_2, 45% CO_2) and two types of packaging (films with different permeability characteristics) over 10 and 17 days of storage. The best combination of gases was 10/45, with negligible losses in antioxidant activity, with no change in organoleptic properties and acceptable microbial development. The conditions used allowed shelf life extension by about four days, without quality and safety concerns.

With regard to the PC vegetables, three atmospheres (% O_2 /% CO_2: 0/40, 2.5 / 40 and 2.5 / 60) were initially tested throughout 20 days of storage. The atmosphere with the best performance (0/40) was tested over a longer period (28 days). Results showed minor

changes, without a consistent trend in the physicochemical and microbiological parameters. This was confirmed by the sensory judges' evaluation.

For data mining and interpretation of experimental results several chemometric tools were used: principal component analysis (PCA) classic, PCA with AutoBiplots.PCA( ) function, canonical variables, canonical correlation analysis, among others. These methods were very helpful to reduce the volume of information, to help previewing product evolution over shelf life and to draw more accurate conclusions. The results indicate adequate, allowing that the studied and proposed preservation techniques are shelf life extension of different king of vegetables.

With this information, the industry and producers may benefit of a few days of storage, which may help rationalizing harvesting and post harvesting procedures which will facilitate logistics operations. At the end of the food supply chain, the consumers will have more variety of vegetables, with high standards of freshness and nutritional quality and, hopefully, food waste is reduced.

Keywords: ready-to-use vegetables; minimally processed vegetables; pre-cooked vegetables; modified atmosphere packaging; chemometrics; sensory analysis; antioxidant activity; bioactive compounds; texture and microstructure

Publicações e Comunicações no Âmbito deste Trabalho

- ***Capítulos de livros***

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Barbosa C., Oliveira M.B.P.P., Alves M.R.
Editado por: M.B.P.P. Oliveira, I. Mafra, J. Amaral
Kerala, India: Transworld Research Network, **2011**, pp. 237-268.

A Comparison of Several Methods to Present The Displays of Principal Component Analysis, with a Special Reference to The Use of Predictive Biplots and The Automatic Selection of Biplot Axes. In: Handbook of Research on Computational Simulation and Modelling in Engineering
C. Barbosa, M.B.P.P. Oliveira, M.R. Alves
Editado por: F. Miranda, C. Abreu
IGI Global (Aceite para publicação prevista em 2015)

- ***Publicações em revistas com revisão por pares***

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C. Barbosa, S. Rocha, L. Silva, M. R. Alves, M. B. P. P. Oliveira. (2012). Evolução Da Qualidade Sensorial e Atividade Antioxidante de Vegetais Prontos a Usar ao Longo do Armazenamento. No livro de atas do 11º Encontro Nacional de Química dos Alimentos, 16-19 Setembro, Instituto Politécnico de Bragança, Bragança, Portugal.

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- ***Comunicações em Congressos ou Reuniões Cientificas***

Comunicações orais

Carla Barbosa, M.R. Alves, O. Morais, M.B.P.P. Oliveira (2014). Sensory Evaluation of Ready-To-Eat Cabbage and Green Beans in MAP: Correlation among mineral, bioactive and textural composition. 12º Encontro de Química dos Alimentos (12º EQA), 10-12 de setembro, Lisboa Portugal.

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Lista de Abreviaturas mais comuns

AM	Atmosfera modificada
ANOVA	Análise de variância
CFU	Unidades formadoreas de colónias / Colony forming unit
CV	Variáveis canónicas / Canonical Variates
CVA	Análise de variáveis Canónicas / Canonical Variates Analysis
DW	Peso seco / dry weight
DPPH	1,1-diphenyl-2-picrylhydrazyl
EFSA	European Food Safety Authority
FW.	peso fresco / fresh weight
FAO	Food and Agriculture Organization of the United Nations
ISO	International Organization for Standardization
L* , a*, b*	Parametros de cor
m/v	mass/volume
MAP	Modified Atmosphere Package
MeOH	Methanol
MP	Minimamente processado
mspe	Mean standard predictive error / erro preditivo padrão médio
n.d.	not detected
N_2	Nitrogen
PC	Precozidos / Precooked
pH	Hydrogen ion potential
RH	Relative humidty
Rt, RT	retention time
spe.	standard predictive error / erro preditivo padrão
vs.	Versus
WHO	World Health Organization
Wt	Peso composicional /compositional weight

PARTE I – FUNDAMENTAÇÃO TEÓRICA

A Parte I desta tese inclui a motivação, os principais objetivos deste trabalho (capítulo I) e uma revisão bibliográfica de apoio (capítulo II).

A revisão bibliográfica foca, inicialmente, os alimentos prontos a consumir (*ready-to-use*), quer minimamente processados, quer pré-cozidos (pontos 1 e 2). Neste âmbito faz-se referência à problemática da embalagem, sistemas de embalagem e tipos de atmosferas usados (ponto 3). Seguem-se as estratégias de controlo da qualidade através dos parâmetros organoléticos, físico-químicos e microbiológicos (ponto 4). Por fim discute-se a importância do tratamento de dados (ponto 5), valorizando o recurso a técnicas quimiométricas avançadas.

I Motivação, enquadramento, objetivos e organização

De acordo com Rodgers (1) o vazio entre a indústria da restauração e a ciência e tecnologia de alimentos mantém-se apesar dos enormes avanços científicos ao nível das técnicas de deteção e quantificação dos indicadores da qualidade e segurança dos alimentos. A falta de conhecimento dos operadores, ao nível da embalagem, nomeadamente a que recorre a atmosferas modificadas é também uma realidade. Estes factos resultam na restrição da produção em grande escala e na limitação das áreas geográficas alvo. Segundo o autor, é fundamental conseguir uma integração de conhecimentos científicos, tecnológicos e de gestão. E neste sentido, tem-se assistido a um grande esforço por parte dos investigadores na área da conservação de alimentos, recorrendo a técnicas de refrigeração, congelação, embalamento sob condições de vácuo ou em atmosferas modificadas (2-5). Contudo, a implementação destas técnicas na indústria, e no caso específico da produção de refeições em unidades de restauração coletiva, os estudos disponíveis são em regra unidisciplinares, limitando-se, na maioria das vezes, ao controlo de alguns parâmetros microbiológicos. Um exemplo típico é o estudo da qualidade microbiológica e segurança das refeições servidas a utentes de cantinas escolares (6).

No sentido de alterar esta situação foi proposto ao Instituto Politécnico de Viana do Castelo (IPVC) o desenvolvimento do projeto "*Transformação das cantinas em unidades de produção*". Este tinha por objetivo a alteração do conceito de serviço de alimentação coletiva, a partir da integração e racionalização dos recursos existentes numa instituição pública, o IPVC. Ao contrário do que acontece em grandes organizações (grandes hotéis, barcos/paquetes de cruzeiros de luxo que podem sustentar vários *chefs* e o seu alargado *staff*) as instituições de ensino, saúde, geriátricas e estabelecimentos prisionais (ou de correção e inserção social) têm dificuldade em suportar tais custos, pelo que necessitam de otimizar os recursos humanos, recorrendo à tecnologia e à centralização da produção das refeições que disponibilizam (7).

A missão do referido projeto centra-se na melhoria da qualidade nutricional e organolética das refeições servidas nas cantinas e nos bares do IPVC, através da implementação de modelos atuais e/ou inovadores de confeção e disponibilização de refeições. Simultaneamente, visava, também, estimular a prática de uma alimentação saudável por parte da sua comunidade académica. As cantinas escolares passariam a confecionar alimentos durante todo o dia útil de trabalho, conservando-os por diferentes métodos, de modo a que, em qualquer cantina, existam sempre refeições prontas a ser servidas, recorrendo a uma simples regeneração para a temperatura normal de consumo.

Em resumo, este projeto visava o desenvolvimento de receitas e a confeção de alimentos, suportados por um trabalho permanente de investigação, desenvolvimento e avaliação, explorando todas as sinergias possíveis entre serviços e atividades letivas, de investigação e de prestação de serviços existentes, tal como Svetlana Rogers (1,7) sugere nos seus estudos.

O projeto implementa uma nova ótica de gestão com a racionalização das atividades de aquisição, processamento e consumo dos alimentos, gerando mais-valias para a instituição como a redução do desperdício, tão elevado neste tipo de atividades de restauração. Essa racionalização será extensível aos recursos humanos envolvidos no projeto, nomeadamente os já afetos às cantinas e bares, e às instalações que serão igualmente racionalizadas em função das novas realidades. Neste projeto, as atividades de investigação, onde se integra o trabalho desta tese, visavam o aumento do tempo de vida útil dos produtos, recorrendo à embalagem em atmosferas modificadas dos produtos minimamente processados e confecionados.

Alguns trabalhos publicados (8-13) compararam diferentes metodologias de conservação e embalagem de produtos frescos, frutas e vegetais em natureza, ou minimamente processados, destinados a consumidores de médias e grandes superfícies. De uma maneira geral efetuaram avaliações microbiológicas a par de avaliações sensoriais dos produtos, recorrendo a painéis de consumidores, de número reduzido. Estes avaliaram propriedades organoléticas (aspeto geral dos produtos, odor, cor e textura), com suporte em escalas hedónicas, ou simplesmente realizando testes de aceitabilidade.

Com a necessidade crescente de consumir alimentos fora de casa, a procura de refeições económicas e nutricionalmente equilibradas, tem vindo a aumentar. As unidades de restauração pertencentes a grandes cadeias, servidas por uma cozinha central, ou as unidades de restauração coletiva que servem escolas, universidades, hospitais, centros prisionais ou entidades industriais privadas, devem responder às necessidades e simultaneamente desempenhar a sua função na comunidade. É, então importante que os técnicos envolvidos possuam informação acerca das matérias-primas e ingredientes mais apropriados à confeção dos alimentos e, também, como estes reagem após o processamento, para evitar perda da qualidade e segurança dos produtos processados.

Os legumes prontos a usar, na forma fresca, apenas cortados e lavados, minimamente processados (MP) ou pré-cozidos (PC), são habitualmente usados como acompanhamento das dietas atlântica e mediterrânica. Portugal é reconhecido como um país produtor de vegetais de elevada qualidade, sendo os procedimentos de manipulação dos vegetais e da embalagem essenciais para garantir a extensão da vida útil, mantendo a frescura e o valor nutricional característicos.

Na sua maioria, os legumes prontos a consumir estão disponíveis em embalagens com atmosfera modificada (AM). O uso de elevadas concentrações de dióxido de carbono (CO_2) conjugadas com uma percentagem reduzida ou ausência de oxigénio (O_2) poderá favorecer a interação dos micronutrientes com a atmosfera envolvente. Como consequência, alguns parâmetros físico-químicos (pH, teor de humidade, cor, entre outros) bem como as propriedades organoléticas podem ser afetados (14).

Assim, o trabalho desenvolvido nesta tese de doutoramento teve como objetivo principal melhorar as condições de conservação de diferentes legumes (couve coração, cenoura, feijão-verde, pimentos verde, vermelho e amarelo). Foram simuladas práticas industriais de processamento mínimo e de pré-cozinhado, embalamento em AM e armazenamento refrigerado. Para tal foi realizado um estudo da influência de alguns materiais de embalagem e de diferentes misturas de gases para definição das atmosferas modificadas a implementar nas cantinas do IPVC. A qualidade e segurança dos produtos em estudo foram monitorizadas recorrendo a parâmetros sensoriais, físico-químicos, nutricionais, microbiológicos.

Assim, entre os objetivos do trabalho de investigação desenvolvido ao longo do estudo incluem-se:

1- Testar misturas de gases (CO_2, O_2 e N_2) a introduzir na embalagem dos produtos minimamente processados e pré-cozidos (couve coração (*Brassica oleracea* L.), cenoura (*Daucus carota* L.), feijão verde (*Phaseolus vulgaris* L.) e pimentos verde, vermelho e amarelo (*Capsicum annum*). A qualidade foi avaliada ao longo de 10 e 20 dias de armazenamento, respetivamente, através de parâmetros físico-químicos, sensoriais e microbiológicos.
2- Selecionar a atmosfera ideal, com base nos resultados obtidos e avaliação do comportamento ao longo de tempos de armazenamento mais longos (17 e 28 dias, no caso de legumes MP e PC, respetivamente). A evolução da qualidade dos produtos refere-se à qualidade nutricional, microbiológica e sensorial, avaliadas de forma integrada por ferramentas estatísticas de análise multivariada.
3- Seleção e treino de um painel para avaliar as propriedades organoléticas dos vegetais minimamente processados e pré-cozidos armazenados em MAP, através de uma análise descritiva quantitativa. A análise sensorial permitirá também expressar a evolução da qualidade em termos utilizados pelos consumidores.
4- Implementar processos eficazes para proceder à seleção e treino de painéis de provadores para avaliação segundo a metodologia QDA, com resultados aferidos

através de *biplots* aplicados à análise multivariada. Os resultados serão também usados para testar, melhorar e validar as metodologias estatísticas.

5- Estudar a evolução dos compostos bioativos, responsáveis pela atividade antioxidante destes produtos. Foram avaliados os teores totais de compostos fenólicos, flavonoides, antocianinas, carotenoides por espectrofotometria. A caracterização e monitorização ocorreu nos diferentes legumes minimamente processados e pré-cozinhados.

6- Utilização e otimização de métodos de análise estatística multivariada, aplicada a dados químicos e sensoriais, recorrendo a funções escritas em R (R *Project for Statistical Computing*, http://www.r-project.org/, programa de acesso livre).

A tese que se apresenta está organizada em parte teórica e experimental. Na parte teórica faz-se uma revisão bibliográfica e uma abordagem dos principais objetivos do trabalho. Inicia-se com alimentos prontos a consumir (*ready-to-use*) quer minimamente processados quer pré-cozidos. Referem-se a problemática da embalagem, sistemas de embalagem e tipos de atmosferas. Seguem-se as estratégias de controlo da qualidade, quer através dos parâmetros organoléticos, físico-químicos e microbiológicos. Por fim discute-se a importância do tratamento de dados.

Na parte II do trabalho, desenvolvimento experimental, apresentam-se os trabalhos publicados/submetidos acerca dos ensaios efetuados com os diferentes legumes e atmosferas, sempre ao longo do tempo de armazenamento (capítulos IV, V, VI e VII). O capítulo III, é dedicado às técnicas de análise estatística multivariada como ferramentas da quimiometria. Na Figura 4 apresenta-se a planificação do trabalho desenvolvido nesta tese de doutoramento.

Legumes prontos a consumir

Minimamente processados (MP)

Pré-cozidos (PC)

Embalagens com AM, seleção de:
- mistura de gases ($O_2/CO_2/N_2$)
- material de embalagem

Armazenamento refrigerado (T 5 ºC)

Controlo Sensorial

- Treino do painel
  15 provadores
- QDA
  Definição de atributos
  17 atributos relacionados com: aparência, textura, cheiro e sabor
  Escala de intensidades de 13 pontos

Controlo Físico-químico

- pH
- Acidez total
- Cinzas
- Minerais
- Textura
- Cor (L*, a*, b*)
- Análise da microestrutura SEM

Compostos bioativos:
- Atividade antioxidante (EC_{50} - DPPH)
- Fenóis totais (Folin Ciocalteau/ ácido gálhico)
- Flavonoides totais
- Carotenoides totais
- Antocianinas

Controlo Microbiológico

- Contagem de microrganismos aeróbios totais a 30 ºC por *plate count agar*

Análise de Dados

- ACP (Análise componentes Principais)
- *Biplots* obtidos a partir da função autobiplot.PCA , disponível para o R (versão 2.11.1)

Parte experimental

Legumes Minimamente processados

- Quality assessment and acceptability of fresh-cut vegetables stored under modified atmosphere packaging

Legumes Pré-cozidos

- Modified atmosphere packaging of precooked vegetables: effect on chemical parameters, sensory perception and acceptability by consumers

Pimentos em AM

- Active MAP improves shelf life of fresh cut bell peppers as assessed by a broad set of quality parameters

MINERAIS

- Read-to-eat (fresh-cut and precooked) cabbage and green beans: effect of MAP on mineral and microstructure, bioactive compounds and sensory quality

TRATAMENTO DE DADOS

- Chemometrics in food authentication

- A comparison of several methods to present the displays of principal component analysis, with a special reference to the use of predictive biplots and the automatic selection of biplot axes

Figura 1- Planificação do trabalho desenvolvido

II Alimentos prontos a consumir (*ready-to-use*)

Os produtos prontos a consumir são uma opção de compra cada vez mais comum, por serem fáceis de preparar e necessitarem de reduzidos tempos para a sua confeção. O ritmo de vida atual, nível de conhecimentos e a exigência dos consumidores bem como requisitos da restauração industrial (cantinas, *catering, takeaway*, etc.) têm estimulado a evolução da ciência dos alimentos, no que se refere à sua versatilidade, conveniência de utilização, satisfação de consumo, qualidade nutricional e segurança. Atualmente, os consumidores esperam que os alimentos sejam seguros (sem microrganismos potencialmente patogénicos, nem resíduos químicos ou físicos) e ao mesmo tempo, tenham elevado valor nutricional e sensorial.

Os produtos alimentares podem ser comercializados de diversas formas, quer em natureza, quer sujeitos a uma preparação prévia seguida de transformação. Assim, hoje em dia, relativamente ao processamento efetuado, são consideradas cinco gamas de produtos: os produtos de 1ª gama, que são os alimentos naturais sem tratamento; os produtos congelados, ou produtos de 2ª gama, que têm a vantagem de se poder conservar durante períodos longos, mantendo as características próximas das originais; os produtos de 3ª gama ou produtos enlatados/em conserva, que são produtos cozinhados e esterilizados na própria embalagem, prontos a consumir e conservados à temperatura ambiente por períodos de tempo muito longos (superiores a um ano). Os hortofrutícolas de 1ª gama deram origem a produtos de 4ª gama ao serem escolhidos, lavados/desinfetados, cortados e acondicionados em atmosfera modificada. Todo este processamento visa aumentar o tempo de vida dos produtos frescos ou minimamente processados (15, 16). Por último, a 5ª gama industrial diz respeito aos alimentos pré-cozinhados, prontos a consumir como tal, ou após um simples aquecimento, e conservados sem congelação, uma vez que resultam de processos de produção que asseguram suficiente estabilidade após confeção (16, 17).

Legumes frescos e cozidos são muitas vezes consumidos como acompanhamento nas dietas Mediterrânica e Atlântica (18). Em Portugal, pela sua situação geográfica, estão presentes aspetos de ambas as dietas, sendo os legumes frescos consumidos mais frequentemente no verão, e os legumes pré-cozidos preferencialmente em estações mais frias. Os legumes podem ser apresentados aos consumidores minimamente processados (frescos) ou como acompanhamentos na forma de pré-cozidos, embalados em atmosfera modificada (AM) e refrigerados, assumindo assim uma posição no mercado de pronto-a-consumir (4ª e 5ª gama, respetivamente).

Genericamente, a produção deste tipo de alimentos está associada a uma etapa de embalagem com atmosfera modificada. Todo o processo deve ser conduzido a temperaturas baixas e, sobretudo estas mantidas durante o armazenamento. Isto é, acondicionar os alimentos numa embalagem com uma atmosfera modificada, só por si, não é suficiente para garantir a sua segurança. Com efeito, o processo de embalagem em AM deve ser visto como mais uma barreira ao desenvolvimento microbiano e à atividade enzimática e deverá ser conjugado com outros fatores importantes à conservação dos alimentos, como por exemplo, a atividade da água (a_w), o pH e a temperatura (T), naquilo a que se chama usualmente efeito de barreira (16, 19).

A embalagem com AM consiste na substituição da atmosfera normal, dentro da embalagem, por uma mistura de gases com composição em CO2, O2 e N2 diferente do ar (20). O dióxido de carbono tem uma função bacteriostática, dependente da temperatura; o oxigénio é usado em teores baixos para desacelerar a respiração (no caso dos produtos frescos ou MP) e ao mesmo tempo evitar os efeitos negativos de um ambiente anaeróbio (senescência, abaixamento de pH ou mesmo risco de botulismo); o azoto, na maior parte dos casos é usado como gás inerte de enchimento ajudando na dispersão da atmosfera protetora (21). Na revisão bibliográfica efetuada para este trabalho, poucos trabalhos foram detetados descrevendo a utilização de outros gases inertes (árgon e hélio) como alternativa ao azoto (N2), tal como também observou Novak (21). No entanto, muito raramente e nem sempre aplicados a alimentos, são usados outros gases na embalagem com AM, tais como monóxido de carbono, ozono, óxido de etileno, óxido nitroso, óxido propileno, etanol (vapor), néon, árgon, hidrogénio, dióxido de enxofre, e cloro (12, 22).

1. Legumes minimamente processados

O processamento mínimo (Figura 2) envolve a seleção cuidadosa, corte e lavagem/ desinfeção dos vegetais, seguidos do embalamento, usando filmes mais ou menos permeáveis, com ou sem alteração inicial da atmosfera dentro da embalagem, induzindo assim uma atmosfera modificada (AM) ativa ou passiva, respetivamente (23). Designam-se vegetais MP aqueles que foram submetidos a etapas de processamento (não térmico) cujo único objetivo é melhorar/otimizar a sua funcionalidade (metabólica) para que estes mantenham as características de frescura ao longo do armazenamento. Neste tipo de processo apenas se tenta desacelerar os fenómenos de senescência, recorrendo a técnicas que permitam também atrasar a inevitável alteração de aspeto, textura, *flavour* (sabor e aroma) e valor nutricional dos produtos, ao longo do tempo de armazenamento (24-27). Sabe-se que estas alterações são mais rápidas nos vegetais submetidos a operações de

corte e de higienização, que aceleram os mecanismos de defesa do próprio vegetal. Em alguns casos expressa-se em alterações rápidas de aspeto; noutros casos, menos visível mas igualmente importante as alterações surgem ao nível do valor nutricional (28, 29). Para além disto, a vulnerabilidade a contaminações e proliferação de microrganismos aumenta substancialmente (30). Têm vindo a ser sugeridas medidas de prevenção adequadas para evitar perdas precoces da qualidade e aumentar consideravelmente o tempo de vida útil dos produtos (30, 31).

Figura 2 - Fluxograma do processamento mínimo de legumes

Os produtos vegetais uma vez colhidos, iniciam a sua degradação, a qual pode levar à completa senescência ao fim de poucos dias, dependendo essencialmente de condições da temperatura de armazenamento e do cuidado tido nas etapas de pós-colheita. O processamento mínimo seguido de embalamento em AM permite aumentar o tempo de vida deste tipo de produtos pois desacelera o metabolismo e a maturação, reduzindo a perda de peso e massa seca à medida que a taxa de respiração também desacelera (19, 23, 25, 32, 33).

O processamento e as condições de armazenamento podem afetar, de forma diferente, alguns parâmetros físico-químicos, tais como: pH e acidez; microestrutura (parede celular/turgidez) e, consequentemente, a textura; e a atividade enzimática (com eventual acastanhamento provocado pelas polifenoloxidases nas superfícies que sofreram corte). No processamento mínimo, uma etapa indispensável é a lavagem/desinfeção em que os agentes usados podem ter uma ação prejudicial sobre alguns compostos bioativos. No entanto, industrialmente, esta etapa é indispensável no controlo da microflora nativa e da contaminação resultante das etapas de manipulação e corte (28).

Em alguns trabalhos publicados, os termos atmosfera modificada e atmosfera controlada (AC) são muitas vezes usados indistintamente. No entanto têm significados diferentes (34). Apesar de tanto no armazenamento em AC como em AM, a atmosfera benéfica em torno do produto ser diferente do ar, na primeira, a composição do gás é continuamente monitorizada e ajustada, e o produto é normalmente armazenado em câmaras de armazenamento ou contentores de transporte. Em contraste, na embalagem com AM, a composição do gás não é monitorizada (mas pode ser controlada pela utilização de saquetas com absorvedores de gás, por exemplo de O2, etileno,…) e o produto é introduzido numa embalagem, como por exemplo uma bolsa plástica ou uma bandeja selada com um filme.

Industrialmente, a embalagem com AM *passiva,* é obtida por colocação dos legumes minimamente processados em embalagens com filmes de baixa barreira ou em sistemas de embalagem com filmes microperfurados. Este último caso, é apenas aplicável a produtos não submetidos a corte. Uma vez dentro da embalagem, os vegetais irão promover alterações nos níveis de O_2 e de CO_2 da atmosfera inicial (20,95 % de O_2/ 78,09 % de N_2/ 0,93 % de árgon e 0,038 % de CO_2). A percentagem de O_2 vai diminuir e a de CO_2 irá aumentar, sendo certo, que o balanço final será determinado pelas taxas de respiração dos produtos introduzidos e pela permeabilidade do filme aos gases (O_2, CO_2, etileno, …) e vapor de água (23, 35).

No caso da embalagem em AM *ativa*, o ar da embalagem é completamente removido e introduzida uma mistura de gases com uma composição distinta e não ativamente controlada (não é monitorizada nem reposta a concentração de gases definida). Nestes casos os filmes usados, geralmente funcionam como barreiras de elevada impermeabilidade à transferência de gases (ou com uma permeabilidade definida) (22). A partir do momento da selagem, a atmosfera (composição gasosa inicial) no interior da embalagem será alterada, dependendo do efeito da mistura gasosa inicial sobre as taxas de respiração dos produtos embalados, bem como das características de permeabilidade do filme que a constitui (12).

Em ambos os casos de modificação *ativa* e *passiva* da atmosfera, a diminuição dos níveis de O_2 e aumento do teor de CO_2 conduz a uma diminuição na taxa de respiração, atrasando a senescência, diminuindo o escurecimento enzimático, o amolecimento e o crescimento microbiano (função bacteriostática do CO2), mantendo a frescura dos produtos por mais tempo (36-38). Estes efeitos benéficos manter-se-ão enquanto a concentração de CO_2 não for excessiva e o O2 não desaparecer completamente. Em alguns trabalhos está descrito que o CO_2 em excesso e solubilizado no produto, promovendo reações de degradação indesejáveis, como a produção de aromas estranhos (*off-odours*), aumento da acidez com perda consequente de sabor típico, textura e cor característica. Assim sendo, e como o CO_2 difunde através dos filmes plásticos duas a seis vezes mais rapidamente do que o O_2, a embalagem deve ser constituída por um filme de permeabilidade seletiva, tendo em atenção a taxa de permeabilidade CO_2 / O_2 (26, 35, 39-43).

De acordo com Kader et al. (9), Fonseca et al. (44) e Brecht et al. (45) entre outros já referidos, vários fatores podem influenciar a eficácia do processamento mínimo com AM: (i) o estado de frescura inicial; (ii) o cuidado na manipulação; (iii) as espécies dos legumes/variedades; (iv) a forma como as operações de corte, branqueamento, lavagem e desinfeção são realizados; (v) o tipo de corte (por exemplo, pequenas peças cortadas / grandes peças / alimentos intactos); e (vi) o tipo de atmosfera. Nestes trabalhos ficou patente a diversidade de resultados e conclusões usando metodologias de armazenamento em AM semelhantes. Diferentes espécies vegetais/variedades apresentam um comportamento diferente nas mesmas condições de AM, principalmente devido a taxas de respiração específicas diferentes ou diferentes tamanhos das peças ou até diferentes quantidades de alimento por embalagem, etc.

Em relação à composição mais adequada da mistura de gases a usar na embalagem, várias opiniões e orientações contraditórias podem ser também obtidas nos muitos artigos publicados (36, 41, 46). Por exemplo, enquanto alguns autores propõem atmosferas suaves (3-5% de O_2 e 3-10% de CO_2, com N_2 para completar 100%), outros anunciam benefícios da utilização de concentrações de O_2 de cerca de 70% (10, 27, 39). Os diferentes resultados publicados relativos ao comportamento dos vegetais submetidos a tratamento mínimo em embalagens com AM são numerosos e de difícil comparação. Os dados obtidos, para além de influenciados pelas técnicas analíticas, são fortemente condicionados pelas próprias matrizes em estudo, no que diz respeito à sua origem geográfica, época de produção, colheita, estado de maturação, pré-tratamentos, etapas de preparação antes da própria embalagem. Pelo referido, a procura da melhor combinação de gases a usar em AM, continua a ser objeto de estudo de muitos investigadores (24).

O comportamento de produtos cortados, quando submetidos a atmosfera modificada (elevados teores de CO_2 e/ou baixas percentagens de O_2) e a sensibilidade ou reação destes ao corte, pode ser muito diferente do produto inteiro, sujeito à mesma atmosfera. A sensibilidade de produtos minimamente processados é, em geral, maior do que dos produtos intactos (30, 36, 38, 40). Por exemplo, os tecidos de alface cortada são mais sensíveis às altas concentrações de CO_2 do que o pé inteiro, respondendo melhor à AM (40).

2. Legumes pré-cozidos

Os alimentos confecionados e refrigerados têm, habitualmente, um prazo de validade muito curto. A apenas podem ser consumidos até ao final do dia seguinte à data de produção, uma vez que se alteram rapidamente em contacto com o ar. Para diminuir os custos de produção, reduzir o desperdício e simultaneamente, aumentar a qualidade, torna-se indispensável adotar novas tecnologias. Estas devem também permitir a manutenção da qualidade dos alimentos durante mais tempo. É neste contexto que surge o processo de embalagem em atmosfera modificada aplicada a produtos pré-confecionados. A combinação de gases visa proteger os alimentos dos efeitos do ar e da humidade, evitar reações químicas e o desenvolvimento microbiológico, retardando a sua deterioração. A utilização de AM permite uma gestão mais racional do processo de produção e distribuição das refeições pelos diferentes operadores (cantinas, serviços de *catering*, restauração com *takeaway*), para além de permitir uma maior flexibilidade e variedade na oferta ao consumidor.

No caso dos legumes pré-cozidos, prontos-a-comer ou que apenas necessitam de ser submetidos a aquecimento, é também um desafio manter elevados níveis de qualidade. Segundo Galić et al. (19), os alimentos perecíveis, genericamente, têm menos de 14 dias de validade devido à deterioração bioquímica e/ou microbiana. No entanto se forem asseguradas as boas práticas de produção, com tecnologias em condições de assepsia e ainda recorrendo a uma embalagem com AM otimizada, estes produtos podem durar até 90 dias (47). Estes sistemas de embalagem devem ser pensados, considerando a produção e taxa de transferência de vapor de água, O2, CO2 e N2 (dependente da permeabilidade dos filmes selecionados) (19).

Atualmente, há ainda poucos dados publicados obtidos com este tipo de produtos, bem como estudos ao longo do tempo de armazenamento. Tanto quanto se sabe, apenas Murcia e colaboradores (36, 37) relatam trabalhos com legumes pré-cozidos, prontos-a-

comer (sopas, legumes cozidos, salteados, estufados e em sopa e puré) embalados em condições de AM e vácuo e armazenados a temperaturas de refrigeração (frio doméstico). Nestes estudos foram analisados alguns parâmetros indicadores da qualidade dos produtos, tais como: composição proximal (teor de humidade, cinzas, proteína e gordura), atividade antioxidante e crescimento microbiano (microrganismos aeróbicos, mesófilos, psicotróficos, bolores e leveduras).

Outros trabalhos já citados nas secções anteriores limitam-se a discutir a influência da embalagem em AM em alimentos minimamente processados e cozinhados, ou a comparar produtos frescos e sujeitos a um ou outro processamento térmico. Muitos desses estudos têm apenas como base um conjunto limitado de parâmetros (48-53).

No que diz respeito às combinações de gases a aplicar na embalagem com AM, deve ter-se em consideração os parâmetros intrínsecos do produto alimentar a ser preservado (pH, atividade da água, o teor e tipo de gordura), que vão influenciar a sensibilidade microbiana, química e enzimática ao longo do processo de deterioração (47).

Ao contrário dos minimamente processados, os vegetais cozidos são produtos que não respiram (não consomem qualquer O2 durante o armazenamento). Neste caso objetivo é manter a composição da mistura introduzida na embalagem, selecionando para tal um filme de alta barreira. No entanto, estes produtos são sensíveis à deterioração microbiana (desenvolvimento de bactérias Gram-negativas e leveduras). Por isso, este tipo de produtos necessita de uma atmosfera com uma elevada concentração de CO2, que irá retardar o crescimento desses microrganismos. O oxigénio deve ser excluído da mistura de gases, apenas permitindo que uma quantidade residual (inferior a 0,5%) permaneça na mistura para evitar o risco de crescimento de microrganismos anaeróbios (19, 47).

3. Embalagem, sistemas e materiais

Existe uma vasta gama de materiais e tecnologias utilizadas na produção de embalagens para alimentos. Ao longo dos últimos 20 anos tem-se assistido a um crescente interesse no estudo e desenvolvimento de sistemas de embalagem, no sentido de responder às crescentes necessidades e exigências dos consumidores (19). Na Europa, esta tendência também se verifica, particularmente, nas pequenas e médias empresas e ainda indústrias alimentares exportadoras. Tal tendência deve-se à preferência dos consumidores por alimentos minimamente processados e prontos a comer, naturalmente preservados (sem aditivos) mas também porque a indústria alimentar tem de investir na qualidade e segurança dos produtos alimentares que produz e comercializa (19).

A embalagem exerce um papel importante na manutenção da qualidade dos produtos nela contidos, desempenhando essencialmente quatro funções (54, 55):

(i) proteger – é uma das principais funções da embalagem, conferindo proteção contra adulteração ou perda de integridade, quer sejam acidentais quer sejam provocadas, durante o seu transporte, armazenamento e utilização. Confere proteção mecânica e barreira a contaminações, sujidade, partículas de pó, etc;
(ii) conservar – retardando os fenómenos de deterioração/senescência, prolongando o tempo de vida útil dos alimentos. Para tal, deve atuar sobre as influências ambientais tais como calor, luz, a presença ou ausência de humidade, de oxigénio, odores externos, evitando que ocorram reações bioquímicas, enzimáticas e/ou não enzimáticas, crescimento microbiano e alterações organoléticas;
(iii) informar – comunicando através de inscrições num rótulo, estabelece um elo entre o consumidor e o produtor de alimentos. Tem obrigação de transmitir informações tais como; lote, peso, origem (geográfica e do fabricante), ingredientes, valor nutricional e precauções ou instruções de conservação e preparação ou uso. Algumas destas alegações são mesmo impostas pela legislação para segurança e proteção do consumidor e ainda rastreabilidade (indicação do lote de produção). No âmbito desta função incluem-se, ainda, ações de promoção do produto ou de marketing;
(iv) servir – é outra das valências da embalagem que, para além de ser contentor, deve ir de encontro às exigências de conveniência dos consumidores (abertura-fácil, bocal apropriado a bebida em movimento, cozedura na embalagem, etc.).

Para manter a qualidade e segurança dos alimentos embalados em AM, é essencial escolher corretamente o material de embalagem. Na embalagem com AM os materiais mais usados são os plásticos flexíveis e semirrígidos e os plásticos laminados. Os materiais plásticos representam uma grande percentagem do total de materiais usados na embalagem de alimentos. O tipo de filme que forma a embalagem é um fator essencial, pois é necessário minimizar ou controlar as trocas gasosas entre a atmosfera interna e o ambiente externo. Existem diversos filmes com diferentes taxas de permeabilidade ao oxigénio, de modo a controlar a velocidade de troca O_2/CO_2 para prolongar a vida útil do alimento embalado. O material utilizado na embalagem deve ter uma propriedade de barreira adequada ao período de vida útil do alimento e à temperatura de armazenamento (44, 56, 57).

Os produtos hortícolas frescos e cortados, requerem O2 para que o seu processo metabólico de respiração continue, libertando CO2, mantendo assim a sua frescura e qualidade após o processamento. Independentemente do sistema de modificação da

atmosfera (AM *ativa*, AM *passiva* ou *controlada*) usado, esta deve ser equilibrada internamente. Este equilíbrio é total ou parcialmente dependente da taxa de respiração do produto cortado e da permeabilidade dos materiais de embalagem (34, 58). Para que isto aconteça, as propriedades de difusão de gases através do filme, devem ir de encontro aos requisitos fisiológicos dos produtos embalados. Numa revisão feita por Galić et al. (19) pode encontrar-se informação relevante acerca dos filmes, sua adequabilidade e comportamento em sistemas de embalagem para produtos não só submetidos a AM, mas também sujeitos a outros tipos de processamento (irradiação, altas pressões, tratamentos óhmicos, ultrassons, etc.).

Também é importante que a razão entre o volume de gases utilizado e o produto dentro da embalagem seja controlada, garantindo que todo o produto está em contacto com a mistura de gases definida e/ou não ocorra colapso das embalagens, por remoção de ar ou, eventualmente, também, devido à possibilidade de solubilidade do CO_2 nos vegetais com maior teor de humidade. Esta razão deve ser de 2:1 ou 3:1 (59).

A taxa de respiração varia de produto para produto, o que condiciona também o tipo de filme a usar. A permeabilidade aos diferentes gases deve ser apropriada para evitar que se crie um meio anaeróbio (10, 27). Muitos dos sistemas de embalagem usados na indústria são aplicados a uma grande gama de produtos. À luz do que se tem vindo a publicar, muitas vezes esses sistemas não são perfeitamente adequados, não satisfazendo os requisitos que o metabolismo de outro produto possa ter mediante um microambiente criado na embalagem. Por exemplo, um filme composto por um determinado polímero com uma taxa de permeabilidade baixa pode adequar-se a produtos com uma taxa de respiração baixa ou até média. No entanto, se for usado para embalar produtos com taxas de respiração elevadas, pode causar grandes alterações na composição gasosa, modificando drasticamente a atmosfera e promovendo, por exemplo, fenómenos de fermentação (27, 57).

As taxas de consumo e produção de O2 e CO2, respetivamente, devidas à respiração dos produtos são mais elevadas do que as taxas de transferência de massa através da embalagem. Tal vai provocar uma alteração à atmosfera inicial por acumulação de CO2 e diminuição da concentração de O2 (9, 44).

Os sistemas de embalagem para hortofrutícolas frescos, podem ser constituídos por filmes com permeabilidade seletiva, ou microperfurados. A seletividade é a razão entre a permeabilidade da embalagem ao CO2 e O2, ou seja, é igual ao quociente entre a taxa com que o CO2 deixa a embalagem, difundindo através do filme e a taxa de entrada de O2 de novo na embalagem. Assim, a diminuição da concentração de O2 ou aumento da

concentração de CO2 no interior da embalagem depende desta taxa de transferência ou seja da seletividade do filme (57).

A otimização e obtenção do filme plástico com a seletividade desejada é uma tarefa árdua. A seletividade de muitas películas varia entre 2,2 e 8,7. Os produtos com uma elevada taxa de respiração (na sua maioria produtos frescos e/ou minimamente processados) exigem seletividades baixas. A maioria dos filmes não é adequada a menos que a sua seletividade seja reduzida (por perfuração, Figura 3) aumentando a sua permeabilidade global (57). Uma outra alternativa é o recurso a membranas respirantes (*breatheway*®) como explica Clarke (60). Estas membranas são muito permeáveis, e de seletividade adequada.

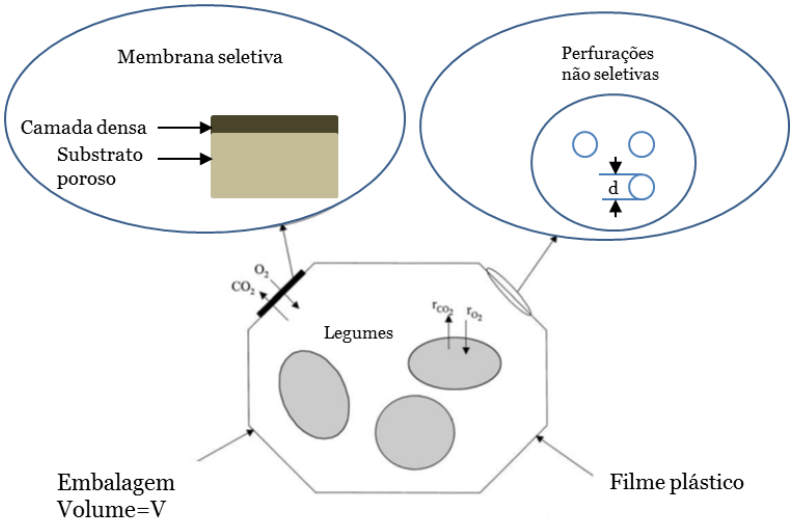

Figura 3 - Esquema de um produto numa embalagem plástica, representando um filme com uma membrana seletiva e uma não seletiva (microprefuração) (56, 57)

Como já foi referido, no caso dos legumes pré-cozidos, prontos a comer, embalados em AM, o objetivo é manter a atmosfera durante o período de armazenamento. Por isso, são usados filmes de alta barreira que, na maioria das vezes, são compostos por laminados, sobrepondo diferentes camadas de polímeros com determinada(s) propriedade(s) (47).

Na realidade, muitas das reações químicas, bioquímicas e biológicas, que decorrem ao longo do período de conservação do produto, podem ser evitadas, controlando a passagem de gases e vapor de água através da embalagem. A maioria dos materiais usados para conter produtos pré-cozidos, é constituída por várias camadas para garantir uma baixa

transmissão de vapor de água, uma elevada barreira aos gases e resistência mecânica ao manuseamento (4).

Os materiais utilizados podem ser simples, extrudidos ou laminados de copolímeros de etileno-álcool vinílico (EVOH), poliéster e polietileno (PE), nylon e PE, cloreto de polivinilo (PVDC), polipropileno (PP), poliamida (PA) e polietileno de tereftalato (PET). Os laminados são projetados de acordo com o tempo de vida atribuído ao produto, devendo a permeabilidade ao O2 ser inferior a 2 ml O_2/m^2 /24 h atm para tempos de armazenamento longos, e inferior a 10 ml O_2/ m^2 / 24 h atm, para tempos reduzidos de validade (<1 semana) (47). O seu custo está diretamente relacionado com a sua maior ou menor capacidade de barreira aos gases. A transparência destes materiais é também um fator importante na escolha, pois alguns produtos podem ser mais sensíveis à luz, podendo optar-se por materiais metalizados ou com filtros UV (4, 12, 47). Os principais materiais usados em embalagens com AM e as suas características estão representados naTabela 1. A Figura 4 resume algumas das características de permeabilidade ao O2 e vapor de água de alguns materiais plásticos comummente usados em embalagens com AM. Os valores apresentados são ilustrativos, pois variam com a temperatura de ensaio, espessura do filme e microestrutura final conferida ao filme (em estado mais amorfo ou cristalino). Em condições ambientais normais (23°C e 50% HR), os valores da taxa de transferência de vapor de água seria cerca de 75% mais baixos (55).

Tabela 1- Principais polímeros usados em embalagem para produtos alimentares (55, 61)

	Designação	Propriedades barreira	Resistência mecânica
LDPE	**Polietileno de baixa densidade**	Muito boa barreira à humidade; má barreira a gases e gordura	Boa resistência à tração e à perfuração/impacto
HDPE	**Polietileno de alta densidade**	Muito boa barreira à humidade; má barreira a gases e média barreira à gordura	Ótima resistência à tração e à perfuração/impacto
PP	**Polipropileno**	Boa barreira à humidade (> PE); fraca barreira a gases e gordura	Variável
OPP	**Polipropileno orientado**	Muito boa barreira à humidade; fraca barreira a gases e à gordura	Ótima resistência à tração e fraca resistência ao impacto/perfuração
PVC	**Policloreto de vinilo**	Média barreira à humidade; fraca barreira a gases e excelente barreira à gordura	Variável
PS	**Poliestireno**	Fraca barreira à humidade; fraca barreira a gases e má barreira à gordura	Muito boa resistência à tração; má resistência ao impacto/perfuração; facilidade de termoformação
EPS	**Poliestireno expandido**	Bom isolamento térmico e excelente acolchoamento	
PET	**Polietileno tereftalato**	Média barreira à humidade; média barreira a gases e excelente barreira à gordura	Excelente resistência à tração e boa resistência ao impacto/perfuração
PA	**Poliamida**	Má barreira à humidade; boa barreira a gases (0% HR) e excelente barreira à gordura	Excelente resistência à tração e ao impacto/perfuração; elevada dureza superficial; reduzido coeficiente de atrito
PC	**Policarbonato**	Fraca barreira à humidade; fraca barreira a gases e muito boa barreira à gordura	Excelente resistência à tração e ao impacto/perfuração
PVDC	**Policloreto de vinilideno**	Excelente barreira à humidade, a gases e à gordura	Não aplicável
EVOH	**Copolímero etileno-álcool vinílico**	Fraca barreira à humidade, excelente barreira a gases (0% HR) e excelente barreira à gordura	Não aplicável

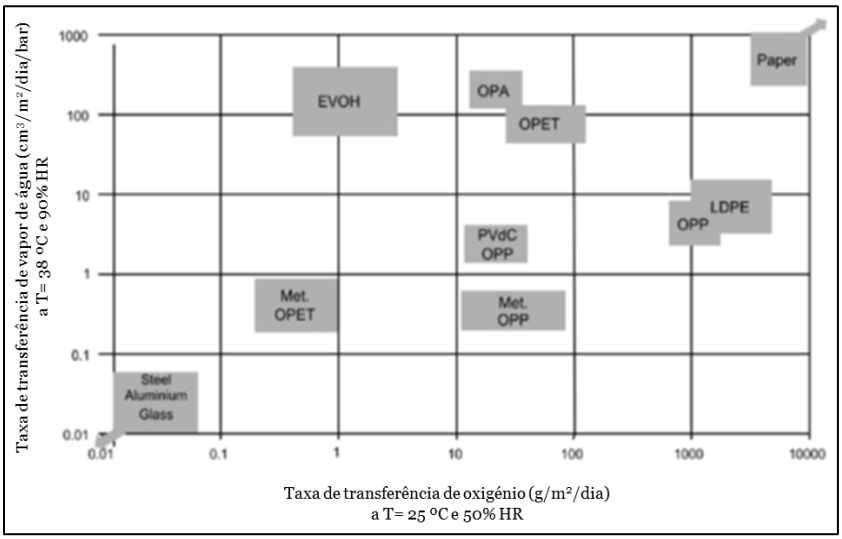

Figura 4- Permeabilidade ao vapor de água e O2 de alguns materiais de embalagem. A escala é logarítmica e os valores da taxa de transferência de vapor de água (TTVA) obtidos em condições tropicais de ensaio (38 °C e 90% HR) requeridas pelas normas industriais (55)

Nesta tese estudou-se a possibilidade de serem usados sistemas de embalagem (filmes e mistura de gases) que servissem uma gama alargada de produtos (couve, feijão verde, cenouras e pimentos verde, vermelho e amarelo), para tentar ir de encontro aos requisitos da indústria, minimizando paragens para troca de sistemas de embalagem. Neste sentido as opções de processamento e embalagem foram testadas em todos os produtos de igual modo.

4. Controlo da qualidade dos legumes prontos a consumir

Como já referido, a conveniência dos legumes prontos a usar, na forma fresca, apenas cortados, lavados (os minimamente processados), ou pré-cozidos, é assegurada pelo sistema de embalagem em AM. AM com elevadas concentrações de CO_2 e percentagem de O_2 reduzida ou ausente (no caso de PC), promovem a interação dos micronutrientes com a atmosfera. Como consequência uma série de parâmetros físico-químicos (pH, acidez, cinzas, humidade, compostos bioativos, textura e cor), propriedades organoléticas e o crescimento microbiano são afetados (14). Neste sentido, é pertinente controlar a qualidade desses produtos e acompanhar a evolução, ao longo do tempo, das diferentes condições de AM (combinação de gases). Deste modo os operadores industriais podem otimizar operações de processamento, incluindo embalagem e materiais de embalagem (filmes de baixa, média e alta barreira) assim como inscrever convenientemente os prazos de validade.

A qualidade dos vegetais é baseada em parâmetros predefinidos que garantem a segurança e disponibilidade. Para o consumidor, a opção de compra é, em primeiro lugar, influenciada por atributos sensoriais, as propriedades organoléticas, essencialmente cor, aroma, sabor e textura aparente. No entanto outros atributos da qualidade como a composição nutricional e a segurança (química e microbiológica) não são desvalorizados (62).

4.1. Parâmetros organoléticos

Os legumes minimamente processados são preparados, cuidadosamente, de modo a manter a frescura típica, que inclui a aparência, o *flavour* e a textura.

A qualidade sensorial dos legumes MP ou PC acabados de preparar, reflete-se na aparência, atributo que à primeira vista irá ser objeto de análise por parte do consumidor. Estes produtos não devem apresentar desidratação superficial nem perda da cor característica. Outros aspetos da qualidade sensorial destes produtos são: presença ou

ausência de defeitos (pontos escuros/acastanhados ou queimados); aroma característico (sem cheiros estranhos); textura característica dos legumes cozidos, sem esfarelar ou demasiado fibroso ou até mole demais. No caso dos MP, espera-se uma textura crocante húmida, sem excesso de suculência e com uma firmeza não elástica e não fibrosa. De uma forma geral, os produtos embalados em AM apresentam níveis elevados de qualidade sensorial quando comparados com outros embalados em ar, pois a probabilidade de senescência é retardada significativamente (63).

No entanto, as alterações metabólicas provocadas pela alteração da atmosfera podem ser prejudiciais, promovendo respostas negativas ao nível organolético. O efeito da supressão de O2 é bem conhecido e provoca a desaceleração da respiração que, em termos microbiológicos, confere mais segurança aos produtos e reduz a senescência (6, 13, 24, 30, 64-67). Contudo, a níveis muito baixos pode ocorrer fermentação (24, 27, 65, 66). Neste caso, ocorre perda de sabor e aroma característicos, entre outros atributos muito relacionados com a tipicidade destes produtos. Os produtos resultantes da fermentação (etanol, acetaldeído, acetato de etilo e lactato) contribuem para o desenvolvimento de cheiros estranhos (*off-flavours*). Com efeito contrário, o CO2 genericamente suprime a produção de *off-flavours,* atuando ao nível da produção de etileno também; com desaceleração do processo de respiração. Compete como substrato, com o O2 nos processos oxidativos. No entanto, ainda se formam alguns aromas, sendo percebidos, sobretudo, no momento de abertura da embalagem (24).

Para além do aroma, outras propriedades organoléticas podem ser afetadas devido aos processos degradativos que ocorrem ao longo do armazenamento. É o caso da cor que é afetada não só pelos processos oxidativos, mas também pelo crescimento microbiano e a atividade enzimática (desencadeia a perda ou reação de pigmentos responsáveis pela cor, clorofilas, antocianinas, carotenos, etc.). A perda de água do produto e a lixiviação de sucos são responsáveis não só pela perda de minerais e pigmentos, mas também faz com que os atributos relacionados com a textura se afastem da tipicidade (ex: firmeza, fibrosidade, aparência esbranquiçada, etc.) (63, 68-72).

A avaliação de produtos alimentares com base na análise sensorial desempenha um papel importante no controlo da qualidade e na avaliação da tipicidade. Para a indústria, e também na investigação e desenvolvimento na área alimentar, a existência de um perfil descritivo (textura, *flavour*, etc.) é essencial pois envolve a avaliação qualitativa e quantitativa de características sensoriais por um painel treinado para o efeito (73, 74).

O método mais completo para caracterizar as sensações desencadeadas pelo estímulo da prova de alimentos (ou outro produto não alimentar) é a análise descritiva (AD) (75,

76). A AD permite descrever os atributos sensoriais associados ao produto e as diferenças entre eles. Esta técnica é amplamente utilizada na indústria alimentar como refere Hildegarde et al. (76). As metodologias de AD usadas, atualmente, são baseadas em três métodos (com patentes registadas): *Flavour Profile* (FP®) desenvolvido nos anos 50; Análise Descritiva Quantitativa (ADQ®) que surgiu no início dos anos 70 e a metodologia *Spectrum*®, desenvolvida mais tarde no final dos anos 70 (76).

A AD é habitualmente realizada por um painel com um número relativamente pequeno de provadores (8 a 15 elementos) que quantificam a intensidade de um conjunto de atributos previamente selecionados. A sua implementação envolve três etapas principais: A *1ª etapa* consiste no desenvolvimento e familiarização de um léxico que descreve, exaustivamente e com precisão, o produto. Esta lista de atributos é, geralmente, conseguida após a apresentação aos provadores de muitas variações dos produtos. No caso do estudo de tempo de vida, podem ser apresentados produtos em diferentes fases de armazenamento e degradação (para pesquisa e reconhecimento de defeitos), sendo gerado um conjunto de termos que podem descrever as diferenças entre produtos. Alguns termos hedónicos são eliminados e reagrupados num único termo os sinónimos ou antónimos; A *2ª etapa* consiste em treinar os membros do painel para padronizar as suas perceções e alinhá-las, visando um consenso em termos de quantificação de conceitos e de sensações. Para tal, geralmente é associada uma definição e uma referência física (padrão) para cada um dos atributos do léxico; Na 3ª etapa decorre a avaliação propriamente dita dos produtos, onde os provadores os classificam com base em cada atributo descritivo, quantificando a sua apreciação numa escala de intensidades. Para alcançar resultados mais precisos, confiáveis e consistentes tanto quanto possível, o desempenho do painel é monitorizado em termos de poder de discriminação, repetibilidade do provador e reprodutibilidade entre provadores (74, 77).

É do conhecimento dos especialistas em análise sensorial descritiva que os avaliadores dão resultados irregulares, decorrentes de diferenças de motivação, sensibilidade e comportamentos psicológicos. Apesar das sessões de treino a que cada painel é submetido, a fiabilidade dos dados recolhidos pode ser afetada por erros associados ao provador e erro de concordância dentro do painel (78).

A implementação desta metodologia acarreta custos que, muitas vezes, inviabilizam a sua aplicação de forma mais ou menos rotineira. Em algumas situações, para suprimir estes custos, não é corretamente executada (73).

Numa AD, o vocabulário e os treinos associados devem ser adaptados a cada indústria ou produto, o que pode levar de algumas semanas a vários meses (em alguns casos anos),

dependendo do produto ou grupo de produtos, até o painel dar respostas fiáveis e/ou um estudo ficar completo. Neste sentido, há uma evidente necessidade de métodos mais rápidos e com custos mais baixos (74). Em resposta, têm sido apresentados vários métodos como alternativas à AD clássica, muitos deles dispensando treinos exaustivos e fornecendo resultados fiáveis, nomeadamente: *flash profile* (FP); *check-all-that-apply* (CATA); *free choice profiling* (FCP); *free sorting task* (FST); *Placing, projective mapping* (PM), *spatial arrangement procedure* (SAP) or Napping®; *Polarised sensory positioning* (PSP), tal como brevemente descrevem Valentin et al. (74), Dehlholm et al. (79) e Albert et al. (80), entre muitos outros (81-83). O último método referido, PSP, trata-se de um método baseado em referências/padrões, adequado às necessidades da indústria e também seguido na avaliação dos produtos em estudo nesta tese. Aplica-se sobretudo quando:

(i) a quantidade de produtos a testar é demasiado grande para ser decorrer numa única sessão;
(ii) um novo produto precisa de ser descrito;
(iii) apenas um produto se encontra disponível num determinado momento, como por exemplo no controlo da qualidade ao longo do tempo de armazenamento.

O objetivo principal desta metodologia é manter um método comparativo, mas em vez de comparar todos os produtos juntos, poder compará-los um a um (ou um conjunto) com uma referência(s) (74, 83, 84).

O princípio subjacente a esta metodologia assenta na materialização de determinados atributos, através da apresentação de protótipos de produtos referência, ou até mesmo produtos em avançado estado de degradação para visualização de problemas que possam surgir nas amostras em estudo, padrões (de cor, texturas ou aromas) que irão atuar como "meta-atributos" das quantificações (84, 85).

No trabalho desenvolvido nesta tese, a análise descritiva teve como base estes princípios. Em todas as sessões de prova, o painel tinha na sua presença uma amostra de produto fresco (quer minimamente processados quer cozidos, no próprio dia da sessão) representativa do tempo zero de amostragem. A avaliação da intensidade dos atributos tinha sempre em conta a evolução desses atributos em relação à intensidade do mesmo atributo na amostra padrão. Na folha de prova (em Apêndice, I) este valor era marcado na escala, e que genericamente se situava no meio.

Seleção e treino do painel de provadores

Para desenvolver estudos de análise sensorial é necessário definir os requisitos para que o laboratório possa, recrutar, selecionar e treinar um painel de provadores e definir os ensaios passíveis de serem realizados. Em controlo da qualidade sensorial o "instrumento" de análise utilizado é o ser humano, que não é facilmente calibrado, sendo esta uma das grandes questões relacionadas com a utilização de métodos de análise descritiva, como a ADQ®. Os provadores treinados constituem um painel de análise sensorial, comparável com um instrumento analítico (75, 86-90). Depois de definido um léxico que, objetivamente, descreve as propriedades organoléticas do produto ou gama de produtos em estudo, o painel treinado passa à fase de identificação e quantificação dos aspetos sensoriais dos produtos, numa prova cega.

Assim, após uma seleção segundo critérios específicos, os provadores são treinados para realizar provas de análise sensorial em geral. Quando é chamado a avaliar um produto, este painel deve ser submetido a sessões de treino mais específicas ou "aquecimento" (refrescar a memória) mais direcionado ao produto a avaliar. O painel, para além das provas a efetuar, deve ser continuamente alvo de um treino, generalizado ou mais específico para cada um dos testes e produtos que terá de avaliar (74).

Nesta tese, o trabalho desenvolvido com o painel de provadores constituiu uma etapa importante deste estudo, sobretudo na fase de treino que foi determinante para a qualidade dos resultados da AD deste projeto. Sem aprovação do painel, os legumes em estudo seriam de imediato considerados impróprios.

Desta forma, no Laboratório de análise sensorial da ESTG-IPVC, desenrolaram-se as fases de seleção e treino de um painel de provadores usado na avaliação dos legumes prontos a comer em AM, recorrendo às técnicas anteriormente descritas. A componente sensorial desta tese teve como objetivos:

(i) recrutar elementos para pertencer a um painel de provadores;
(ii) selecionar e treinar elementos para um painel de provadores, estudando e testando métodos para este fim;
(iii) efetuar um controlo contínuo da prestação do painel de provadores como um todo e de cada um dos elementos do mesmo durante o treino;
(iv) confrontar o painel com provas de produtos alimentares propriamente ditos e avaliar a sua *performance*;
(v) relacionar as sessões de prova com as provas com consumidores (aceitabilidade);

(vi) perceber e analisar as dificuldades inerentes a todo o processo de avaliação de produtos distintos, no entanto englobados numa mesma prova;
(vii) quantificar diferenças entre os diferentes tempos de armazenamento no estudo do tempo de vida dos legumes.

Estudos do tempo de vida útil – métodos sensoriais de estimativa

O tempo de vida útil de um alimento é definido como o período de tempo em que o produto pode ser armazenado, antes de se tornar inaceitável do ponto de vista microbiológico, nutricional e/ou sensorial. O prazo de validade é função do tempo, de fatores ambientais e da suscetibilidade do produto, os quais promovem alterações bioquímicas ao longo da cadeia de distribuição alimentar (desde o armazenamento até ao consumo) (70).

De acordo com o IFST (Institute of Food Science & Technology (1993)) define-se como o tempo em que o produto, enquanto armazenado em condições normais:
(i) se mantém seguro;
(ii) retém as características sensoriais, químicas, físicas e microbiológicas desejadas;
(iii) obedece à informação da rotulagem nutricional;

Esta definição identifica os fatores chave e alerta para o armazenamento apropriado, contudo, mantém um termo subjetivo "características desejadas".

Em muitos produtos, as alterações das características sensoriais ocorrem, em grande parte, antes de ser comprometida a segurança e antes de haver qualquer risco para a saúde dos consumidores (75, 91). Uma vez que se trata de um parâmetro do controlo da qualidade e fator decisivo na opção de compra/ou escolha dos consumidores torna-se pertinente a previsão do tempo de vida útil por métodos sensoriais. Além disso, é pertinente no que toca à redução do desperdício alimentar (http://www.tristramstuart.co.uk/FoodWasteFacts.html, (92)).

O desperdício alimentar tem implicações económicas e ambientais (92), para além das questões morais que, frequentemente, são levantadas tendo em conta a fome no mundo. Muito deste desperdício é devido à influência de regras de "estética" adotadas pelos grandes grupos distribuidores de alimentos, má gestão de compras e produção no caso das cantinas e ainda à influência da rotulagem, mais propriamente prazos de validade inscritos nas embalagens, desajustados ao produto ou à técnica de conservação subjacente ao seu processamento (70).

A rotulagem exata do prazo de validade (otimizado) pode contribuir para uma gestão mais eficaz dos *stocks* (tanto no retalho como ao nível doméstico), contribuindo para o aumento dos níveis de rentabilidade ao longo de toda a cadeia de distribuição. Tal é do ponto de vista financeiro interessante considerando as pequenas margens tradicionalmente associadas à indústria alimentar (93). Neste sentido, a fim de prolongar os tempos de comercialização, garantindo simultaneamente frescura do produto, as empresas de alimentos deve contar com metodologias precisas para estimativa do tempo de vida útil (*shelf-life)* (70).

O desenho experimental para determinação/estimativa do tempo de vida dos vegetais é importante para minimizar o tempo e custos deste tipo de análise sensorial. A abordagem ao desenho experimental para estimar o tempo de vida pode dividir-se em duas estratégias a seguir para armazenar e avaliar os produtos e que se denominam por *desenho básico* ou *escalonado de Gacula* e *desenho invertido* (70, 94, 95).

O primeiro (Figura 5) é o mais comum. Neste caso, o mesmo produto (mesmo lote) é armazenado ao mesmo tempo e sob as mesmas condições de teste. Ao longo do tempo de armazenamento são retiradas e avaliadas as amostras. Esta abordagem é habitualmente acompanhada pela avaliação físico-química nos estudos de monitorização da qualidade. A análise físico-química deve seguir a mesma estratégia de amostragem, sendo executadas análises a replicados do mesmo lote (75). Neste desenho experimental, a evolução vai sendo acompanhada e o número de amostras aumenta à medida que o produto se mantém dentro dos limites da qualidade mínima ou estipulada.

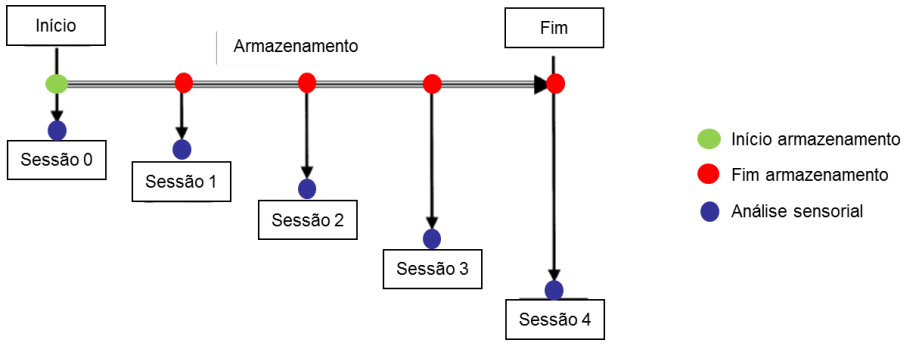

Figura 5- Esquema do desenho experimental escalonado de Gacula para determinação do tempo de vida útil (95)

No trabalho desenvolvido nesta tese, a análise sensorial dos legumes em AM foi conduzida de acordo com este desenho experimental.

Segundo Hough (94), para obter melhores resultados, a análise sensorial deve ser feita com todas as amostras dos diferentes tempos, em simultâneo, de acordo com o desenho experimental *invertido*. Neste caso existem três procedimentos possíveis (94, 95):

1- O produto de diferentes lotes vai sendo armazenado ao longo do tempo para que, ao fim de um dado tempo final, as amostras (com diferentes tempos de armazenamento) possam ser todas avaliadas em simultâneo (Figura 6). Esta metodologia foi seguida na preparação do painel de avaliadores dos produtos em estudo nesta tese, com o objetivo de treinar os provadores na perceção dos atributos relacionados com a perda de frescura.

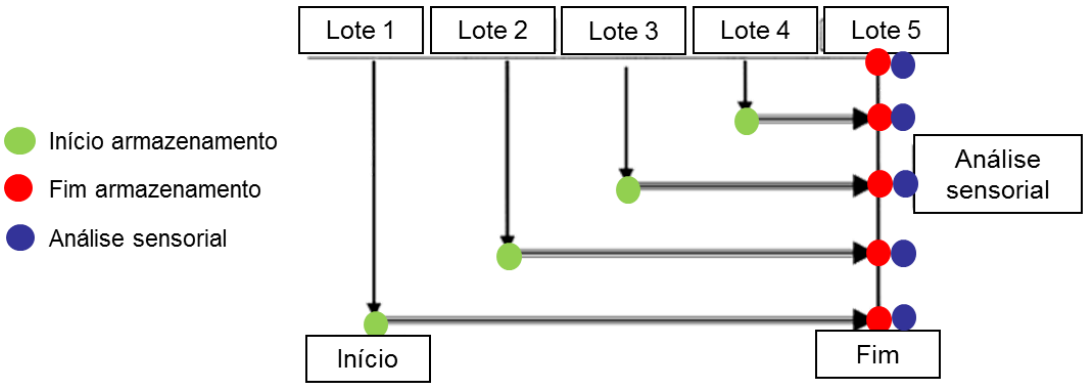

Figura 6- Esquema do desenho experimental invertido para determinação do tempo de vida útil (95)

2- Inicialmente o mesmo produto (todo de um mesmo lote) é congelado e aos tempos de amostragem definidos é descongelado e armazenado nas condições desejadas de teste. A análise sensorial das amostras é feita ao mesmo tempo (Figura 7).

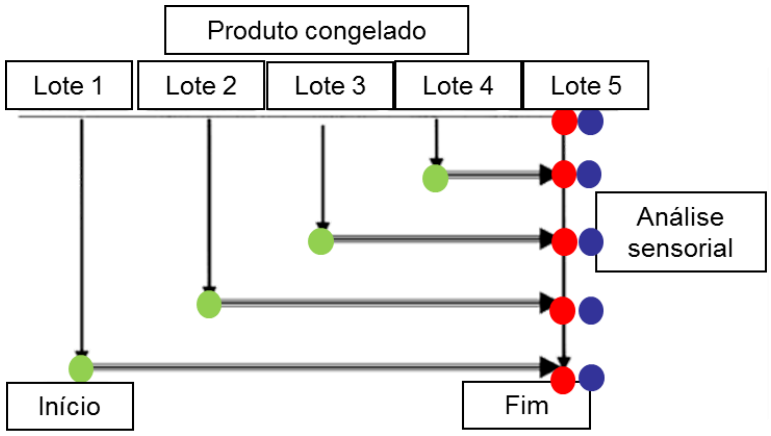

Figura 7- Esquema do desenho experimental invertido considerando a congelação das amostras antes do estudo para determinação do tempo de vida útil (95)

3- O produto é todo armazenado nas condições desejadas de teste e a cada tempo de amostragem definido é congelado. A análise é feita descongelando todo o produto e toda avaliação realizada ao mesmo tempo (Figura 8).

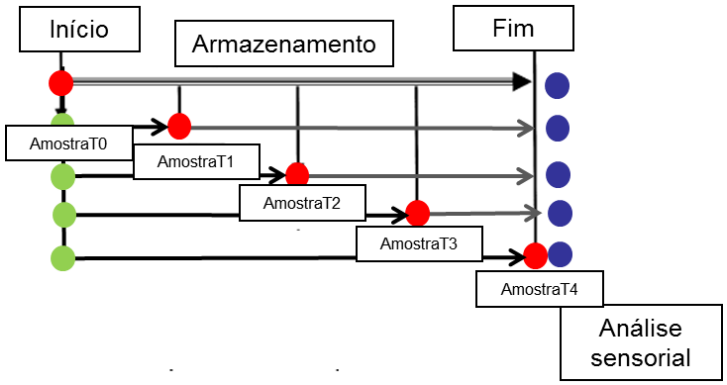

Figura 8- Esquema do desenho experimental invertido considerando a congelação das amostras correspondentes a cada tempo de amostragem, para determinação do tempo de útil (95)

Aceitabilidade e consumidores

O tempo de vida útil dos produtos não é uma característica própria, mas sim uma característica dependente da interação entre estes e o consumidor. Por isto, o prazo de validade sensorial deve também ser determinado, recorrendo a consumidores que avaliam os produtos segundo as suas expectativas. Muitas vezes os dados recolhidos com painéis treinados e consumidores não são coincidentes. Alguns trabalhos que baseiam a previsão do tempo de vida útil recorrendo apenas a paneis treinados, chegam a resultados em que os produtos estão validados e com grandes margens de segurança. No entanto quando submetidos à apreciação de consumidores, estes consideram a qualidade do produto inferior à esperada (pontuações baixas na aceitabilidade). Esta posição pode, futuramente, conduzir a uma rejeição de compra/escolha do produto. Pode então dizer-se que o tempo de vida útil do produto, sob o ponto vista sensorial, é o período de tempo durante o qual o produto ainda é considerado pelo consumidor final, com aceitabilidade que reflete um nível de qualidade esperado (70). Este nível correlacionar-se-á tanto melhor (ou pior) com os métodos usados com painéis treinados (na avaliação de intensidades de atributos), dependendo de conclusões analíticas mais ou menos restritivas e/ou conservadoras extraídas da apreciação desses resultados (70).

Para que a correlação de dados seja válida e realista, é importante suprimir problemas que, vulgarmente, ocorrem na implementação das técnicas de análise sensorial. Apesar da falta de treino dos provadores limitar e condicionar muitas vezes a validade dos

resultados/informação obtida em análise sensorial descritiva (ou mesmo discriminativa), em estudos do tempo de vida, o que frequentemente acontece e prejudica os estudos é a confusão instalada acerca do tipo de provadores a usar em cada uma das provas. Pode citar-se como exemplo, provadores treinados a emitir opiniões hedónicas em vez de objetivamente apreciar a intensidade de determinado atributo (70). Especialistas reconhecidos como Lawless e Heymann (75) e Stone e Sidel (90) recomendam que provadores treinados não devem participar em provas com questões do tipo gosto/ não gosto, pois a sua opinião hedónica não é representativa da perceção de consumidor desavisado (*naif*).

Neste sentido, é importante a sensibilização para o uso correto das ferramentas de análise sensorial, com vista à obtenção de informação realmente válida e fiável, da evolução das propriedades organoléticas ao longo do tempo de armazenamento dos produtos prontos a consumir.

No que se refere ao limite de aceitabilidade, não há regulamentação, pois trata-se de um parâmetro que geralmente, não é monitorizado nestes produtos pelos agentes reguladores. Este parâmetro é estabelecido, geralmente, pelos investigadores ou ao nível da indústria, dos técnicos do processamento em consonância com os técnicos dos departamentos da qualidade e I&D.

A aceitabilidade reflete um juízo feito pelo consumidor, integrando as características sensoriais percebidas de um determinado produto e a sua adequação ao uso pretendido. É geralmente pedido ao consumidor que classifique o alimento quanto ao grau de gostar (não gosto / gosto), usando uma escala hedónica (75). A escala tradicional com 9 pontos (

Figura 9) desenvolvida por Peryam e Pilgrim (96) é a escala hedónica mais amplamente utilizada para avaliar a aceitação de produtos alimentares (70).

Nesta metodologia, para a estimativa do tempo de vida útil sensorial, com base no estudo do limite de aceitabilidade, os consumidores são confrontados com conjuntos de amostras com diferentes tempos de armazenamento, sendo solicitado que registem o seu nível de apreciação global utilizando a referida escala hedónica de 9 pontos.

Assim, recorrendo também a uma abordagem baseada em estudos de aceitabilidade, a previsão do tempo de vida útil dos produtos fica mais completa.

Figura 9- Escala hedónica típica usada em estudos de aceitabilidade com consumidores (96)

4.2. Parâmetros físico-químicos

Humidade, pH e acidez, cinzas e minerais

A perda de água nos legumes é resultado de reações metabólicas, como a respiração/transpiração (no caso específico dos produtos minimamente processados), crescimento microbiano ou alterações físicas (sobretudo flutuações de temperatura durante o armazenamento e transporte). O aspeto e textura características do produto fresco manter-se-ão, se o teor de humidade do produto for mantido, e enquanto os tecidos conseguirem reter esta água. A suculência que lhes é característica é devida à grande quantidade de água que estes possuem (mais de 80 %). À medida que a água do alimento se vai libertando e chegando à superfície, são arrastados com ela nutrientes e micronutrientes (fitoquímicos e vitaminas), potenciado o crescimento microbiano, para além das já referidas alterações no aspeto (enrugamento, perda de firmeza, crocância, suculência e cor). A perda de água por transpiração e consequente condensação do vapor de água na embalagem é, assim, um dos principais processos que contribuem para a deterioração comercial e fisiológica dos produtos hortofrutícolas (97, 98). No caso dos alimentos cortados, a área superficial exposta ao ar é muito maior que nos produtos inteiros, pelo que as perdas podem ser mais acentuadas e o ritmo das reações metabólicas e/ou de degradação acelerado. A condensação pode ser evitada, selecionando o material de embalagem com adequada permeabilidade ao vapor de água e também mantendo um *headspace* de forma a que a humidade relativa no interior da embalagem se mantenha mais baixa que o nível de humidade (cerca de 95%) causado pelo processo de transpiração ou condensação, devido a flutuações de temperatura (97).

O pH de um alimento é a medida da acidez ou alcalinidade do produto. Assim, quanto ao valor de pH, os alimentos são classificados como ácidos (pH <4,5), pouco ácidos (4,6 <

pH < 7,0), neutros (pH = 7,0) ou alcalinos (pH >7,0). Os vegetais são ligeiramente ácidos, embora os preparados à base de vegetais, essencialmente sumos, tenham pH≤3,7. A maior parte dos vegetais são não-ácidos com pH que pode chegar aos 6,6 (62).

O pH dos alimentos é um fator preponderante no crescimento dos microrganismos contaminantes, já que estes não possuem mecanismos próprios para controlar o seu pH interno. A maioria dos microrganismos cresce melhor a um pH perto da neutralidade; as bactérias patogénicas não conseguem desenvolver-se a um pH de 4,5 e as que alteram os alimentos dificilmente crescem a um pH inferior a 3,5 (62). Desta forma, este parâmetro é usado para determinar os requisitos de processamento e a aplicação da regulamentação e de recomendações relacionadas com as *Boas Práticas de Processamento*. Para além dos aspetos relacionados com a segurança, a monitorização do pH pode revelar o estado de alteração de fitoquímicos responsáveis por inúmeras reações que, consequentemente, provocam alterações fisiológicas no produto e que dependendo da sua extensão, serão percebidas pelos consumidores. Por exemplo, as antocianinas alteram a sua cor em resposta a uma alteração no pH do meio. A pH baixo (pH <3), estes pigmentos são laranja, passando por vermelho e à medida que vai aumentando o pH a cor púrpura/violeta acentua-se. O mesmo acontece com os carotenoides que sofrem isomerização, passando de configuração *trans* a *cis* em meios ácidos, promovendo alterações de cor nos vegetais, uma vez que as duas configurações apresentam propriedades espetrais diferentes (99).

O teor de cinzas é vulgarmente associado ao teor de minerais num alimento (100). A composição mineral dos vegetais é um parâmetro de particular interesse, não só pelo importante aporte nutricional mas também quando se pretende compreender a evolução de outros parâmetros como a textura e o sabor (101-103) ao longo do tempo de armazenamento de produtos hortofrutícolas, como os legumes MP e PC, objeto de estudo desta tese.

É amplamente reconhecido o valor nutricional dos vegetais e que apresentam teores consideráveis de minerais, entre muitos, o potássio (K), ferro (Fe), sódio (Na), magnésio (Mg), fósforo (P), enxofre (S) e cálcio (Ca). Nos vegetais, os minerais desempenham funções biológicas, integrando-se no metabolismo de proteínas, lípidos e hidratos de carbono e também funções estruturais, ligando-se a moléculas da parede celular e ainda a importante função de regular o equilíbrio osmótico e reações ácido/base (104). No homem, são essenciais por motivos semelhantes aos referidos anteriormente. São micronutrientes com função de regulação de processos físico-químicos, de importância vital para o metabolismo, o normal crescimento e desenvolvimento. O potássio, o mais abundante nos vegetais, é reconhecido por ajudar na redução do risco de AVC (acidente vascular cerebral); o magnésio tem uma função relevante na síntese proteica e no normal

funcionamento do coração; o fósforo é essencial na mineralização óssea para o correto funcionamento da célula e o cálcio, como é do conhecimento geral, essencial também na formação óssea e prevenção da osteoporose (105).

O processamento mínimo (lavagem, corte e embalamento) de vegetais promove o aumento da taxa das reações metabólicas, conduzindo a uma mais rápida deterioração, com consequente perda de propriedades organoléticas características e de valor nutricional. O período de colheita e as práticas pós-colheita bem como o amadurecimento influenciam também a estabilidade mineral dos vegetais frescos (106). No entanto, a maiores perdas ocorrem geralmente quando os vegetais são submetidos a operações de processamento térmico, motivadas pela lixiviação para a água de fervura (nas operações de cozedura). Os tratamentos térmicos que minimizam estas perdas são os tratamentos com vapor (branqueamento ou cozedura), micro-ondas e fritura (48).

Os minerais (Ca, P, Mg, Na, K, Fe, Cl, S entre outros) e a microestrutura onde se encontram podem ser avaliados recorrendo a técnicas de microscopia eletrónica de varrimento (SEM – do inglês *scanning electron microscopy*). A análise elemental é recorre a um detetor de raio-X depois de obtidas as imagens da microestrutura por SEM. O princípio de funcionamento desta técnica para deteção dos minerais tem por base a estimulação dos átomos, pelo feixe de eletrões de energia uniforme, que instantaneamente imite raios-X de determinado nível energético, característico de cada elemento. O espetro de raio-X emitido é convertido em teores de determinado elemento por comparação com uma base de dados que um *software* adequado armazena. Esta emissão de radiação fornece informação acerca da composição elemental da amostra analisada. Os resultados são habitualmente expressos em percentagem de peso na composição total (Wt %) de Ca, P, Mg, Na, K, Fe, Cl ou S normalizada para o total do sinal emitido pela totalidade dos elementos detetados.

A composição mineral determinada por SEM é uma metodologia prática e rápida. Tratando-se de uma análise semi-quantitativa, a concentração aparente de elementos de número atómico baixo, tende a ser estimada por defeito (ligeiro) e no caso dos elementos de número atómico elevado acontece o contrário, sendo a estimativa da concentração aparente ligeiramente superior (107). No entanto, realizando um número adequado de determinações (réplicas) e recorrendo a uma análise estatística apropriada, pode rapidamente obter-se informação válida acerca da distribuição de minerais ao longo da microestrutura e, por comparação entre amostras, obter informações acerca de perdas ou ganho devido a etapas de processamento e/ou armazenamento.

Compostos bioativos e atividade antioxidante

Segundo Gry et al. (108), compostos bioativos são constituintes não nutritivos inerentes aos produtos vegetais, que estão associados a efeitos benéficos e promoção da saúde quando ingeridos.

A importância destes compostos é amplamente reconhecida pela sua contribuição na prevenção de doenças cardiovasculares e degenerativas, entre outras, devido à sua atividade anti-inflamatória, antiviral, antimicrobiana e antialérgica (108-110). O teor em compostos fitoquímicos é afetado não só pelo grau de maturação e do genótipo dos produtos, mas é também influenciado pelas operações de processamento e condições de armazenamento a que os produtos prontos a comer são submetidos. Como consequência, a atividade antioxidante pode ser afetada, e no final, a atividade biológica destes compostos ser também comprometida (53, 111).

Nesta tese foram monitorizados, ao longo do tempo de armazenamento, alguns compostos bioativos, com a finalidade de estudar a influência do processamento e da embalagem em AM adotadas na conservação dos legumes. Vários autores (49, 51, 64, 67, 110, 112-122) associam aos ensaios dos produtos conservados em AM a quantificação destes compostos, para monitorização da qualidade nutricional do alimento, mas também pela sua importância na manutenção das propriedades organoléticas características (cor e *flavour*) (123, 124).

Estes compostos fitoquímicos são classificados de acordo com a sua estrutura química, por exemplo: polifenois, carotenoides, compostos organossulfurados, alcaloides e compostos azotados (124). Outras classificações podem ser consideradas, como por exemplo segundo o tipo de extração como refere Bessada (125) na sua monografia sobre o potencial antioxidante de plantas da família *Asteaceae*: (i) Compostos hidrofílicos ou polares (extração, por exemplo, com metanol e/ou água). Os compostos fenólicos são exemplos bem conhecidos; (ii) Compostos lipofílicos ou apolares (extração, por exemplo, com éter de petróleo ou hexano). Alguns exemplos são os carotenoides (vitamina A, β-caroteno, licopeno), alcaloides, terpenoides, ácidos gordos, ou a vitamina E.

Compostos fenólicos

De uma forma geral os legumes são ricos em diferentes tipos de compostos fenólicos que incluem ácidos fenólicos, flavonoides (flavonas, isoflavonas, flavanonas, flavanois, flavonóis, antocinaninas, entre outros) e amino-fenóis (

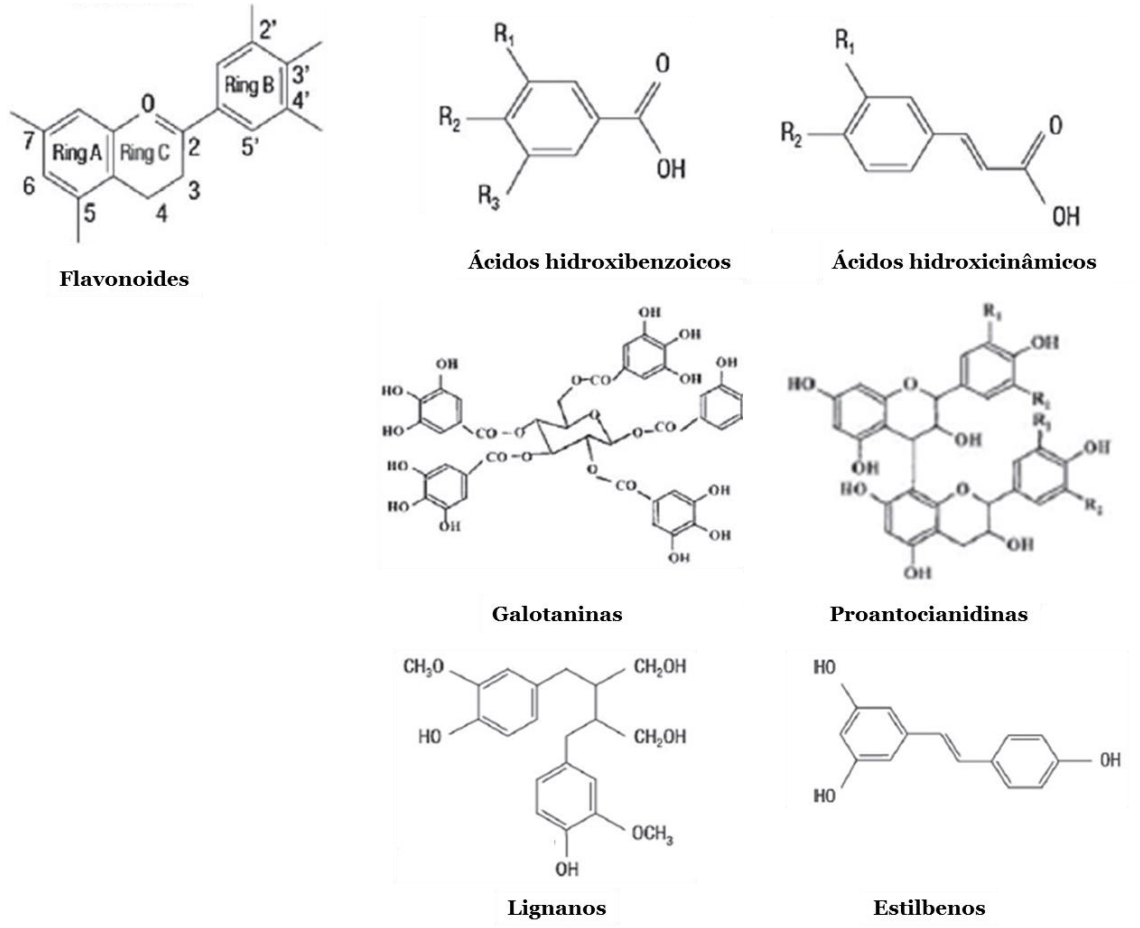

Figura 10) (126)

Figura 10- Estruturas químicas das principais classes de compostos fenólicos (126)

Os compostos fenólicos são vitais, muito devido à sua atividade antioxidante associada à sua capacidade de captar agentes pro-oxidantes desencadeadores de doença crónica (47, 127). Estes compostos podem prevenir lesões causadas pelos radicais livres através dos seguintes mecanismos (124, 128):

i. Captação direta de espécies reativas de oxigénio
ii. Ativação de enzimas antioxidantes;
iii. Atividade quelante de metais;
iv. Redução dos radicais α-tocoferilo;

v. Inibição das oxidases;
vi. Atenuação do *stress* oxidativo causado pelo óxido nítrico;
vii. Sinergia de propriedades antioxidantes.

Como existe um elevado número de compostos fenólicos, muitos trabalhos, sobretudo de monitorização e controlo da qualidade, reportam resultados em teores totais (127). A composição fenólica dos legumes é dependente da cultivar, estado de maturação e das condições pós-colheita.

Os compostos fenólicos são solúveis em água, logo suscetíveis à lixiviação. O processo de branqueamento, muitas vezes utilizado como pré-tratamento no processamento de conservas, na congelação e em produtos prontos a consumir, inativa as enzimas que provocam a oxidação destes compostos (129). A degradação química pode ainda ocorrer durante o armazenamento, dependendo do oxigénio disponível e a exposição à luz. Além disso, os compostos fenólicos, bem como outros fitoquímicos, encontram-se em maior quantidade nas camadas mais superficiais (casca) dos hortofrutícolas, de modo que ocorre sempre alguma perda durante as etapas de corte e desinfeção (123, 124).

Flavonoides

Os flavonoides são a maior classe de fitoquímicos presentes nos hortofrutícolas. Estão presentes em todas as plantas vasculares, sendo reconhecidos como os pigmentos responsáveis pela cor (várias tonalidades de amarelo, laranja e vermelho) dos vegetais em geral. A atividade bioquímica dos flavonoides e dos seus metabolitos depende da sua estrutura química e da orientação relativa dos grupos nos diferentes anéis. As variações na estrutura do anel central dão origem a outras classes como: flavonóis, flavonas, flavanonas, flavanóis (ou catequinas), isoflavonas, flavanonóis e antocianidinas. Na Figura 11 apresentam-se as estruturas químicas das principais classes de flavonoides. As flavonas e os flavonóis são os mais abundantes e estruturalmente diversificados (124, 126, 127).

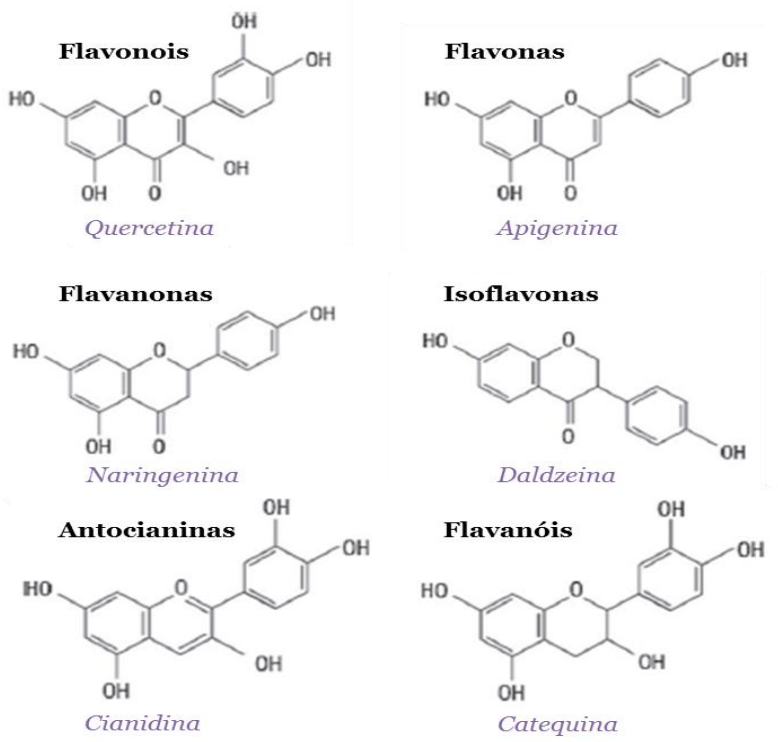

Figura 11- Estruturas químicas das principais classes de flavonoides (126)

Os flavonoides, também designados bioflavonoides, têm despertado interesse devido aos seus efeitos benéficos na saúde, sendo descritos como possuidores de atividade antioxidante (14, 126, 130), efeito protetor na própria planta contra a radiação UV, contaminantes fúngicos e microrganismos patogénicos. Alguns deles têm um poder antioxidante superior ao da Vitamina C e E (131).

Antocianinas

As antocianinas estão presentes na grande maioria dos tecidos vegetais, sendo particularmente evidentes nas frutas e flores, onde são responsáveis pelas cores vermelha, azul e roxa, dependendo do pH (132). Estão presentes nas folhas, caules, sementes, tecidos e raízes. É o maior grupo de pigmentos hidrossolúveis da família dos flavonoides. A estrutura química base das antocianinas são as antocianidinas (ou aglíconas). Estas estruturas são formadas por um anel aromático (A) ligado ao anel heterocíclico C que contém uma molécula de oxigénio que, por sua vez, está ligado a um outro anel B através de uma ligação C-C (126). Estas aglíconas encontram-se maioritariamente ligadas a uma molécula glicosídica (monossacáridos: xilose, arabinose, ramnose, galactose e glicose), constituindo a forma glicosilada, denominando-se antocianinas. Os açúcares podem, por sua vez, surgir acilados com ácidos aromáticos ou alifáticos.

Os legumes são fontes importantes de antocianinas. A sua estabilidade é facilmente afetada por diversos fatores: pH, temperatura de armazenamento, estrutura química, concentração, luz, presença de O2, solventes e pela presença de enzimas, proteínas e iões metálicos.

Tal como acontece noutros fitoquímicos, as aglíconas e a forma glicosilada (antocianina) possuem diferentes atividades biológicas. O controlo pode ser realizado monitorizando o teor de antocianinas monoméricas, ou identificando e quantificando as principais classes (pelargonidina, cianidina, peonidina, delfinidina, petunidina e malvidina) (52, 117, 131) . Sabendo que se trata de compostos importantes do ponto de vista da saúde e também da qualidade físico-química e organolética dos legumes, torna-se pertinente a sua quantificação. Sendo reconhecida a sua instabilidade e sensibilidade a fatores externos, a monitorização ao longo do tempo de vida dos legumes é também um parâmetro da qualidade a integrar nos estudos de tempo de vida.

Carotenoides

Os carotenoides são pigmentos naturais presentes nas plantas, animais e microrganismos. São pigmentos que conferem cor laranja, amarela e vermelha e são sintetizados pelo mecanismo fotossintético das plantas superiores e algas. Por vezes, a expressão destes pigmentos é mascarada pela cor verde das clorofilas, presentes sobretudo nos vegetais de folha verde (120, 124). Os carotenoides mais abundantes nos vegetais são os β-carotenos, alocados nos cloroplastos. Noutros vegetais (frutos, flores e raízes) é mais comum o licopeno, armazenados nos cromoplastos (99, 133).

Os carotenoides são lipossolúveis, com um esqueleto isoprenoico comum de 40 carbonos, de cadeia longa conjugada de ligações duplas. Dependendo dos átomos ligados à molécula base, os carotenos dividem-se em dois grupos:

- carotenos - que contêm apenas carbono e hidrogénio (ex.: β-carotenos e licopeno),
- xantofilas - que contêm moléculas de oxigénio (grupos ceto, hidroxilo, epóxi, metoxi ou acido carboxílico; ex.: luteína, zeaxantina e astaxantina).

Os primeiros apresentam polaridade mais baixa do que os segundos, derivados oxigenados com polaridade mais forte (99). As moléculas menos polares têm como solventes adequados o hexano e o éter de petróleo, ao contrário das xantofilas que se dissolvem melhor em solventes apolares, como por exemplo, álcoois (metanol) (124).

As cores características deste pigmento são devidas à estrutura carbono-carbono da molécula de duplas ligações conjugadas, pelo menos sete vezes, lhe conferem determinada cor. A tonalidade e intensidade da cor estão relacionadas com o tipo de molécula, a sua

concentração e o seu estado físico. Estes pigmentos são sensíveis ao calor, pH do meio, luz e oxigénio, o que os torna instáveis. São amplamente usados na indústria alimentar como corantes naturais (99, 124, 133)

Nos tecidos, os carotenoides mais abundantes surgem na forma de isómeros *trans,* pois trata-se da configuração mais estável. A configuração *cis* também existe, mas em quantidades mais reduzidas, como já referido anteriormente, devido a diferentes condições de pH, luz e presença de O2. A diversidade de carotenos é devida a alterações que ocorrem na estrutura da molécula base, como por exemplo a ciclização dos terminais, com a introdução de grupos funcionais com oxigénio. Estas alterações conferem à molécula do pigmento, para além da cor, propriedades antioxidantes diferentes. Dos diferentes carotenoides, o que apresenta maior capacidade antioxidante é o licopeno mas o β-caroteno é o mais abundante nos legumes de folhas verdes e mais bioativo, tanto como precursor da vitamina A como na proteção da saúde humana (99, 124, 134).

O sistema conjugado de duplas ligações é responsável pela atividade antioxidante dos carotenoides, captando oxigénio ou radicais livres (135). A presença dessas ligações também facilita a oxidação dos carotenoides, o que provoca uma perda da coloração nos alimentos.

A degradação precoce destes pigmentos é provocada pela exposição à luz durante a campanha de pós-colheita (incluindo processamento e armazenamento). A senescência dos legumes favorece também a sua deterioração. No entanto, dos fitoquímicos, os carotenoides são os mais estáveis e resistentes a etapas de processamento e armazenamento. De todo o modo variações excessivas de temperatura excessiva exposição à luz ou atmosfera inadequada afetam os seus teores totais. Alguns estudos indicam que a capacidade antioxidante dos carotenoides e consequente bioatividade aumentam após etapas de cozedura de alguns vegetais (ex: a cenoura), como relatam Miglio et al. (136), Rickman et al. (134) e Shahidi et al. (124). Relativamente a vegetais em AM há ainda poucas certezas e até alguma discordância entre trabalhos publicados (99, 134, 137).

Neste sentido, a monitorização do teor total de carotenoides é um parâmetro importante, não só para o controlo nutricional, mas também na compreensão das alterações da cor avaliada, quer instrumental quer sensorialmente, ao longo do tempo de vida útil dos legumes embalados em AM.

Atividade antioxidante

Os vegetais minimamente processados ou pré-cozidos têm um papel importante nas dietas Mediterrânica e Atlântica, consumidos como acompanhamentos ou incluídos na sopa. Para além de colorirem o prato e serem apreciados pela frescura, texturas e sabores característicos, são considerados alimentos saudáveis por serem fontes de micronutrientes e de uma grande variedade de bioativos. Estes compostos são reconhecidos pela sua atividade biológica, não só na defesa da própria matriz, preservando as propriedades organoléticas (14), mas também na proteção da saúde, na prevenção de doenças causados por *stress* oxidativo, como referido anteriormente (138).

O processo respiratório é a atividade metabólica que providencia a energia necessária às reações bioquímicas das células. A respiração (aeróbia), envolve a desagregação/ quebra de macromoléculas como hidratos de carbono, lípidos e ácidos orgânicos, em moléculas mais pequenas e mais simples através de reações oxidativas, com libertação de energia (44). Estas reações levam à formação de radicais livres, espécies reativas de oxigénio, como o peróxido de hidrogénio e o anião superóxido, que causam *stress* oxidativo devido ao desequilíbrio entre espécies antioxidantes e oxidantes (130, 137). A ocorrência de reações de *stress* oxidativo causa dano ao organismo e contribui para o aparecimento de doenças, tais como: inflamação, tumores, Alzheimer e doenças cardiovasculares, bem como aceleram o processo de envelhecimento (135). Assim, as células humanas necessitam de uma certa capacidade antioxidante para manter o equilíbrio e assim prevenir os efeitos prejudiciais de radicais livres e espécies reativas de oxigénio. A atividade antioxidante é conferida, em grande parte, pelos compostos bioativos presentes nos vegetais, tais como: carotenoides, compostos fenólicos, especialmente flavonoides entre outros (algumas vitaminas), que desempenham um papel específico na captura de radicais livres, constituindo a rede de defesa *in vivo* (Figura 12) (139).

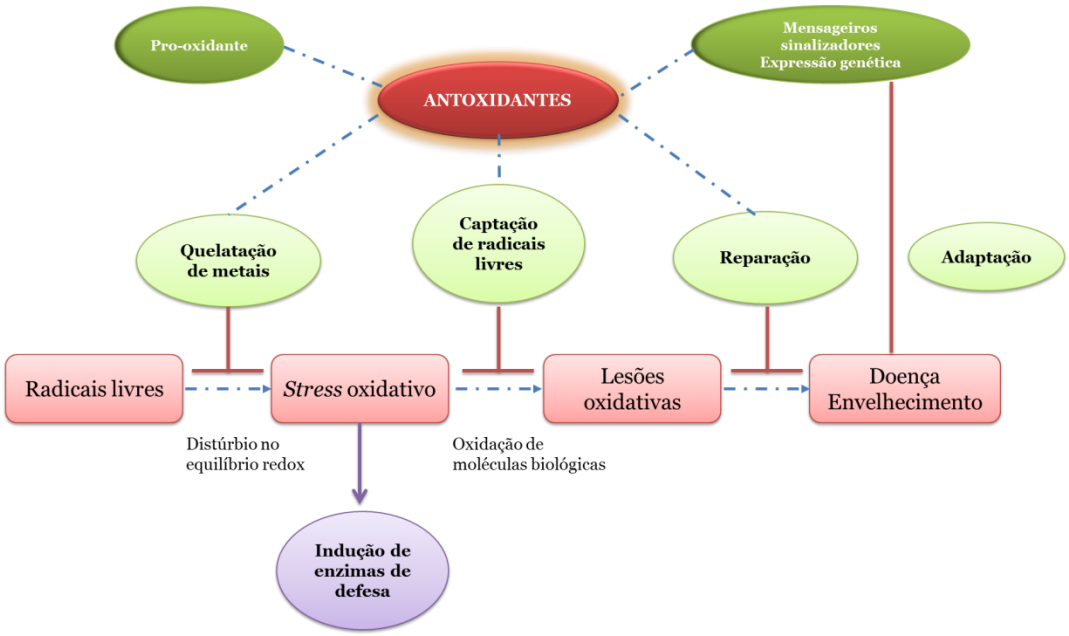

Figura 12- Sistema de defesa *in vivo* contra o *stress* oxidativo (139)

No entanto, como já tem sido referido, o teor em compostos bioativos disponíveis e consequente atividade antioxidante depende da história do produto desde o momento da colheita e, sobretudo, durante operações de armazenamento e processamento (14, 36, 136, 140). O estudo do efeito de pré-tratamentos (lavagem e desinfeção e/ou térmicos), corte e ainda o armazenamento refrigerado em embalagens com AM no potencial antioxidante é relevante, uma vez que são métodos importantes para a conservação do produto e da sua integridade nutricional (19, 140).

Neste sentido, a monitorização da atividade antioxidante dos legumes é importante e, em certa medida, um indicador da qualidade nutricional destes. A captação dos radicais livres é o principal mecanismo de ação dos antioxidantes nos alimentos. Foram desenvolvidos muitos métodos em que se mede a capacidade antioxidante através da captação dos radicais livres sintéticos em solventes orgânicos polares, por exemplo metanol, à temperatura ambiente. Vários métodos têm sido estudados e implementados com sucesso para a determinação da atividade antioxidante, nomeadamente DPPH.

O método DPPH (1,1–difenil–2–picril–hidrazilo) é um dos métodos mais usados na determinação da atividade antioxidante em vários legumes. O DPPH é um radical livre estável, que se torna numa espécie não radicalar dificilmente oxidável quando aceita um eletrão ou um átomo de hidrogénio. O radical DPPH (forma oxidada) tem cor violeta forte e o DPPH não radical (forma reduzida) é amarelo. No ensaio espetrofotométrico a 515 nm,

a absorvência diminui como resultado de uma alteração na coloração violeta característica para amarelo. O radical DPPH na presença de antioxidantes dadores de átomos de H, é reduzido formando a molécula estável DPPH-H (141).

$$DPPH^{\bullet} + AH \rightarrow DPPH - H + A^{\bullet}$$

Alguns antioxidantes, por exemplo o α-tocoferol, reagem rapidamente com o radical DPPH. No entanto, uma série de reações secundárias mais lentas podem causar uma diminuição progressiva da absorvência, devendo-se, na maior parte dos casos, medir depois de um determinado tempo (previamente otimizado) após o início da reação. Os resultados são normalmente expressos como EC_{50}, que corresponde à concentração de antioxidante necessário para captar 50% do radical DPPH, num determinado período de tempo (53, 111, 142). Podem ser usados outros métodos na determinação da capacidade antioxidante, tais como: reação com o padrão Trolox, cuja atividade é usada como equivalente à capacidade antioxidante (TEAC – do inglês *Trolox Equivalent Antioxidant Capacity*); quantificação da absorvência de radicais livres de oxigénio (ORAC – do inglês *Oxygen Radical Absorbance Capacity*) e determinação da capacidade de redução do ião férrico (FRAP- do inglês *Ferric Reduction Antioxidant Parameter*). Segundo a revisão bibliográfica feita, os resultados publicados relativamente à atividade antioxidante de diversos hortofrutícolas frescos, minimamente processados ou processados termicamente, são divergentes. Segundo Sulaiman et al. (143), tal deve-se não só à diversidade natural das matrizes analisadas cuja composição depende da sazonalidade, estado de maturação, práticas de pós-colheita, como já referido anteriormente, mas também depende dos métodos de extração (tipo de solvente usado) e de quantificação usados. Segundo este autor, a extração com metanol é aquela que, em termos de quantificação de capacidade antioxidante total, exibe valores mais elevados na captura do radical DPPH.

São poucos os estudos, até ao momento, que avaliaram a estabilidade de compostos antioxidantes em vegetais durante o armazenamento e processamento doméstico ou mesmo em grande escala, em restaurantes e cantinas escolares (136). Neste sentido, torna-se pertinente a monitorização deste parâmetro, que ajuda a perceber evolução da qualidade dos produtos armazenados e correlacioná-la com os restantes parâmetros monitorizados, nomeadamente: propriedades organoléticas, textura e cor.

Nesta tese apenas se determinou a atividade antioxidante pelo método de captação de radicais livres do DPPH.

Textura

A textura é uma das propriedades dos alimentos que mais influencia a aceitabilidade e preferência dos consumidores, seguindo-se o aroma e o sabor (71, 72, 144). A melhor forma para descrever os parâmetros de textura é a análise sensorial, pois uma série de sensações de boca são percebidas aquando da mastigação. A análise sensorial permite, também, como já referido, descrever de forma completa/integrada os produtos e, ainda permite quantificar estas sensações com precisão, recorrendo a um painel treinado (89). No entanto, este treino dos provadores é moroso, pois nem todos os parâmetros de textura são simples de aprender e quantificar. Mais, nem sempre existe compatibilidade entre a disponibilidade de amostra e do painel ou mesmo disponibilidade de amostra suficiente para treino e sessões de prova. Na tentativa de ultrapassar estas dificuldades tem-se assistido a uma evolução contínua de técnicas de análise instrumental que visam prever atributos sensoriais.

A textura é uma propriedade organolética complexa que suscita uma grande variedade de sensaçõeTabela 2). Um teste mecânico não conseguirá explicar de forma totalmente abrangente todas as nuances da textura do alimento percebidas aquando do ato de comer. Os equipamentos analisadores de textura conseguem detetar e quantificar determinados parâmetros físicos, que posteriormente o técnico irá interpretar à luz da perceção sensorial (145).

Apesar da definição de textura englobar uma série de atributos, como os listados na (Tabela 2)Tabela 2, os relacionados com alterações mecânicas e estruturais são suficientes para conseguir uma boa aproximação instrumental em termos de controlo da qualidade e de informação. É assim possível adequar a metodologia ao produto, minimizando danos decorrentes de práticas de pós-colheita, do transporte, do processamento (MP ou PC) ou ainda alterações durante o armazenamento (146).

Vários autores publicaram trabalhos de avaliação instrumental da textura em alimentos. Alguns deles propõem correlações com a avaliação sensorial a partir da avaliação instrumental desses atributos (144, 147, 148). Muitos trabalhos quantificam atributos como firmeza, crocância, fraturabilidade, adesividade, elasticidade num só teste de compressão, conseguindo precisão em pouco tempo de trabalho. Muitos autores afirmam também que pequenas diferenças na firmeza são mais facilmente detetadas quando comparadas com um painel treinado, que precisa de pelo menos 6-8 N, para perceber tal diferença (144).

Tabela 2- Terminologia mais comum usada na análise sensorial da textura em alimentos (146)

Termos relacionados com o comportamento do material sob compressão ou tensão				
firme / firm	borrachento /rubbery	adesivo / adhesive	espesso / thick	quebradiço / brittle
duro / hard	elástico / elastic	húmido / tacky	fina / thin	friável / friable
macio / soft	plástico / plastic	pegajoso /gooey	fibroso / chewy	grumosa /crumbly
resistente / tough	pegajoso /sticky	glutinoso /glutinous	elástico springy	crocante / crunchy
tenro / tender	floculento / short		viscoso / viscous	estaladiço / crisp
Termos relacionados com a microestrutura da matriz: tamanho da partícula e forma				
suave / smooth	grumoso / lumpy	fino / fine	farinhento / powdery	
pulverulento / chalky	granulado / mealy	arenoso / gritty	grosseira / coarse	
Termos relacionados com a microestrutura: forma e organização estrutural da matriz				
escamosa /flaky	celular / cellular	vítreo / glassy	cristalina / crystalline	
fibrosa / fibrous	gaseificado / aerated	gelatinosa / gelatinous	esponjoso /spongy	
filamentoso /stringy	expandido / puffed	espumoso /foamed	polposo / pulpy	
Termos relacionados com sensações de boca				
paladar / mouthfeel	molhado / wet	cera /waxy	quente / hot	borracha / chewy
residual /getaway	aguado / watery	suculenta / juicy	frio / cold	pastosa /mushy
corpo / body	seco /dry	oleosa /oily	fresca / cooling	cremosa / creamy
viscoso / slimy	húmido / moist	gorduroso / greasy		

A resposta sensorial a um estímulo mecânico é não-linear e pode ser afetada não só pelo treino (ou falta dele) mas também pela adaptação e fadiga. No caso dos equipamentos, a resposta/os resultados interpretam-se à luz das sensações sensoriais. No entanto, essa resposta apenas depende da carga aplicada e das propriedades reológicas dos tecidos (149). As microestruturas do órgão vegetal submetido a uma determinada força (por exemplo de compressão) irão reagir de forma concertada, conferindo ao produto uma resistência a essa força que lhe será característica (Figura 13). Esta resposta pode ser comparável com a perceção sensorial (146).

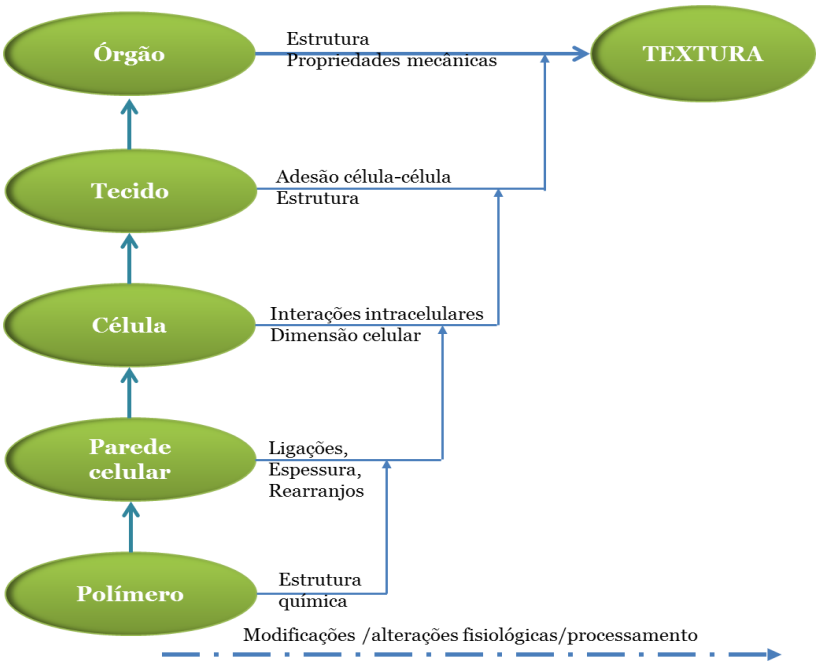

Figura 13- Níveis de interação das estruturas que contribuem para a definição dos parâmetros texturais de um vegetal (146)

A deformação durante um teste de compressão (ou mastigação) depende das propriedades mecânicas nos diferentes níveis de estrutura e da sua interação, verificando-se uma hierarquia estrutural (Figura 13). A forma como as células estão organizadas na matriz do produto, os espaços existentes entre elas e o tipo de membrana que as une, determinam as suas características texturais. Do mesmo modo, durante etapas de pós-colheita, processamento e armazenamento e no final durante a mastigação, a forma como se dá a separação das células, a sua rutura e a libertação dos componentes intracelulares (amidos, proteínas, lípidos, açúcares e sais), são também fatores que irão influenciar a textura destes produtos. Além disso há fenómenos de osmose que condicionam os movimentos da água através da parede celular que, por seu lado, contribuem para a manutenção da estrutura túrgida ou mais macia/mole do produto. Este estado da matriz é dos mais

importantes na definição das propriedades mecânicas da célula, conferindo aos produtos uma textura característica (146).

A avaliação das propriedades texturais/reológicas dos alimentos, segundo a teoria de Scott-Blair, pode ser dividida em três tipos de métodos instrumentais (146, 150, 151):

(i) *métodos fundamentais* - avaliam (quantificam) propriedades físicas bem definidas, independentemente do tamanho da amostra, dimensão e teste, como por exemplo a determinação da viscosidade, recorrendo a um reómetro ou viscosímetro;

(ii) *métodos empíricos* - avaliam uma determinada propriedade, atributo ou comportamento quando o alimento é submetido a condições experimentais de teste bem definidas, por exemplo avaliar a resistência a uma determinada deformação, recorrendo a expressões matemáticas empíricas para correlacionar as grandezas físicas;

(iii) *métodos imitativos* – são aqueles que melhor se correlacionam com a análise sensorial, numa tentativa de simular o que acontece a um alimento quando este é sujeito à mastigação ou outros fenómenos que ocorrem na boca.

Estes últimos, estão descritos como melhor correlacionados com a análise sensorial, pois mimetizam a mordida e a mastigação num alimento. Para tal, é vulgarmente usada a técnica de avaliação do perfil de textura do alimento (TPA – do inglês *Texture Profile Analysis*) que simula duas dentadas, quantifica a reação deste em termos de força necessária para romper a estrutura, e respetiva recuperação (se existir). A partir desta informação podem ser calculadas outras propriedades (elasticidade, coesividade, fraturabilidade, etc.), de forma a caracterizar o perfil de textura do alimento e, assim, correlacionar com alguns dos atributos sensoriais. A aplicabilidade e a evolução destas técnicas e equipamentos têm sido tal que, atualmente, não só permitem a avaliação do efeito da força, mas também avaliam o efeito da saliva no alimento, o som que produz, etc (151).

É possível, também, avaliar a evolução da textura ao nível da microestrutura celular, recorrendo a técnicas de SEM. Com esta técnica, podem observar-se os fenómenos de perda de turgidez, quer na comparação de produtos frescos com produtos processados termicamente, quer durante o armazenamento, na tentativa de determinar o momento, a partir do qual, se verificam alterações/degradação da microestrutura. No trabalho desenvolvido nesta tese, todos os legumes em estudo foram observados, recorrendo a técnicas de SEM, e cujas fotografias servem de auxílio à discussão dos resultados obtidos noutras análises (fenólicos totais, minerais e sensoriais).

Cor

A cor é um dos atributos mais importantes nos alimentos e, sendo um dos aspetos que define a aparência do produto, é um dos primeiros indicadores da qualidade considerados pelo consumidor (152). O modo como a cor dos vegetais evolui durante o período de armazenamento pode determinar a sua aceitação pelo consumidor, pois uma grande alteração em relação à cor inicial poderá levar à rejeição do produto (62, 153). Genericamente, a evolução da cor nos legumes é acompanhada por alterações nos seus pigmentos corados naturais (clorofilas, flavonoides, carotenoides, antocianinas,...) (62), ou por reação enzimática, sobretudo no caso dos legumes minimamente processados. A matriz celular destes produtos possui enzimas (PAL- fenilalanina amónia liases, PPO- polifenoloxidases, PO- peroxidases) que se libertam e em contacto com a atmosfera envolvente ou com os seus substratos, que inicialmente estavam em organelos diferentes ou indisponíveis, reagem e promovem alterações de cor (154). Muitas destas reações têm início logo durante as etapas de processamento (lavagem, corte e desinfeção) e estimulam o metabolismo dos compostos fenólicos como mecanismo de defesa (154, 155).

As alterações de cor são avaliadas sensorialmente pelo consumidor ou por um painel de provadores. A avaliação sensorial da cor com precisão é uma tarefa extremamente complexa, pelo que é vulgarmente um dos atributos de maior dificuldade na obtenção de dados precisos com painéis de provadores. Questões relacionadas com as diferentes interações do meio ambiente e com o modo como a visão (olhos) interpreta a reemissão da luz vinda de um objeto, por meio de ondas eletromagnéticas no espectro do visível (400 a 700 nm), fazem com que a evolução da cor (e suas *nuances*) não seja facilmente transposta para uma lista de atributos. A avaliação instrumental, conforme já referido, pretende simular a perceção humana da cor. A retina do olho humano possui dois tipos de recetores: os bastonetes, que permitem visão em ambientes de baixa intensidade de luz e os cones que atuam em ambientes de maior intensidade de luz. Estes possuem os três tipos de recetores (células fotorrecetoras) sensíveis aos tons primários, a luz vermelha (comprimentos de onda longos), verde (comprimentos de onda médios) e azul (comprimento de onda mais curtos), do inglês Red, Green, Blue ou mais comummente conhecido por RGB. Esta sensibilidade tricromática compõe a perceção da cor pelo olho humano. Instrumentalmente, a perceção tricromática é conseguida à custa de filtros que simulam os *tristimulus* colorimétricos, um para cada uma das referidas cores primárias. Para além desta caracterização, pode ainda determinar-se o nível de intensidade com que cada produto reflete a luz e a saturação que é a intensidade da cor (diferença ou afastamento de um tom cinzento) (152).

No entanto quando pequenas diferenças não são percetíveis pelos provadores ou para abreviar as metodologias analíticas, é possível recorrer à avaliação instrumental. Para medição da cor em alimentos podem ser usados métodos instrumentais em que a cor é expressa de acordo com o sistema da *Commission Internationale d'Eclairage* (CIE) (http://www.cie.co.at/). Existem vários sistemas de cor reconhecidos pela CIE e entre eles o sistema $L^*a^*b^*$, desenvolvido em 1976 por Judd e Hunter e cujas coordenadas estão diretamente relacionados com as coordenadas X, Y e Z (152, 153).

O sistema de cor $L^*a^*b^*$ é o que melhor representa a perceção de cor pelo olho humano. Estas coordenadas apresentam-se num sistema tridimensional, um eixo representa a luminosidade (L) variando entre 0 (ausência de luminosidade, preto) e 100 (máximo de luminosidade, branco), as coordenadas cromáticas posicionam-se nos eixos que representam a variação de verde (-a) a vermelho (a) e de azul (-b) a amarelo (b). Assim, neste sistema, qualquer cor corresponde a um ponto posicionado num gráfico tridimensional (155).

Genericamente a avaliação instrumental da cor é expressa em termos das coordenadas L*, a* e b*, individualmente ou através da combinação entre elas para avaliação da tonalidade (h, eq.1), saturação (*chroma, C*, eq.2*) ou até mesmo variação total de cor (ΔEab, eq. 3).

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad \text{(Equação 1)}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \qquad \text{(Equação 2)}$$

$$\Delta Eab^* = \sqrt{(a^* - a^*{}_0)^2 + (b^* - b^*{}_0)^2 + (L^* - L^*{}_0)^2} \qquad \text{(Equação 3)}$$

Na Equação 3, a variação total de cor permite perceber a variação total das propriedades cromáticas das amostras de alimentos antes e após um processo, ou ao longo do tempo de armazenamento. L_o, a_o e b_o são os valores das coordenadas correspondentes ao alimento no tempo zero (ex: início do armazenamento). A Figura 14 esquematiza estes três atributos cromáticos.

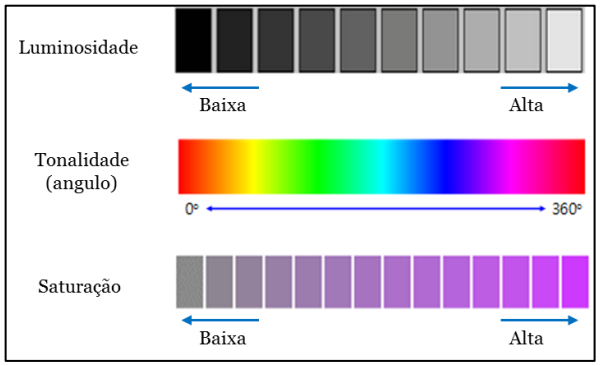

Figura 14- Atributos cromáticos, resultantes da combinação das coordenadas L, a e b: luminosidade, tonalidade e saturação (156)

4.3. Parâmetros microbiológicos

Com já referido anteriormente, a tecnologia de conservação em embalagem com AM e refrigerada, é reconhecida como eficaz na preservação da qualidade dos alimentos prontos a consumir, retardando os efeitos da senescência. Um dos fatores que promove a senescência é o crescimento de microrganismos, o qual pode ser controlado recorrendo à substituição da atmosfera. É amplamente conhecida ação antimicrobiana dos gases habitualmente usados na conservação com AM de hortofrutícolas MP, carnes e peixe fresco, refeições prontas a comer ou outros prontos a consumir (46, 64, 157-161). Estes estudos de uma forma geral concluem que o crescimento microbiano em produtos armazenados em AM com concentrações de CO_2 elevadas e reduzida percentagem de O_2, quando comparado com produtos apenas refrigerados, é mais lento e sobretudo o crescimento de bactérias patogénicas chega mesmo a ser inexistente. Esta inibição é devida ao efeito bacteriostático, não seletivo, do CO_2 (162).

De acordo com a revisão feita por Caleb et al. (162), o efeito bacteriostático do CO_2, também coadjuvado pela competição entre a microflora nativa e os microrganismos patogénicos mais frequentes de cada alimento. Os hortofrutícolas apresentam características químicas diferentes, que se refletem na composição da microflora presente em cada um. Os legumes contêm elevada quantidade de água, nutrientes e pH neutro. Assim, as bactérias tornam-se os microrganismos preponderantes nestes alimentos. Entre as bactérias, as *gram* negativas são as predominantes, sendo a maioria pertencente às famílias *pseudomonacea* e *enterobacteriaceae*, principalmente os géneros *Pseudomonas sp.* e *Erwinia sp.* O género *Pseudomonas sp.* apresenta atividade pectinolítica e não resiste a altas concentrações de CO_2. As bactérias lácticas crescem sob condições

moderadas de CO2 (66, 162, 163) e a maioria dos bolores e leveduras são sensíveis ao CO2 (47).

Os alimentos em AM apresentam normalmente um pH favorável à formação de ácido carbónico devido à dissolução do CO_2 presente na atmosfera da embalagem. Este ácido altera ligeiramente o pH do meio e retarda a proliferação microbiana. No entanto, este facto não é amplamente aceite, pois outros ácidos poderiam provocar o mesmo efeito acidificante, embora sem o poder bacteriostático promovido pelo CO_2 (159). Este efeito resulta do poder penetrante do CO_2 nas células, cerca de 30 vezes mais rápido que o O_2. Aquele, juntamente com os iões bicarbonato, afeta a estrutura da membrana celular, desidratando-a e aumentando a sua permeabilidade a outros iões, alterando o equilíbrio do meio intracelular dos microrganismos. Este mecanismo contribui para o efeito inibitório do CO2 sobre as enzimas do metabolismo energético dos microrganismos. Altas concentrações de CO2 inibem a ação da oxaloacetato descarboxilase, da fumarato e succinato desidrogenases e citocromo c oxidase, aumentando a formação de succinato. Eleva, também, a atividade da ATPase mitocondrial que resulta numa redução da energia necessária ao crescimento microbiano (159). No entanto, este efeito inibidor varia de microrganismo para microrganismo. Isto porque o CO2 inibe alguns tipos de microrganismos, mas não apresenta efeito direto sobre outros (162, 164). Ainda segundo Caleb et al. (162), Hintlian e Hotchkiss (164), qualquer que seja a atmosfera presente existe risco microbiológico potencial, e portanto, o enriquecimento da atmosfera não dispensa a refrigeração. A embalagem em AM pode inibir o crescimento de microrganismos deterioradores, mas permitir a proliferação de patogénicos que, sem os sinais de degradação comuns, podem ser ingeridos com os alimentos. Por isso, o aumento do tempo de vida dos alimentos minimamente processados deve ser criteriosamente estudado.

A realização de análises microbiológicas ao produto final faz parte dos procedimentos de verificação dos limites críticos dos critérios de higiene dos processos e permite saber se o produto final está próprio para consumo. Esta avaliação, salvo alguma disposição legal, segue as orientações dos valores guia definidos pela *Health Protection Agency* (HPA) *"Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market"* (165). Este guia fornece os parâmetros microbiológicos que permitem avaliar a segurança de alguns alimentos prontos-a-comer e respetivos critérios da qualidade. São recomendações baseadas no conhecimento biológico e epidemiológico dos microrganismos presentes nos alimentos, respeitando os critérios exigidos na regulamentação comunitária (Regulamento (CE) nº 1441/2007).

A análise microbiológica, por si só não garante a segurança final de um produto, mas dá informações válidas quando aliadas e integradas num sistema com implementação de medidas preventivas. Nem sempre a presença de microrganismos significa um perigo para a saúde dos consumidores, pelo que é importante determinar que microrganismos estão presentes e em que quantidade (166). Neste trabalho, o controlo microbiológico consistiu apenas na contagem de microrganismos aeróbios totais a 30 ºC, em *Plate Count Agar* (PCA). Não pretende ser uma avaliação da segurança, mas apenas um indicador da evolução microbiológica, para que as provas de análise sensorial não colocassem em risco os provadores. Segundo estes valores guia, os produtos prontos a comer são aceitáveis, do ponto de vista microbiológico quando a contagem de microrganismos aeróbios totais não seja superior a 10^8 ufc/g (unidades formadoras de colónias por grama de amostra) (165).

5. Tratamento de dados

A variedade de parâmetros considerados importantes na determinação da qualidade dos legumes MP e PC é grande. São comummente realizadas análises químicas (determinação de bioativos e atividade antioxidante, pH e acidez, teor de humidade, cinzas e minerais); físicas (cor e textura) e ainda sensoriais. Todos estes parâmetros foram anteriormente descritos como relevantes e, de alguma forma, justificada a sua importância no controlo da qualidade. No entanto, a análise destes parâmetros não fica completa, sem que se proceda a uma tentativa de correlação entre eles, ou mesmo tentar compreender como interagem na evolução e com a senescência do produto. Só assim se obtém informação que permita prever o comportamento ao longo do seu tempo de vida útil. Neste contexto, é importante referir as ferramentas quimiométricas que permitem explorar os dados, de forma a compreender e explicar tais inter-relações, correlações, etc.

A química é uma ciência que se dedica ao estudo de sistemas complexos, que acabam por não ser explicados totalmente pela teoria. Apenas recorrendo ao estudo experimental é possível compreendê-los. Mais difícil se torna quando em presença de sistemas com interfaces entre a química e biologia/bioquímica, estas com a tecnologia e ainda mais com interações do mundo real do mercado e dos consumidores. Assim a teoria fornece informação acerca de: o que e como medir/quantificar, orienta na implementação das técnicas que, conjuntamente com a informação extraída dos dados experimentais, podem ajudar a compreender os fenómenos num espaço multidimensional (167).

No entanto, haverá sempre algumas dimensões deste espaço que serão parcialmente desconhecidas. São exemplo, a avaliação rotineira de propriedades como a atividade

enzimática, o sabor, ou a atividade bacteriostática ainda que bem correlacionadas com, por exemplo, a configuração molecular de um composto antioxidante, ...

Segundo alguns investigadores, a estatística pode ser vista como a arte de tirar conclusões a partir de dados e tomar decisões na presença de variabilidade. A investigação nesta área tem sido muito produtiva nas questões que lidam com essa variabilidade, mas não há ainda muitos livros de texto com abordagens simples sobre erros e a sua modelação, bem como os próprios programas estatísticos existentes apresentam muitas limitações relativamente a este aspeto (167).

Na revisão bibliográfica realizada para apoiar o desenvolvimento do trabalho experimental e a discussão dos resultados obtidos, verificou-se que, apesar de alguns autores estarem de acordo, observa-se uma grande discrepância de resultados entre autores. Muito deste desacordo deve-se, possivelmente, ao facto de: (i) diferentes autores estudam isoladamente cada um destes parâmetros; (ii) estudam conjuntos de parâmetros diferentes; ou ainda (iii) analisam os seus dados recorrendo a ferramentas de estatística também distintas na conceção do modelo, distribuição e/ou tolerância de erro. É assim importante conseguir realizar estudos completos, que envolvam o maior número possível de parâmetros, verificar a sua correlação e com ajuda da análise multivariada, perceber qual a informação realmente importante e assim prever com segurança o comportamento dos produtos em estudo.

Pelo exposto noutras secções deste capítulo, parece evidente a necessidade de um estudo abrangente no âmbito das propriedades físico-químicas, organoléticas e microbiológicas para perceber o comportamento dos legumes MP e PC em AM. Para atender a esta necessidade, surge uma outra que é, como lidar com tão grande volume de dados. A resposta pode ser dada pelas ferramentas disponíveis para exploração de dados e numa palavra pela quimiometria.

A quimiometria recorre a ferramentas de estatística e a modelos matemáticos (álgebra) para, genericamente, resolver questões/problemas de química, tendo aqui a origem da sua designação, cuja definição remonta a 1971 por Svante Wold (168, 169). Atualmente, o nome mantém-se mas a sua definição é mais abrangente bem como o campo de aplicação. Inclui-se a componente de informática e destina-se agora não só ao tratamento de dados químicos, mas também ao desenho experimental e extração da maior quantidade de informação útil, integrando diversas áreas da ciência e do mundo real (169, 170). Para tal é necessário usar técnicas quimiométricas sofisticadas, baseadas na estatística multivariada (171), que lidem com grandes volumes de dados e, que cada vez mais frequentemente resultam de poucas observações para um grande número de

variáveis. Nesta tese, esta necessidade foi sentida devido ao grande número de parâmetros (variáveis - referidas em secções anteriores) avaliados num número reduzido de observações (as amostras - produtos em MP ou PC ao longo to tempo de armazenamento), o que despoletou o interesse pelo recurso a estas técnicas de estatísticas e outras mencionadas nos capítulos subsequentes.

A análise de componentes principais (ACP) é a ferramenta mais popular entre os investigadores que recorrem à análise multivariada. Numa ACP, são definidos uma série de vetores ao longo dos quais a variância dos dados é maximizada. A ACP é um método estatístico que tem por objetivo determinar as principais estruturas existentes nos dados. Entende-se por estrutura, um conjunto de variáveis correlacionadas entre si, isto é, um conjunto de variáveis com um padrão de variação interligado. Para tal, a ACP determina um número de componentes que é, em geral, igual ao número de variáveis iniciais (desde que o número de unidades analisadas seja superior ao número de variáveis). As componentes, correspondendo a grupos de variáveis correlacionadas entre si, organizam-se por ordem decrescente de importância. Uma vez que a informação importante se concentra nas primeiras componentes, as últimas componentes concentram a informação irrelevante, erros das análises, etc. Deste modo, desprezando as últimas componentes, reduz-se a dimensão dos dados, mas não se perde informação relevante (172).

Na maior parte das referências consultadas, assiste-se a um descuido na utilização dos *outputs* gráficos da ACP, geradas pelos programas habitualmente usados no tratamento estatístico de dados, como por exemplo: (i) apresentação gráfica com escalas diferentes em cada componente (como no caso de trabalhos apresentados por Koley et al. (117) e por Santos et al. (173)) ou (ii) sem a preocupação de colocar a informação acerca das variáveis realmente importantes e que sobre as quais deve recair (só e apenas) a discussão. Em relação ao primeiro problema (i) note-se que cada vetor próprio é semelhante a um vetor unitário que define uma reta de regressão ortogonal; cada componente principal é semelhante às coordenadas (projeções) das unidades sobre uma reta de regressão ortogonal; para que esta projeção seja feita corretamente aquando da análise das estruturas formadas no gráfico que opõe as componentes principais (CP) um e dois, este gráfico deve ser perfeitamente quadrado com escalas iguais tanto na CP1 como na CP2. Em relação ao segundo caso (ii), a questão prende-se com o facto de, muitas vezes, os investigadores não conseguirem decidir o que realmente é importante. Na ACP, as componentes representam conjuntos de variáveis bem correlacionadas. Esta informação é fornecida pela matriz de vetores próprios, que para cada componente expressa a intensidade da relação de cada variável com cada componente principal. É ainda importante o conhecimento da informação fornecida pela matriz de valores próprios,

também habitualmente, designados por *eigen values*, que classifica as componentes por ordem de importância e, consequentemente, os grupos de variáveis correlacionadas entre si. Descartando as componentes de menor importância, consegue-se reduzir o volume de dados construindo os gráficos das componentes principais, é possível visualizar em apenas um ou dois gráficos a informação relevante num dado estudo (172). Ainda segundo Alves (172), não é suficiente interpretar os gráficos apenas olhando para as posições das estruturas que surgem posicionadas em determinado ponto do gráfico, sugerindo que *"são mais qualquer coisa"* numa variável e que outras unidades que surgem em posição opostas, "*terão menos"* dessa variável. Nem sempre esta situação reflete a realidade (na verdade, na matriz de dados originais, podem não ser assim tão diferentes). Para garantir que as conclusões extraídas dos gráficos correspondem à realidade é preciso, durante a sua análise, ter em consideração não só a matriz de vetores próprios, mas também a matriz de correlações.

Muitas vezes, a utilização da quimiometria introduz, ainda, uma maior confusão, devido ao facto de que estas análises são complicadas e, ao mesmo tempo, não são isentas de erro, na medida em que cabe ao investigador escolher os parâmetros que explicam as variações observadas. Mais, na maior parte das vezes os investigadores não possuem os conhecimentos de matemática e estatística, a que isso obriga. Estes limitam-se a utilizar programas incluídos em *software* que operam num "ambiente quimiométrico", isto é, os investigadores terão apenas que inserir uma matriz de dados, seguida da seleção da ferramenta estatística a utilizar. O restante trabalho fica a cargo do *software* que produz um *output* de resultados em gráfico ou tabela, a partir dos quais o operador terá apenas que fazer a sua interpretação, que é na maior parte dos casos de acordo com as suas expectativas e nem sempre atentando aos erros de previsão do modelo de análise. Estas dificuldades foram reconhecidas por Gabriel (174) que associou pela primeira vez às técnicas quimiométricas, até então trabalhadas, o termo *"biplot"*.

Os *"biplots"* efetuam uma projeção das variáveis iniciais nos planos das componentes principais, equipadas com escalas de valores iguais aos valores iniciais das variáveis, para que se possa, a partir do gráfico, verificar quais eram os valores iniciais de cada uma das unidades que foram analisadas (*biplots* preditivos), ou colocar no gráfico novas unidades analisadas (*biplots* interpolativos) (172, 175). O objetivo da utilização desta ferramenta é precisamente ajudar os investigadores na interpretação dos seus dados, recorrendo apenas à análise dos *outputs* gráficos (174).

No entanto, estas representações gráficas com *biplots* muitas vezes tornam-se confusas, sobretudo no caso onde o tipo de dados/resultados a tratar é complexo e volumoso (175). Na tentativa de melhorar a aparência gráfica e ao mesmo tempo

contribuir para o rigor da sua interpretação, muitas vezes questionável, Alves (175), em 2012, desenvolveu o conceito de erro preditivo padrão médio (*mspe* – do inglês *mean standard predictive error*). O *mspe* é uma nova medida de ajustamento dos dados que permite o desenho de *biplots* cujo *mspe* está abaixo do definido pelo investigador, evitando assim sobrecarregar o gráfico com eixos de varáveis de baixa correlação e evitando a más interpretações e, consequentemente, conclusões erradas.

Tal como Wold (167) comentou, a estatística é a arte de desenhar conclusões a partir de dados experimentais, e tomar decisões atendendo à variabilidade destes. No entanto, não acontece bem assim, pois nem sempre estão corretas, pois não reconhece a importância dos erros dos modelos aplicados. Apesar do estudo da estatística estar continuamente a procurar responder a este problema, pouca informação tem sido veiculada quer ao nível da literatura, quer ao nível da implementação nos habituais programas de estatística. No entanto em 2012 Alves (175) criou uma função para o R (AutoBiplot.PCA ( )), que tem por base algumas das funções já existentes no referido programa (funções para o R). Esta função automatiza o processo, permitindo que o utilizador decida o grau de precisão da análise real. O método baseia-se na definição do *mspe* de uma variável, como grau de precisão no processo de projeção dos valores originais nos *biplots*, que é comparado com um valor de tolerância pré-definido (T_{axis}) e assim decidir se o eixo correspondente é desenhado no *biplot*. O erro preditivo padrão (*spe* – do inglês *standard predictive error*) é calculado para cada unidade, em relação a cada eixo projetado no *biplot* do gráfico que opõe cada duas componentes e comparado com um valor de tolerância pré-definida (T_{units}) para decidir quais unidades cuja projeção ortogonal ao eixo deve ser encarada como valor discrepante (*outliers*) (175).

A análise estatística realizada recorrendo à função AutoBiplot.PCA ( ), permite uma abordagem mais precisa à avaliação dos dados. As unidades em estudo (amostras/produto) são projetadas no eixo da variável correspondente, permitindo determinar o valor inicial da variável e, assim, ajudar interpretar os dados em função dos valores iniciais, e não apenas em função das componentes principais, como acontece numa ACP tradicional.

Os dados gerados no trabalho desta tese foram analisados recorrendo a diferentes programas estatísticos e diferentes modelos, que facilitaram a discussão e clarificação de dúvidas não só em relação ao comportamento dos legumes ao longo do tempo de armazenamento, mas também em relação a alguns parâmetros (importância na descriminação de amostras e correlações entre eles) usados no controlo da qualidade.

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PARTE II – DESENVOLVIMENTO EXPERIMENTAL

A parte II desta Dissertação é constituída pelos capítulos III a VII.

Cada capítulo, à exceção do capítulo III que inclui dois capítulos de livro (um publicado e outro aceite para publicação), é constituído por um artigo submetido em revistas científicas internacionais. Por isso estão em inglês e formatados de acordo com a respetiva revista. Estes artigos mostram os resultados obtidos na execução experimental do trabalho conforme planificação apresentada na Parte I. Em cada capítulo, antes da apresentação do artigo, é incluído um pequeno resumo, em português que destaca o produto ou produtos em análise e os principais resultados obtidos durante a monitorização dos parâmetros em estudo, ao longo do armazenamento.

No capítulo III faz-se uma abordagem às técnicas quimiométricas usadas na autenticação de produtos. Estas mesmas técnicas foram usadas na discussão dos resultados obtidos, que apoiaram e permitiram chegar a conclusões assertivas. No ponto dois deste capítulo, é feita uma comparação entre a análise de componentes principais (ACP) clássica, e a ACP resultante do uso da função AutoBiplot.PCA( ), que permite elaborar os *biplots* de forma automática.

III Análise Estatística Multivariada como Ferramenta da Quimiometria

1. Chemometrics in Food Authentication

Carla Barbosa, M. Beatriz P.P. Oliveira e M. Rui Alves

Chemometrics in Food Authentication é um capítulo do livro ***Current Topics in food Authentication***. Este livro aborda a problemática da autenticidade de produtos, relacionando questões de controlo da qualidade, rastreabilidade e conformidade de alegações, sobretudo na rotulagem de produtos alimentares. Mitiga estas questões de forma clara e útil aos técnicos da indústria e investigadores na área dos alimentos.

O papel dos diferentes métodos físico-químicos usados na determinação da qualidade dos produtos alimentares, abordados ao longo do livro, é importante. No entanto, tem-se tornado indispensável o recurso a ferramentas de análise multivariada, na tentativa de obter correlações e descartar informação assessória, dado o volume de informação.

O capítulo ***Chemometrics in Food Authentication*** trata de métodos úteis para a análise exploratória de dados e da forma como os dados experimentais podem ser usados na modelização, classificação e calibração, com especial enfoque na autenticidade de produtos.

Acompanhadas de estudos de caso, as técnicas estatísticas univariadas, bivariadas e multivariadas, são descritas em jeito de revisão, tendo sempre em atenção uma aplicação muito prática das mesmas.

Neste capítulo, é feita, em primeiro lugar, uma abordagem simples às técnicas univariadas (paramétricas e não paramétricas) de apoio à modelização e classificação. Os sistemas bivariados são referidos na apresentação das técnicas mais usadas no estudo de correlações e covariâncias. Seguem-se as técnicas multivariadas sem supervisão, com uma referência especial à análise de componentes principais e análise de dados agrupados e hierarquizados (*clusters*). As técnicas supervisionadas mais importantes, como por exemplo as funções Bayes de classificação discriminante e análise de variáveis canónicas, são também brevemente explicadas neste capítulo, e discutidas ainda as técnicas de regressão múltipla para calibração. O capítulo termina com uma breve apresentação de algumas ferramentas estatísticas mais específicas e referências que se julgam importantes nas tendências futuras da quimiometria aplicada a autenticidade de alimentos.

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11. Chemometrics in food authentication

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Abstract. Discussions about food authenticity and the role of many sophisticated chemical and physical methods to determine the quality of food products have been carried out in previous chapters.
This chapter deals with methods useful for exploratory data analysis and with the way experimental data can be handled for modelling, classification and calibration techniques in food studies with relevance for authentication. Univariate statistics, parametric and non-parametric, are first described and used as a simple approach for modelling and classification. Correlation and covariance are studied in bivariate problems. Multivariate unsupervised techniques, with a special reference to principal component analysis and hierarchical cluster analysis are presented, followed by important supervised techniques, as is the case of Bayes discriminant classification functions and canonical variates analysis. Multiple regression approaches for calibration are discussed. All discussions include examples with an emphasis on graphical outputs. A special attention is paid to predictive and interpolative biplots as good alternatives to aid interpretation of statistical outputs and to enable a practical use of these outputs for classification purposes.
The chapter ends with a brief presentation of some more specific statistical tools and references that may give some idea of the main trends in future developments as applied to food authentication.

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INTRODUCTION

Many definitions of chemometrics can be found in the literature. A simple one is given by Fiorina et al. [1], (...) *chemometrics is the science that uses mathematics, statistics and informatics to: a) select or design optimal procedures and experiments; b) to obtain the maximum useful information from the experimental chemical data*. According to this definition, chemometrics uses normal statistical and mathematical tools to solve problems in the field of chemistry. But the literature review clearly shows that some statistical approaches for modelling, classification and calibration widely used today, in reality developed in a special environment that can be called the chemometrics environment. This was due to the need to have statistical tools that, in opposition to classical statistics, could handle data with a few observations and a very large number of variables, as it happens, e.g., with NIR (Near Infrared) and NMR (Nuclear Magnetic Resonance) spectroscopies. The books by Martens and Naes [2] and by Esbensen [3], the latter related to the software Unscrambler [4], are probably the best examples on how chemometrics evolved in a very specific way. However, there are no sharp boundaries separating chemometrics from classic statstics, both using a large number of common techniques.

The reviews by Arvanitoyannis et al. [5,6], although concerned with particular aspects of food authentication related to potato tubers and honey, are typical examples and good starting points to evaluate the state of the art on the application of chemometrics in the field of authenticity. Analysis of variance, principal component analysis, partial least squares regression and soft independent modelling of class analogies are, by far, the most popular statistical tools to aid in authentication problems. The books edited by Martens and Russwurm in 1983 [7] and by Piggott in 1986 [8] give a good insight into the evolution of chemometrics during the last three decades. The future is probably moving towards three-way analysis, since modern equipments and team networks will provide large data sets of many types (chemical, physical, sensory) to model and classify food data. In this aspect the book by Smilde et al. [9], is mandatory. Although the statistical methodologies are well developed to handle all these types of very complex data, they will gain wide acceptance only when available in the most common statistical packages, which may not be the case today. Most up-to-date methodologies can be found in statistical software like R [10,11,12], which is probably popular among statisticians, but not among chemists.

This chapter will, therefore, try to focus on a few selected univariate and multivariate analyses that will be described with some detail, together with example applications. Other methods will only be explained briefly.

In what concerns data analysis, the authenticity of foods can be seen in three main important areas of statistics: (i) developing models, (ii) classification and (iii) calibration [13]. Developing models regards the way a given product is described, being synonymous of characterisation. One aims to find the parameter, or group of parameters, and their respective expected values and admissible variations, that may be used to describe a given food product. In statistical terms, modelling is the definition of classes, each class describing a group of items, or food products, that share some common patterns, i.e., that are characterised by the same expected levels of the parameters used. Therefore, modelling is answering the question: "what are the characteristics of the product?"

In the field of authentication, it may be necessary to model a product in such a way that it can be distinguished from other similar products, i.e., products that although sharing some properties that make them look alike, are known to come from different origins, to have different formulations, to be made under different processing conditions or with different raw materials, to have been subjected to adulteration, etc. Such models may be slightly different from the previous ones, since one is looking for the parameters and respective levels that can be used to discriminate among samples. Therefore, in authenticity studies, modelling is answering the question: "what are the characteristics of the product that make it unique?" And the answer to such a question is usually everything but an easy task.

The second area, classification, comes after modelling. Classification is the evaluation of whether or not a new sample (usually referred to as an unseen observation), fits in a given model. Once modelling has carried out successfully, a new sample (usually referred to as an unseen observation), can be classified in one of the modelled classes.

The problem with the definition of a given product authenticity is the need to build up a model that provides a clear picture of the product characteristics and a simultaneous discrimination from the other products of interest. Authenticity is therefore a two-sided problem: a) the technical side, i.e., the search for the parameters that can be used to provide the distinction between similar products; b) the statistical side, i.e., the search for the best combination of those parameters that provide model building and classification with a reasonably low degree of uncertainty.

The technical evolution led to the development of equipments that provide a huge number of parameters, or analyses over broad spectra leading to the same huge number of

parameters, increasing statistical complexity. This complexity increases when, due to the difficulties associated with authenticity, tables of parameters of different nature are used in conjunction.

Classical statistics stipulated that when classification was difficult, increasing the number of observations could lead to successful discrimination. As a consequence the experimenter ended up with a few parameters and very large samples. On the other hand, modern statistics, developed to deal with results from new equipments, stipulate that if the parameters analysed do not provide a clear solution, then it is necessary to look for other parameters with better modelling/classification abilities. And the experimenter ends up with a few items analysed and a very large number of variables.

The third area of statistics, important for food authenticity, is related to the indirect estimation of quantities of a given substance, i.e., application of regression techniques to determine the levels of one or more variables of interest (usually called **Y** variables) based on the levels of one or more explanatory parameters (usually called **X** variables). In chemometrics, these problems are usually referred to as calibration, since one wants to find the best equipment and working conditions, and a mathematical equation that relates the explanatory variables to the observed response.

NOMENCLATURE AND EXAMPLES

In order to make the text more clear, the following notations will be used. The initial data is a matrix $\boldsymbol{X}$, i.e., the set of results organised in P column vectors and N row vectors. The column vectors are referred to as $\boldsymbol{x}_p$ [$p=1...P$] and represent the initial, declared variables (parameters analysed). The N rows, referred to as $\boldsymbol{u}_n$ [$n=1...N$], represent the sample units. Caution must be taken in order to understand the difference between the "chemical sample" and the "statistical sample", the former corresponding to an analytical unit, the latter to a group of analytical units. It is important to note that any matrix is represented with a capital, bold letter, vectors are small bold letters, scalars are capital, italic letters and indices are small, italic letters. The symbol "^{t}" will be used to indicate a transposed vector or matrix.

Unless otherwise specified, the examples used in this chapter were extracted from a PhD thesis of one of the authors [14] or were specifically prepared for this purpose, and will not be referenced again.

UNIVARIATE MODELING AND CLASSIFICATION

The simplest models are univariate and based on the normal distribution. These models address simple and useful problems, which involve only one variable, usually representing a chemical or physical parameter. Before assuming a normal distribution of the data, it is advisable to check if there are reasons to consider that the observed variation is indeed normal. This can be done by appropriate statistical tests or by visual inspection.

As an example, consider a study carried out to determine the content of palmitic acid (C16) in sunflower oils. After a considerable amount of analytical work examining 54 samples, a summary of the results is expressed in Figure 1 and Table 1, produced with the software Minitab [15].

Table 1. Descriptive Statistics and normality tests of C16 content in 54 sunflower oils

n	$\bar{x}$	***s***	x_{min}	$\tilde{x}_{.25}$	$\tilde{x}_{.50}$	$\tilde{x}_{.75}$	x_{max}	r_q	**Kurtosis**	**Skewness**
54	6.2001	0.2881	5.2500	6.0500	6.2225	6.3725	6.8200	0.3225	1.54	-0.49
Anderson-Darling: value = 0.404, p = 0.344 Kolmogorov-Smirnov: value = 0.084, p > 0.15 Ryan-Joiner (similar to Shapiro-Wilks): value = 0.983, p > 0.10										

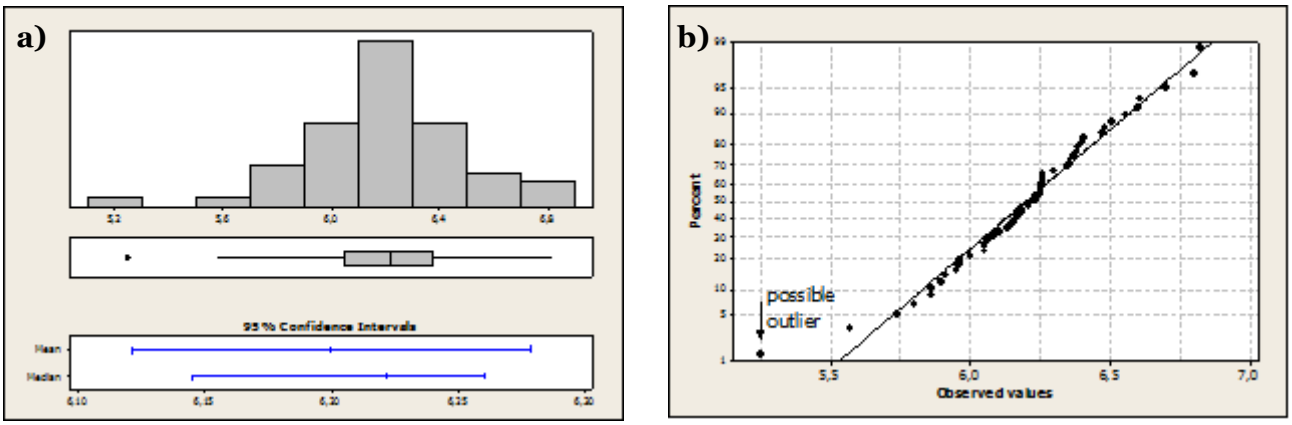

Figure 1. Graphical aspects and tests of C16 content in 54 sunflower oils. a) Histogram (above) and box-plot (below); b) normal probability plot.

Common tests for normality are the Anderson-Darling, the Kolmogorov-Smirnov and the Ryan-Joiner or the Shapiro-Wilks tests [16]. For all of them, the null hypothesis is H_0: *"the data is normal"*, which will be accepted with 95% confidence if the test result has a probability of occurrence expressed as p>0.05. This seems to be the case in the example. Skewness and kurtosis should be in the interval ±0.5, which is not the case. The kurtosis

value together with the discrepancy between mean and median may indicate some distortion in the data.

Carrying out a visual inspection of the data accomplished by means of a histogram, a box plot (box-&-whisker's plot) and a normal probability plot, it seems that there is an outlier. Outliers may be defined in several ways [16, 17]. A common way is to consider an observation x as a mild outlier if its value is $x< \tilde{x}_{.25}-1.5\times r_q$ or $x> \tilde{x}_{.75}+1.5\times r_q$, and as a severe outlier if $x< \tilde{x}_{.25}-3\times r_q$ or $x> \tilde{x}_{.75}+1.5\times r_q$, where $\tilde{x}_{.25}$ and $\tilde{x}_{.75}$ are the first and third quartiles and r_q is the inter-quartile range. Taking into consideration these equations, it is seen that there is a mild outlier in the data. If there are plausible reasons to remove this outlier observation, then it can be deleted from the data and the analysis redone. Quite often the data becomes normal as it is shown in Figure 2 and Table 2.

Table 2. Statistics and normality tests of C16 sunflower oils after removal of one outlier

n	$\bar{x}$	***s***	x_{min}	$\tilde{x}_{.25}$	$\tilde{x}_{.50}$	$\tilde{x}_{.75}$	x_{max}	r_q	**Kurtosis**	**Skewness**
53	6.2181	0.2587	5.5700	6.0500	6.2300	6.3750	6.8200	0.3250	53	6.2181
Anderson-Darling: value = 0.404, p = 0.344 Kolmogorov-Smirnov: value = 0.084, p > 0.15 Ryan-Joiner (similar to Shapiro-Wilks): value = 0.983, p > 0.10										

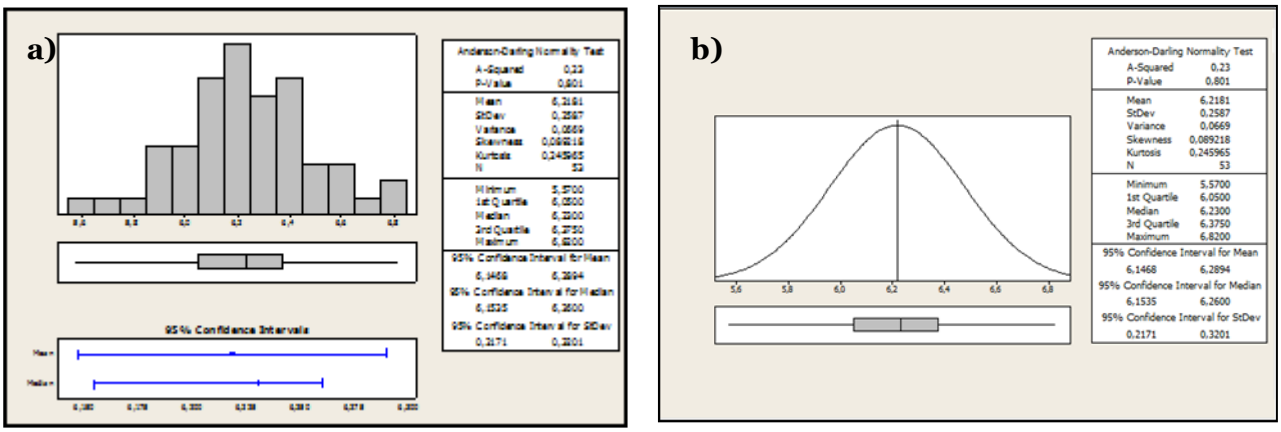

Figure 2. Graphical aspects and tests of C16 in sunflower oils after removal of one outlier. a) Histogram and box-plot of observed data; b) the normal model for C16 in sunflower oil.

To validate the model, it would be important to have some more observations in order to carry out tests for the mean, the variance and the distribution. Also, two very important issues must be taken into consideration:

1) To remove an outlier, a study must be carried out to assure that it is really an atypical observation. One cannot remove it just because the analysis becomes better. This decision must be taken on the basis of sound technical knowledge or field work;
2) Similarly, it cannot be assumed that the model found is a good model, unless the sample is random and comprehends all variations expected to be found in the food product under study. Caution is necessary because food products may vary according to region, year, season, producer, etc., and modelling may become a tricky question.

The majority of chemical, physical and sensory measurements are continuous and therefore the variability observed in repeated measurements is usually well described by the normal distribution. When the normal distribution applies, the mean and the standard deviation of parametric measures (respectively $\bar{x}$ and s) can be used to describe data. The former is the measure of central tendency and the latter is the measure of dispersion. The non-parametric measures are used where the normal distribution does not apply. The main statistics are the extreme values x_{min} and x_{max}, the quartiles $\tilde{x}_{.25}$ and $\tilde{x}_{.75}$, and the second quartile, named median $\tilde{x}_{.50}$ or simply $\tilde{x}$. The measure of dispersion is the range, $r=x_{min}-x_{max}$, or the inter-quartile range, $r_q=\tilde{x}_{.25}-\tilde{x}_{.75}$.

Models are important when used for classification purposes. Figure 3a represents a bimodal distribution, corresponding to at least two different oils. As a matter of fact, it corresponds to the same 54 sunflower oils plus 54 idealised almond oils. In this situation, two models are necessary, as depicted in Figure 3b (gray shade), sunflower oil with $\mu=6.22$ and almond oil with $\mu=7.0$, with a common $\sigma=0.3$, μ and σ representing the population mean and standard deviation (or model parameters). Once the models have been defined, they can be used for classification of new samples. But there is an evident problem: models for almond and sunflower oils are overlapped.

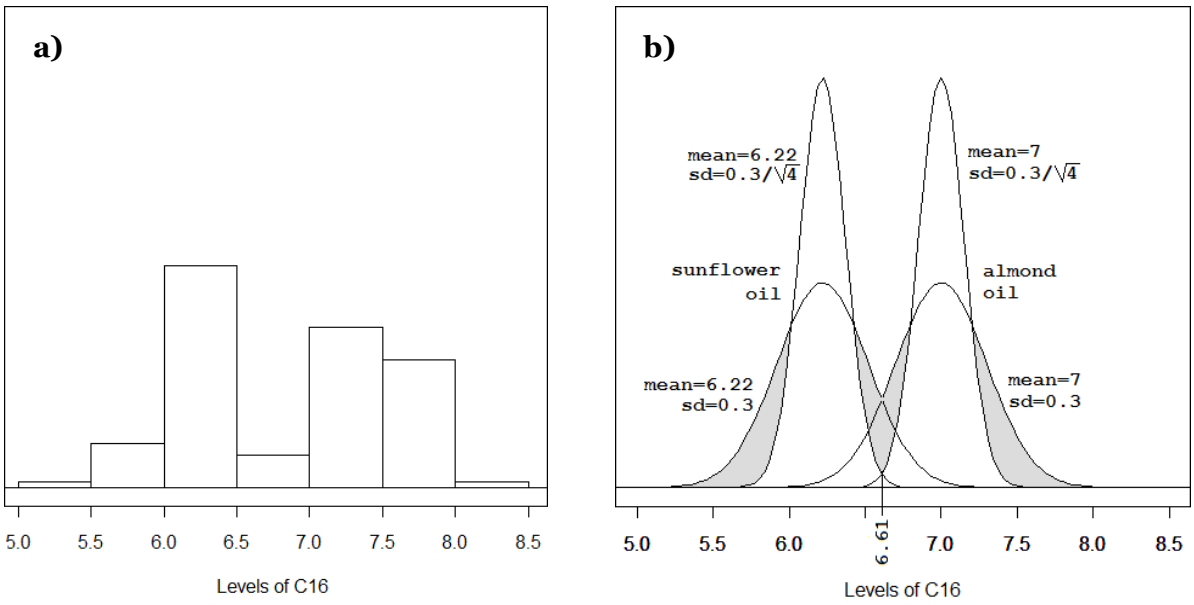

Figure 3. Univariate models for two classes and classification. a) Models for individual observations; b) models adapted for sample averages.

To solve this situation, we can force the models to become apart, to a given extent, using sample means instead of individual observations, since sample means follow a normal distribution with mean μ and standard deviation $\sigma/\sqrt{n}$. Therefore, sample means will lie in the interval $\mu \pm z\sigma/\sqrt{n}$. Equating the maximum limit for sunflower oil with the minimum limit for almond oil, $6.22+2.58\times0.3/\sqrt{n} = 7-2.58\times0.3/\sqrt{n}$, it can be seen that a sample of size $n=4$ will be enough to guarantee that probabilities of misclassification will be reduced to less than 0.5%. As shown in Figure 3b, the value of 6.61% is the boundary, any oil with less than 6.61% being classified as sunflower oil, and classified as almond oil otherwise.

It is therefore seen that models are useful for description purposes, while some modifications may be needed, involving the definition of specific values, or boundaries, necessary for classification purposes. The previous reasoning can be modified in order to formulate many different hypotheses.

BIVARIATE MODELS AND CLASSIFICATION

In the same way as we can build a univariate model, a bivariate model is also possible. While in univariate statistics a model is confounded with confidence limits, in a bivariate model ellipses of equal probability represent the model [18]. It is important to see that in bivariate situations, if the **X** matrix is centred to mean 0, the covariance matrix is

calculated as $\mathbf{S}=\mathbf{X}^t\mathbf{X}/(N-1)$, and if **X** is further standardised to unit variance, the correlation matrix is easily calculated as $\mathbf{R}=\mathbf{X}^t\mathbf{X}/(N-1)$.

With *N* being the sample size, *P* the number of variables, **μ** the vector with means, *F* the value of the *F* distribution that gives the desired 1–α confidence level, then the coordinates of any point over the ellipse are given in vector ***x*** [19]:

$$\{[(N-P)\times N]/[P\times(N-1)\times(N+1)]\}\ (\mathbf{x}-\boldsymbol{\mu})^t\ \mathbf{S}^{-1}\ (\mathbf{x}-\boldsymbol{\mu}) = F_{(\alpha,p,n-p)}$$

Figure 4a shows an example of a bivariate histogram representing real data: levels of stearic acid (C18) and arachidic acid (C20) in 54 sunflower oils. If this data is validated in relation to underlying distributions and outliers, a bivariate model can be assumed as the one shown in Figure 4b. In this case, the model was represented by ellipses of 50%, 90% and 95% confidence levels. One is therefore describing what is expected for a sunflower oil. The model can be used for classification, e.g., a new oil will be classified as a sunflower oil, with 95% confidence, if the point representing the combined levels of C18 and C20 falls inside the outer ellipse. Otherwise, the hypothesis that a new oil is sunflower oil will be rejected at the significance level α=0.05.

In Figure 5, another aspect is shown: a scatter plot of four oils (peanut, sunflower, soybean and corn), and models (95% confidence ellipses) based on samples of size 10. It is clear that a new sample *A* will be classified as peanut oil, sample *B* as an outlier and sample *C* as soybean or sunflower oil.

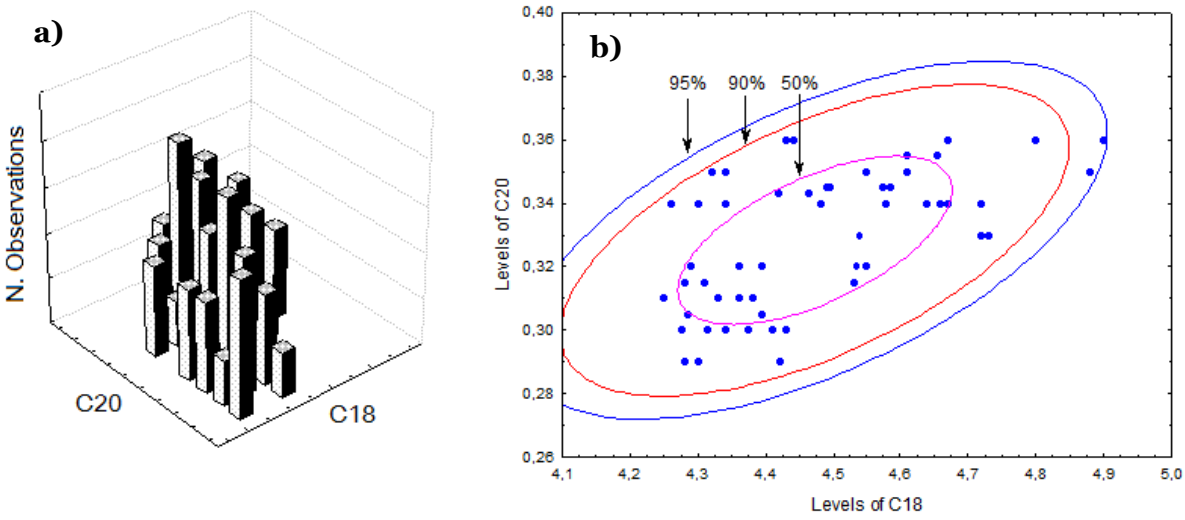

Figure 4. Analysis of real data and transition from real data to a model, in relation to the levels of C18 (stearic acid) and C20 (arachidic acid) in 54 sunflower oils. a) Bivariate histogram of real data; b) scatter plot of real data and model ellipses.

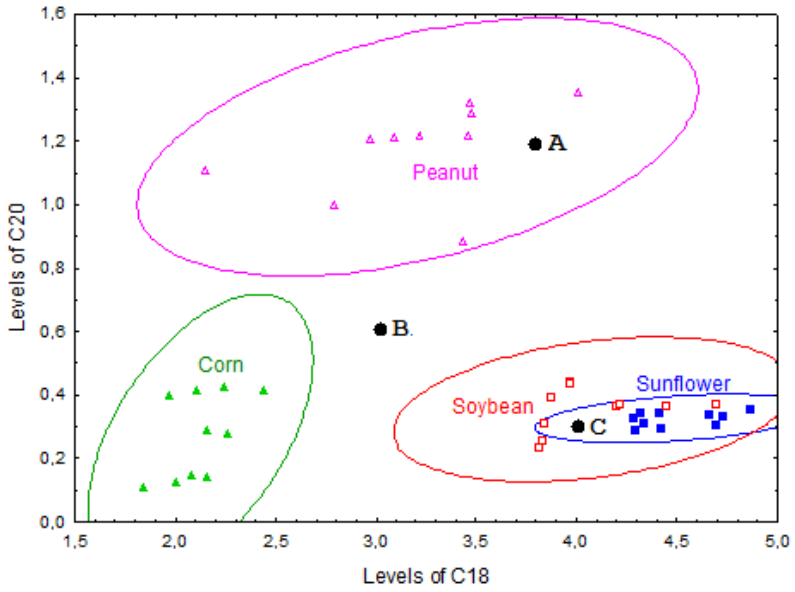

Figure 5. Bivariate scatter plot of the levels of C18 and C20 in four edible oils (peanut, corn, soybean and sunflower) based on samples of size 10. In the scatter plot each point represents a single oil analysed, and therefore refers to actual data. 95% Confidence ellipses represent class models.

Univariate and bivariate statistics are used in profusion, since they address simple and very common problems. For example, if one has a data base with two or more variables and N observations divided in M groups, the analyses of variance ANOVA and MANOVA can be used to check if there are reasons to suspect that groups in a given data set are different in one or more variables, and, in case of significative differences, student's t tests or Hotelling T^2 tests can be used to determine which are the different groups. For example, in the case of Figure 5, a MANOVA could be used to determine if the four oil groups are different, and a Hotelling T^2 would confirm which are the different groups [18].

MULTIVARIATE MODELS AND CLASSIFICATION

With modern equipments and techniques any authenticity study may involve a large number of parameters. As a consequence, the results obtained are condensed in complex data matrices that need special statistical treatments to help organising and evaluating the information.

Unsupervised methods

Unsupervised methods are those that are used for general data inspection, without imposing any restrictions or previous knowledge on the data units. They can be used to inspect the data quality, if there are possible groups, possible outliers, errors, etc.

Cluster analysis (CA)

Cluster analysis represents, in multivariate analysis, a similar role to histograms in univariate analysis, i.e., they constitute a simple way of inspection of a data matrix, looking for natural groupings and eventual outliers. CA is usually based on Euclidean or Mahalanobis distances between the rows of the data matrix $\boldsymbol{X}$. For example, if $\boldsymbol{u}_i$ is the i^{th} row of $\boldsymbol{X}$, and $\boldsymbol{u}_j$ is the j^{th} row, the squared Euclidean distance between the two rows is given by $d_{ij}^2=[(\boldsymbol{u}_i-\boldsymbol{u}_j)]^t[(\boldsymbol{u}_i-\boldsymbol{u}_j)]$. Calculating these distances between all possible pairs of rows of $\boldsymbol{X}$, one ends up with a symmetric matrix $\boldsymbol{D}$, of dimension $N\times N$.

To proceed with CA, looking for the smallest distance in $\boldsymbol{D}$, the two corresponding rows are merged in one cluster, e.g., averaging the two rows. $\boldsymbol{D}$ is recalculated, with N-2 units and one cluster. A new cluster will be formed and the process repeated until a final cluster merging all units will be computed. Typically, a graphical representation will be produced, called dendrogram, like those presented in Figures 6 and 7. The dendrograms indicate a cluster that has been formed through a horizontal link, and the clustering linkage distance through a vertical line [18,20].

CA may yield different results depending on the merging methods (the most popular are the nearest neighbour, average neighbour, furthest neighbour and Ward's methods) and on the distance measures and scaling procedures. Each technique will be more specific to a given shape of clusters, and in many practical situations, several procedures must be tried in order to look for particular relationships in the data.

Figure 6 shows the dendrogram applied to a matrix of 54 sunflower oils ($N=54$ units) and 11 fatty acids ($P=11$). Units are shown in the horizontal axis and linkage distances in the vertical axis. Figure 7 presents the results of the same methods applied to a matrix of 40 edible oils ($N=40$), 4 types (peanut, corn, soybean and sunflower) in samples of 10 units each, also analysed according to the levels of 11 fatty acids ($P=11$). Comparing both figures, it becomes obvious that in the case of sunflower oils there is no clear evidence for the existence of clusters. Unit 51 may be an outlier and deserves a special analysis. In the

second case (Figure 7), a linkage distance of around 3.5 (represented by a dotted line), clearly separates four distinct clusters, corresponding to the four oil types.

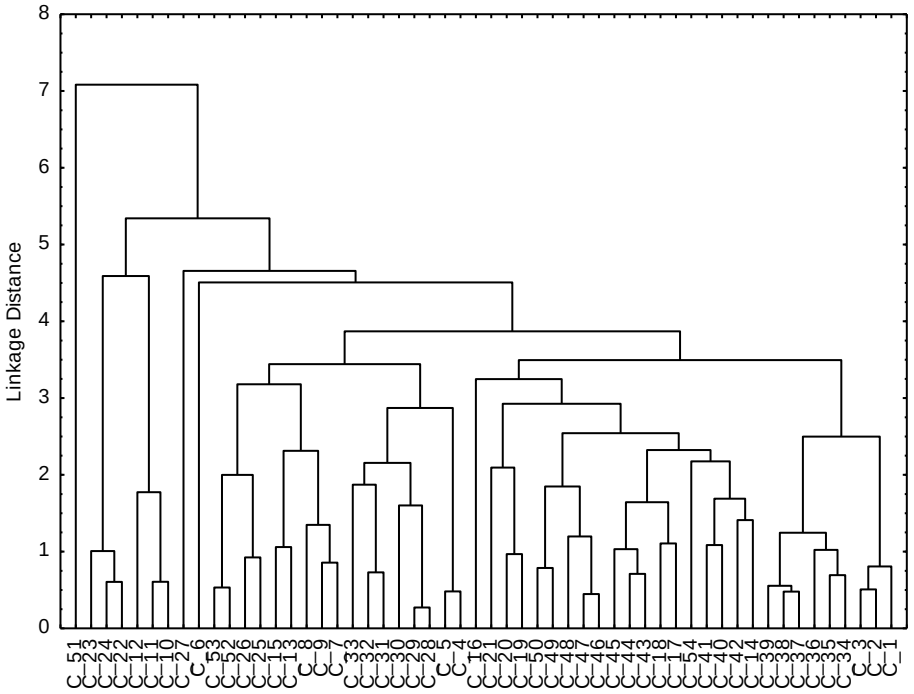

Figure 6. CA of a matrix of 54 sunflower oils and 11 fatty acids, with unweighted pair-group average merging method and Euclidean distances.

The cluster analyses referred herein are called hierarchical because at each step one unit must be assigned to one cluster and the process cannot be reversed. Other methods exist, called non-hierarchical, which try to be more flexible, as is the case with the fuzzy clustering algorithms, which do not seem to have found application in the area of food authenticity.

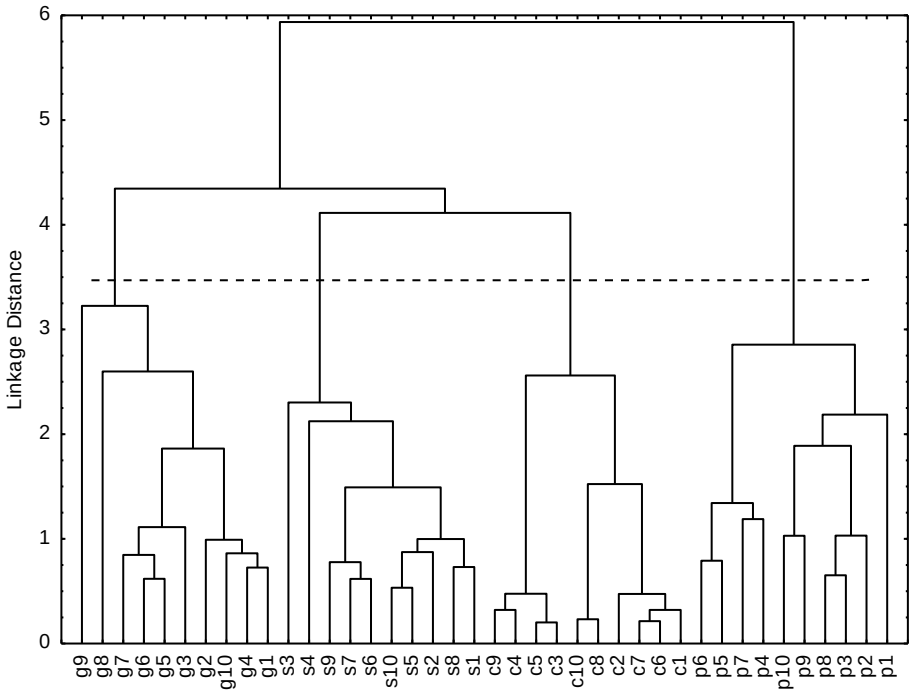

Figure 7. CA of a matrix of 40 oils (p-peanut, c-corn, s-soybean and g-sunflower) and 11 fatty acids, with unweighted pair-group average merging method and Euclidean distances.

Principal component analysis (PCA)

PCA is the most important multivariate analysis (MA) tool. It is important to understand the way it operates because many of the principles apply to other MA techniques [14,18,21]. PCA is the definition of a set of new P directions along which the data variance is maximised. Terminology varies according to authors or software packages. The mathematics behind PCA are quite simple:

a) With $\boldsymbol{X}$ standardised to mean zero and unit variance, the correlation matrix is $\boldsymbol{R}=\boldsymbol{X}^t\boldsymbol{X}/(N\text{-}1)$.
b) The spectral decomposition of $\boldsymbol{R}$ is carried out as $SD(\boldsymbol{R})=\boldsymbol{V\Lambda V}^t$.
c) The $\boldsymbol{X}$ matrix is projected onto the directions of $\boldsymbol{V}$, as $\boldsymbol{Y}=\boldsymbol{XV}$.
d) Providing in $\boldsymbol{X}$ the number of sample units is relatively large, i.e., $P \leq N+1$, $\boldsymbol{Y}$ will have dimension $N \times P$ and $\boldsymbol{V}$ and $\boldsymbol{\Lambda}$ will have dimension $P \times P$.
e) Graphs of pairs of columns of $\boldsymbol{Y}$ and of $\boldsymbol{V\Lambda}^{½}$ are plotted to enable visualisation and interpretation.

The following points are important:

1) The columns of $\boldsymbol{V}$, are usually called eigen or latent vectors of the correlation matrix, or factor structure. Each element is the cosine of the angle between an initial variable

(row) and the new direction (column). In $\boldsymbol{V\Lambda^{½}}$, the cosines are replaced by correlations, making interpretations easier.

2) The columns of $\boldsymbol{Y}$ are the relationships of all sample units with these new directions and are usually called factor scores, component scores or just principal components (*PC*).
3) The elements of the diagonal matrix $\boldsymbol{\Lambda}$ are eigen or latent values of the correlation matrix and give the variance, or relative importance, of each *PC*.

In $\boldsymbol{Y}$, $\boldsymbol{V}$ and $\boldsymbol{\Lambda}$, columns are organised by decreasing importance. It is up to the experimenter to decide how many of the first, most important columns, to retain. This is called reduction in dimensionality or data compression, and leads to the deletion of the last components, which usually contain errors and irrelevant information. If Q dimensions are found to be important, then $\boldsymbol{Y}$ will have dimension $N \times Q$, $\boldsymbol{V}$ and $\boldsymbol{\Lambda}$ will have dimension $P \times Q$.

To illustrate PCA, we return to the data matrix $\boldsymbol{X}$ with 54 oils (units) and 11 fatty acids (variables). Figure 8 presents the graphs of PC1 vs PC2 (The two first columns of $\boldsymbol{Y}$) and of transformed eigen vectors 1 vs 2 (The two first columns of $\boldsymbol{V\Lambda^{½}}$). No clusters are observed in accordance with the dendrogram of Figure 6. An example of interpretation is as follows: oils located in the upper left part of the graph (25, 26, 19, 27) have high levels of C18:1 and C16:1 and lower levels of C18:2, while the opposite happens with oils 10, 11 and 12 (Figure 8).

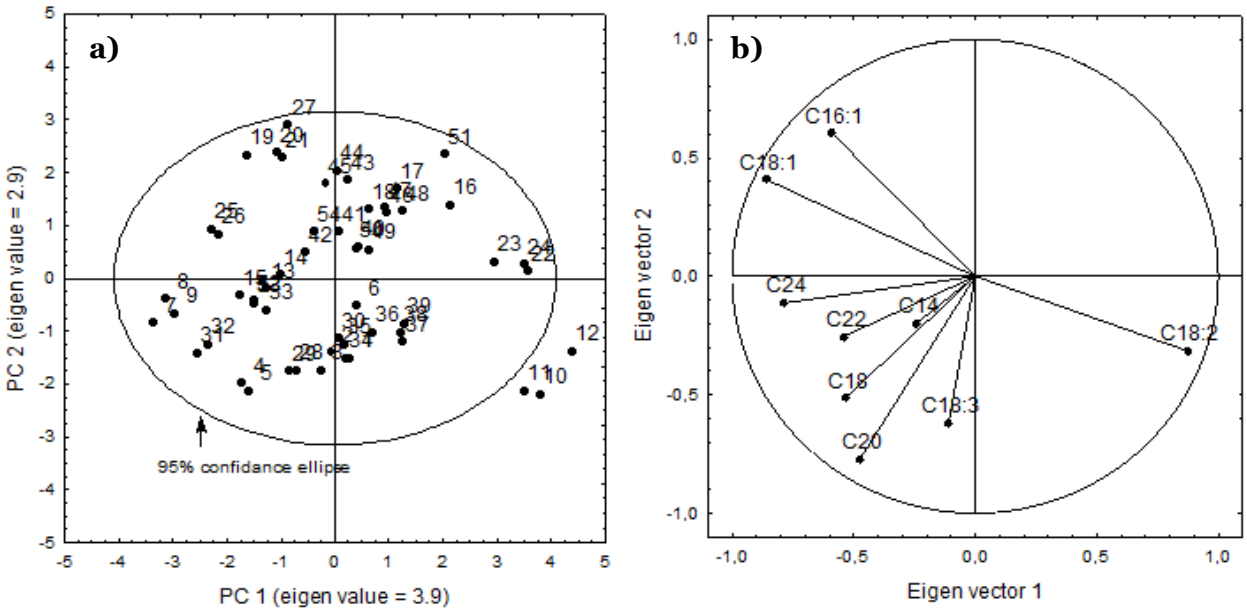

Figure 8. Principal components 1 vs 2. a) Sample scores; b) variable correlations.

An ellipse of 95% confidence was drawn in the PC plot. If these characteristics of sunflower oil are validated, then that ellipse can be seen as a model, interpreted through the eigenvector's plot. A matrix $\boldsymbol{X}_e$ with an extra set of oils of unknown origin could be projected on the same space, through $\boldsymbol{Y}_e=\boldsymbol{X}_e\boldsymbol{V}$, for classification purposes. Samples falling within the ellipse would be classified as sunflower oil, samples falling outside would be classified as non-sunflower oils, admitting a 5% probability error.

Predictive biplots can be very helpful when used for the interpretation of results, while interpolative biplots can be useful to position new samples in the graph. These types of biplots are shown in Figure 9. The former are used to carry out interpretation, reading directly from the graph the actual initial values of each sample unit (Figure 9a). For example, unit 12 had 0.3% C20, 62.5% C18:2 and close to 25% C18:1. For classification purposes, different axes are drawn (Figure 9b), which enable correct positioning through the vector sum method [22,23,24].

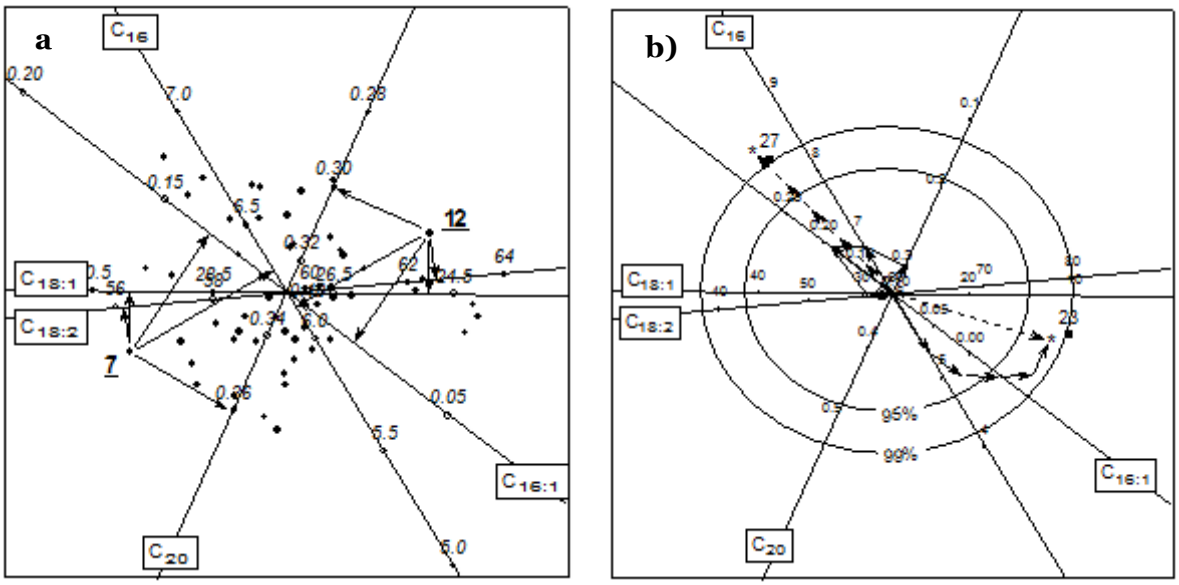

Figure 9. Predictive (a) and interpolative (b) biplots applied to the plane of PC1 vs PC2. Arrows on the left indicate how interpretation is carried out (examples applied to sample units 7 and 12). Arrows on the right indicate how new samples are positioned in the graph for classification purposes (examples applied to sample units 23 and 27).

Applying PCA to the data matrix $\boldsymbol{X}$ with 40 oils of four different origins and 11 fatty acids (Figures 10 and 11), some clustering is observed, in accordance with the dendrogram of Figure 7. However, as it can be easily seen, only peanut oil is well separated from the other

oils, which means that this PCA model would not be adequate for classification, and different would be required.

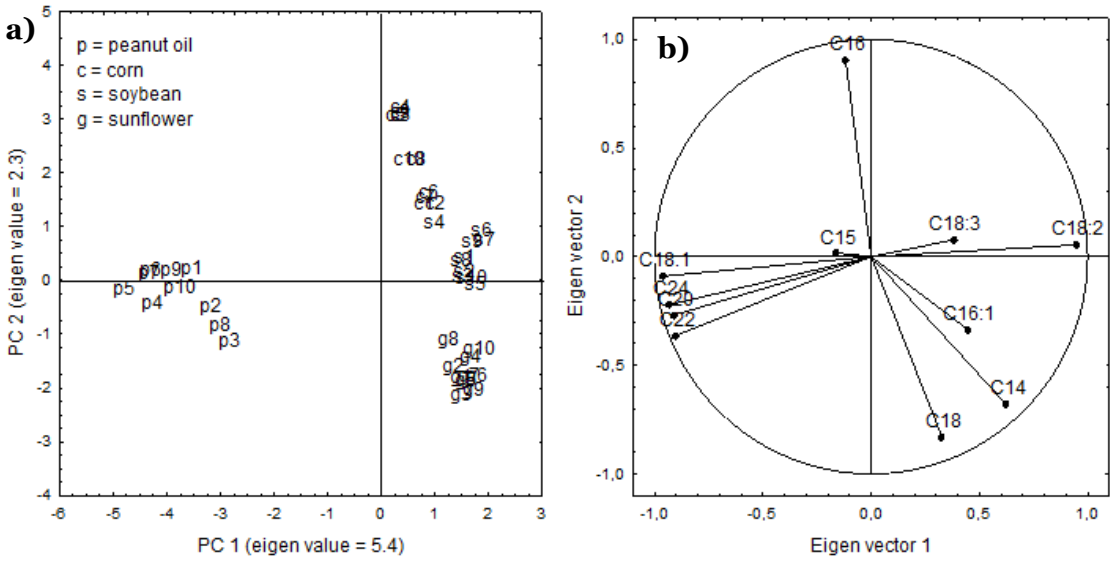

Figure 10. Usual representation of principal components 1 vs 2. a) Sample scores; b) eigen vectors modified to express correlations.

Observing Figure 11, which represents the same aspects as in Figure 10, but using biplots, one can see that predictive biplots are very interesting for interpretation since it can be read directly from the graph that peanut oils have only between 20 and 30% C18:2, while the levels of this fatty in the other oils ranges between 50 and 60%. The same can be done in relation to the other fatty acids. On the other hand, the interpolative biplots enables to print the PCA result and position a new oil in the graph "by hand". Confidence ellipses can inform on how precise interpolations can be. It is seen that this biplot can only classify correctly new peanut samples, while the others overlapping with each other. For example, Figure 11 shows that sample unit $\boldsymbol{u}_{15}$ could be classified as soybean and as sunflower oil with the same probability.

With modern equipment and techniques, like NMR, FTIR, NIR, chromatography, etc, it is common to use small samples (e.g., 10) and a large number of variables (e.g., from 20 or 40 to 1000). In that case, PCA may lead to the development of unstable models because it is difficult to determine the correct number of components to keep. To overcome this problem, some method for the validation of the number of components is necessary [2,3,25,26]. If possible, PCA is carried out with a training set and validation is accomplished via a different test set. If these two sets are not possible, PCA can be carried

out with all training samples less 1, the remaining one being left for validation (leave-one-out method).

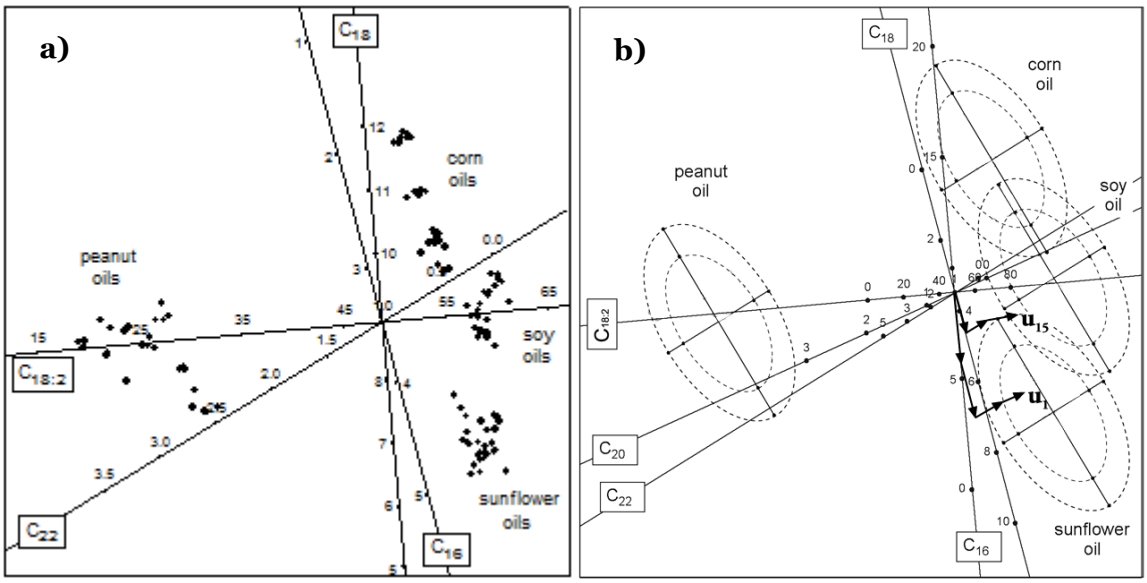

Figure 11. Predictive biplots (a) and interpolative biplots (b) with confidence ellipses for classification purposes. Arrows on the right indicate how to carry out interpolation of new sample units, as exemplified with units 1 and 15.

The procedure considers the ability of a given number of components (say A) to reconstitute the original data with a given error, called the residual prediction error. Since $\boldsymbol{Y}=\boldsymbol{XV}$, it follows that $\boldsymbol{X}=\boldsymbol{YV}^t$. Thus, if $\boldsymbol{V}$ is reduced to A dimensions, then $\boldsymbol{Y}_A = \boldsymbol{XV}_A$, the subscript A meaning the number of dimensions in $\boldsymbol{Y}$ and $\boldsymbol{V}$, then the original $\boldsymbol{X}$ matrix can only be approximated by the reduced number of dimensions as $\boldsymbol{X}_{pred}=\boldsymbol{Y}_A\boldsymbol{V}_A^t$. The difference $\boldsymbol{X}$-$\boldsymbol{X}_{pred}$ is the matrix of residuals, which can be squared and added up to obtain the residual mean square error of prediction (RMSEP). Therefore, if there is a validation set, $\boldsymbol{X}_{val}$, the RMSEP is calculated based on the residual matrix $\boldsymbol{X}_{val}$-$\boldsymbol{X}_{val}\boldsymbol{V}_A\boldsymbol{V}_A^t$. Increasing the number of dimensions (increasing A), the RMSEP will decrease to a certain point, due to a decrease in underfitting, and then starts to increase, meaning overfitting problems. This minimum in RMSEP indicates the correct number of dimensions to keep in the model.

If there is no validation set, the leave-one-out method may be employed. The process is as described for the validation set, but for each number of components, it is cycled, calculating a model with A components based on N-1 units, the extra unit being used to calculate the RMSEP for that unit. Cycling through all units, an average RMSEP is obtained for each number of A dimensions. The processe stops when the minimum RMSEP

is encountered. The cycled procedure for the determination of the RMSEP is facilitated by the NIPALS algorithm and enables different strategies for the detection of outliers [2,26].

Modelling and supervised classification

In supervised methods, the analyst defines a set of *M* classes of interest. Samples are needed to represent each class. The group of samples is the training set. The resulting data matrix $\boldsymbol{X}$ has *P* columns (one for each parameter) plus an extra column, usually called factor, defining the class membership for each sample unit. This information on class membership will be used by algorithms to build up models and/or carry out classification. The definition of the *M* classes may also be derived from a cluster or a PCA analysis, when these exploratory techniques indicate the possible existence of distinct groups in the data set. Two methods will be seen in detail, the Bayes classification functions and canonical variate analysis.

Bayes classification functions

Once all sample units are identified in relation to group membership, the simplest way to classify a new sample unit $\boldsymbol{u}$ as belonging to one particular group is through the Bayes classification method. With $\boldsymbol{G}_m$ [$m=1...M$] indicating a partition of matrix $\boldsymbol{X}$ corresponding to group *m*, this method involves the calculation of: a) the covariance matrix for each group as indicated before, and denoted as $\boldsymbol{S}_m$; b) the group mean vector $\bar{\boldsymbol{g}}_m$; c) the prior probability π_m. The prior probabilities are computed directly from the data or from previous knowledge. When this knowledge is not available, or it is assumed that it is not reasonable to calculate it from the data, prior probabilities are set equal to M^{-1}, i.e., equal for all groups. Then, the Bayes classification function for group $\boldsymbol{G}_m$ is given by [18,27]:

$$L_m = (\boldsymbol{u} - \bar{\boldsymbol{g}}_m)^t \, \boldsymbol{S}_m^{-1} \, (\boldsymbol{u} - \bar{\boldsymbol{g}}_m) + \log|\boldsymbol{S}_m| - 2\log(\pi_m).$$

It is important to see that if the prior probabilities π_m are set equal to all groups and if it is also assumed that the covariance matrices of the groups are equal, as it may happen in many works, then the determinant also vanishes from the equation, and the Bayes classification function reduces to the Mahalanobis distance. This is a measure of distance between the row vectors $\boldsymbol{u}$ and $\bar{\boldsymbol{g}}_m$, which is scaled in each dimension by the total variability observed in that same dimension. Therefore, applying the Bayes function to all

groups, sample unit $\boldsymbol{u}$ will be classified as belonging to the class for which it has the smallest L_m value or the lowest Mahalanobis distance. In practice, algorithms use transformed Bayes functions [14], in the form of a row vector $\boldsymbol{f}_m$, that enable classification through simple algebra operations, like $L_m = \boldsymbol{u}\boldsymbol{f}_m^t$, and caution must be paid in order to check the rules for classification.

Table 3 shows four classification functions developed for peanut, corn, soybean and sunflower oils. One oil, *p1=(0.03, 0.01, 8.937, 0.05, 2.147, 41.09, 38.58, 1.107, 2.093, 3.357, 2.043),* is used to illustrate how these functions work: each value of *p1* is multiplied by the corresponding function coefficient, all products added, plus the function constant, obtaining the L_m for each group (shown in the bottom line of Table 3). As a result, the oil is classified to the peanut oil group. This example was produced with the Statistica for Windows [28] (statistical software package), which also calculates posterior probabilities, Mahalanobis distances, etc.

Table 3. Modified Bayes classification functions for four edible oil types. Each column is a classification function, and any new oil is classified to the group for which the L_m is higher. The bottom line is the result of the application of each function to an example (see text for details).

Fatty acids	**Groups (edible oils)**			
	peanut	**corn**	**soybean**	**sunflower**
C14	7496.4	5274.2	5811.5	5300.8
C15	-1596.8	2061.6	1672.3	1694.9
C16	487.5	429.9	424.2	386.6
C16:1	-4697.1	-3207.3	-3352.6	-2790.6
C18	93.6	66.5	94.4	99.4
C18:1	226.5	232.6	220.6	221.3
C18:2	200.5	222.4	206.4	212.3
C20	1301.7	913.6	934.5	789.9
C18:3	138.4	118.6	154.3	117.1
C22	335.3	-340.1	-75.3	-112
C24	141.6	512.1	249.4	259
Constant	-12337.5	-12033.7	-11039.8	-10926.5
L_m for p1	12382.03	11271.38	11618.94	11334.81

Bayes classification functions have two limitations. The first is that they assume that data is normal, which may not always be the case. Second, they assume that variability is

similar in all dimensions. If this latter assumption is not met, because the functions use the inverse of the covariance matrix, classification may be very dependent on the variables with the lowest variance, leading to overfitting. Hence, the importance of a validation data set. However, this problem can be avoided by carrying out a previous data compression using PCA or by a previous deletion of variables with low discriminating power [29,30,31].

Canonical variate analysis

A second important method for classification is known as canonical variate analysis (CVA), or Fisher linear discriminant analysis [18,27,30]. As previously, the data matrix $\boldsymbol{X}$ is divided in M groups. Instead of calculating the covariance matrix for each group, only the sums of squares matrices, $\boldsymbol{SS}_m$, are calculated, and summed to obtain the within-groups variation, usually indicated by $\boldsymbol{W}$. The sums of squares between group averages, i.e., between the $\bar{\boldsymbol{g}}_m$, are also calculated, and referred to as the between-groups variation, $\boldsymbol{B}$. The spectral decomposition of the ratio matrix $\boldsymbol{BW}^{-1}$ yields a set of Q dimensions, $Q=min(P,M-1)$, along which the ratio of the between- to the within-groups variability is maximised. Thus, CVA can be seen as the counterpart of PCA for grouped data. The spectral decomposition *of* $\boldsymbol{BW}^{-1}$ yields equals $\boldsymbol{V\Lambda V}^t$, as seen before. If the group means are condensed in a matrix $\boldsymbol{G}$, then all group means can be projected in the dimensions defined in $\boldsymbol{V}$ (the latent vectors), yielding the canonical variates $\boldsymbol{Y}_G$ (or group average scores). Sample units can also be projected as $\boldsymbol{Y}_X=\boldsymbol{XV}$.

An application of CVA to the data set of four oil types is shown in Figure 12. Comparing with Figures 10 and 11, it becomes clear that CVA is very powerful when classification purposes are required. In the plane of CV 1 vs CV 2 (Figure 12a), it is seen that peanut oil is separated from the others, while in the plane of CV 2 vs CV3 (Figure 12b), the remaining three oils are clearly separated. To see which are the important fatty acids for these results, it is necessary to interpret the $\boldsymbol{V}$ matrix (the eigen vectors with the relationships between initial $\boldsymbol{x}$ variables and the canonical dimensions).

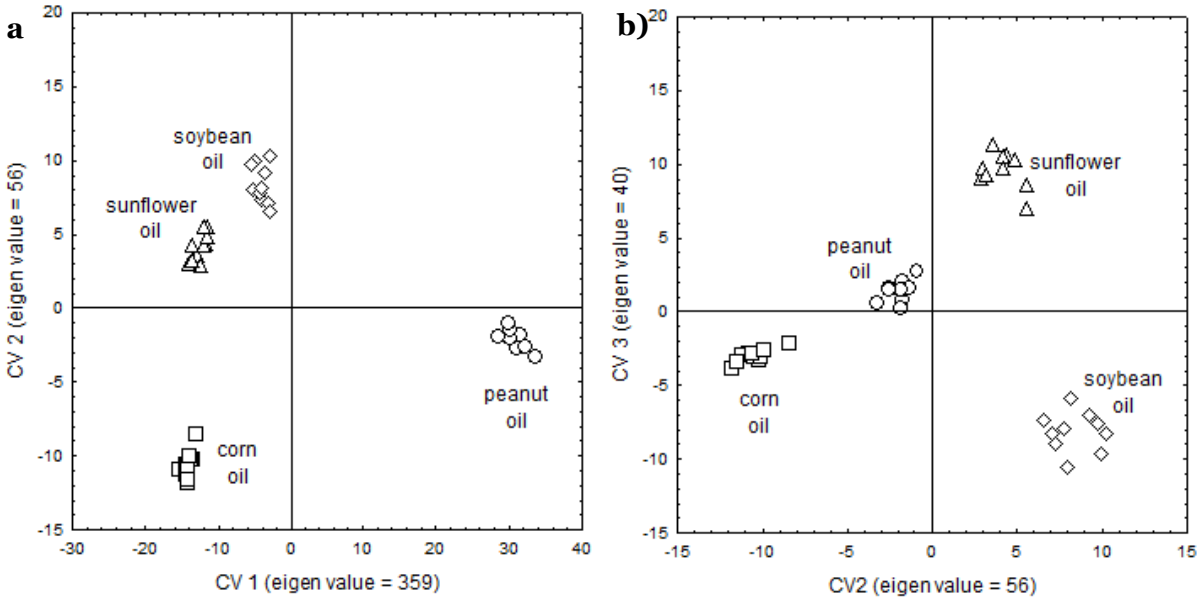

Figure 12. Canonical variates 1 vs 2 (a) and 2 vs 3 (b).

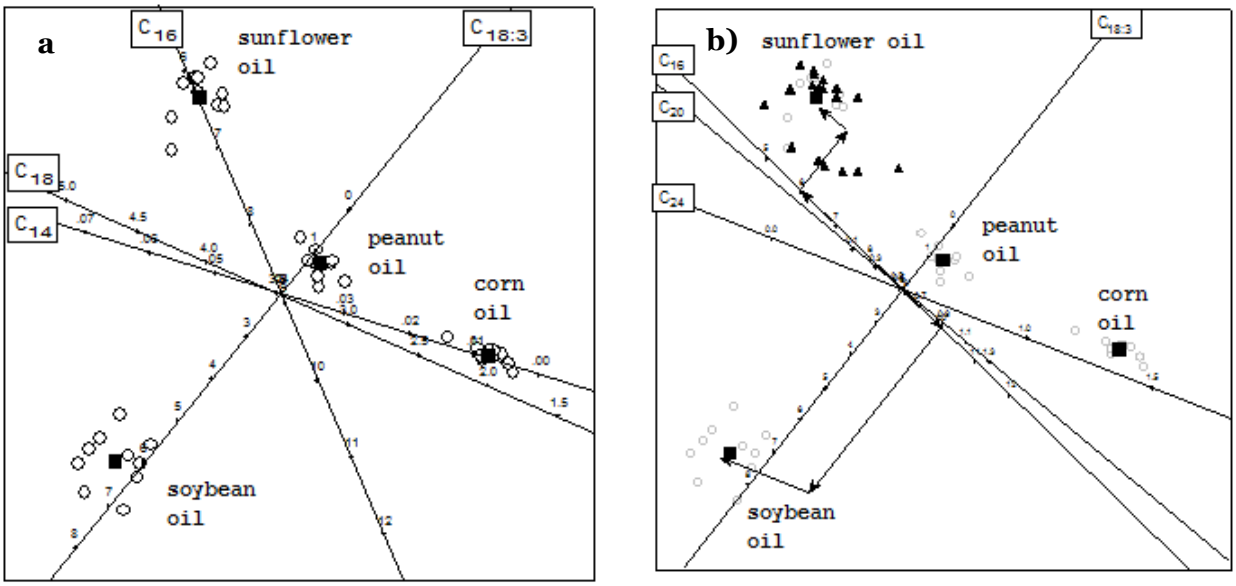

Figure 13. Examples of predictive (a) and interpolative (b) biplots applied to the CVA plots of the four oil types. In interpolative biplots the method for interpolation is illustrated.

If one wants to carry out classification of a new sample unit $\boldsymbol{u}$, it suffices to project it as $\boldsymbol{y}_u=\boldsymbol{uV}$, where $\boldsymbol{y}_u$ represents the coordinates of the row vector $\boldsymbol{u}$ in the set of Q canonical dimensions. Drawing confidence circles is possible to aid classification [18].

As it was mentioned for the Bayes classification functions, dangers of overfitting also exist in CVA due to the inversion of matrix $\boldsymbol{W}$, which may lead to create models that attribute the highest importance to the least important variables. This may be overcome by carrying

out a CVA on a reduced set of parameters selected by means of other types of discriminant analyses, like forward selection or backward elimination stepwise analyses, or by performing CVA on the compressed data by means of a previous PCA [29,30,31]. The problem with this latter procedure is that interpretation of CVA results is carried out judging relationships between sample units and principal components, which in turn are explained in terms of relationships between sample units and groups of correlated variables. This problem can be solved by applying predictive biplots, making sure that all translations and scalings applied to the original data in both analyses are also applied to the initial variables, projecting them in the final PCA/CVA plots (Figure 13), with appropriate scales for measurement. This enables a less cumbersome interpretation in terms of chemical parameters and raw measurement values [31].

As it was already mentioned, ANOVA and t tests, and MANOVA and Hotelling T^2 tests may be useful to evaluate differences between groups in supervised techniques [32].

Calibration

Calibration is used for quantification purposes. The simplest calibration is a regression line relating the observed values of variable ***y*** (referred to as dependent variable) with those of an explanatory variable ***x*** (named independent). Usually, the values of ***y*** are the levels of a given food compound evaluated by some standard method, and variable ***x*** contains the levels of the same compound estimated through an alternative method, or the levels of another component highly correlated with ***y***. Since both variables are supposed to be highly correlated, a regression equation of the form $y=a+bx+\varepsilon$ will enable to predict the value y, given the observed (measured) value of x, affected by a certain degree of uncertainty enclosed in ε.

In many cases, better predictions can be made if instead of a single parameter ***x***, a set of P parameters $\boldsymbol{x}_p$ $[p=1...P]$ are used. This would be the case where a matrix ***X*** is available and it is desired to predict the values of ***y*** through a multiple regression equation of the form $y=b_1x_1+b_2x_2+...+b_Px_P+\varepsilon$.

The determination of the regression equation, i.e., the estimation of all parameters b_p, is straight forward. However, it happens quite often that a very complex regression model may indicate that close to 100% of the variation in ***y*** may be explained by the variables in ***X***, as expressed by a coefficient of determination R^2 [33], but the prediction ability of the model, evaluated with unseen observations (a test set) may be very poor. This is usually a

consequence of overfitting due to the existence of co-linearity in the ***X*** variables. To solve this problem, stepwise multiple linear regression (SMLR), principal component regression (PCR) and partial least squares regression (PLSR) can be used [2,3,25,27].

Stepwise multiple linear regression

In SMLR, variables are added to (forward selection) or deleted from (backward elimination) the model, step-by-step. Forward selection SMLR tries to find the ***x*** variable, among all ***x*** variables, that better explains ***y***, then searches for a second variable that explains ***y***, given that the first is already in the model, then looks for a third ***x*** variable, and so on. The process is controlled via an "*F-to-enter*" value defined by the experimenter. Backward elimination SMLR starts with all variables in the model and eliminates the one whose removal has the least impact on the explained variance, then removes a second variable following the same principals, and so on, the process being controlled via an "*F-to-remove*" value. The major problem with SMLR is that once a variable is included/excluded in the model, its importance is not re-evaluated in the presence/absence of other variables. Trying to overcome this problem, many statistical packages allow the scientist to try several variable combinations, a process that may be too dependent on luck.

Principal component regression

PCR calculates the principal components of ***X***, producing a data compression: all ***x*** variables are compressed to a few important PC. A normal multiple linear regression is carried out afterwards, trying to explain ***y*** in terms of the main components of ***X***. Since all components are uncorrelated, the danger of overfitting is reduced to a minimum. As a matter of fact, collinearity is used to obtain more stable models, since a model based on relationships between ***y*** and groups of correlated ***x*** variables uses more information than models that only take into consideration the individual information in each ***x*** variable.

Partial least squares regression

PLSR is similar to PCR, in the sense that regression is not done directly on the ***x*** variables, but on latent variables, i.e., on combinations of ***x*** variables. As a consequence, both PLSR and PCR overcome the problems due to collinearity and produce more stable models. The

difference between PLSR and PCR is that, while the former calculates the PC with disregard of the response variable ***y***, the latter develops one intermediate set of latent variables produced with information coming from explanatory (***x***) and response (***y***) variables. Hence, the name of soft modelling is used to refer to several PLSR variations. The disadvantage of PLSR is the production two sets of latent variables that are more difficult to explain. The advantage is the higher flexibility, mainly when the ratio of ***x*** variables to the number of sample units is very high, e.g., when a wave has to be sliced in several hundred wavelength segments. Although being more complex than PCR, PLSR has gained more popularity among chemists.

Validation

Validation is very important in these statistical methods, mainly when the number of sample units used for modelling is very low. Two sets of samples are the better choice for modelling, one used for developing the model (the calibration set) and another used for testing it (the validation set). But, as *N* is usually low, scientists tend to use all samples as the calibration set and carry out cross-validation afterwards. The method for validation or cross-validation was explained above in relation to PCA. In any case, once the model is put into practice, it is important to a new sample set and re-test the model.

An example with SMLR and PCR

In this very simple example, original data ***X*** is an average matrix of sensory experts judging *N=12* potato chips using *P=19* sensory descriptors. An average acceptability (***y*** variable) was obtained for all sample units averaging consumer's responses using the 9 point hedonic scale. Descriptors are shown in Table 4. Sample units are also shown in Table 4 together with the respective acceptability results. The name *ind* means "producer of industrial type" and *hmt* stands for "home-made type", *0.5*, *1*, *2*, *3*, *4*, *5* refers to shelf-life times in months and *a*, *b*, *c* indicates a different producer. The aim of this example is to illustrate how to calibrate (predict) acceptability for different types of potato chips based on the sensory expert appraisals, taking into consideration samples with different shelf-life times and known frying oil quality [34].

Table 4. Principal component analysis of sensory experts and acceptability data. Results are shown for the only significant (first) principal component. Important descriptors to explain the meaning of PC1 are highlighted in bold.

Latent vectors	
descriptors	**EV 1**
dark colour	-0.43864
shiny	-0.79522
transparent	-0.76804
defects	0.212448
wavy shape	-0.7441
visible oil	-0.76953
odour_intensity	**-0.94123**
rancid odour	**-0.91659**
attypical odour	-0.72162
crunchy	0.053782
keeps crunchy	0.218221
fat smell	-0.76899
visible salt	0.650552
salty taste	0.732568
perceived fat	**-0.90775**
industrial type	-0.81019
burnt	-0.47898
rancid taste	**-0.86227**
bitter	-0.64644

Principal components		**Acceptability**
sample units	**PC 1**	
ind-a5	-0.59904	4
ind-a4	-0.21356	4.25
ind-a3	0.67832	5.25
ind-a1.5	1.02142	7.25
ind-a0.5	1.0078	5.5
hmt-a1	-1.10153	3.25
hmt-a3	-1.41751	2.75
hmt-b1	-0.98208	3.5
hmt-b3	-0.5373	2.75
hmt-c	-0.45895	5.25
ind-b0	1.344	4.25
ind-b3	1.25842	6.75
Eigen_Value	9.325399	

Starting with SMLR, Table 5 presents the main results. The *adjusted* R^2 shows that over 80 % of the observed variability in consumer's acceptability results can be explained in terms of five sensory descriptors, mainly related to fat content and perceived fat quality. It must be understood that reducing the *F-to-enter* value, more descriptors would be included and the value of R^2 would always increase. However, variable p-levels are very high, almost all above *0.05*, indicating that the model may be overestimated, and predictions may lack precision. The values of *beta* or those of *b* and *intercept* (the regression coefficients) shall be used in order to build the regression equation based on, respectively, the standardised or raw data.

Table 5. Results from a forward selection SMLR applied to sensory data (results from sensory experts) in the prediction of acceptability (results from consumer data)

Forward selection SMLR - Regression Summary with a minimum F-to-enter = 1.0 R=0.95 R²=0.91 Adjusted R²=0.82929229 F(5.6)=11,688 p<0.00475 SE of estimate: 0.60730						
descriptors	**beta**	**Std.Err.**	**b**	**SE**	**t(6)**	**p-level**
Intercept	----	----	11.94	2.031	5.882	0.001
rancid odour	-0.28	0.259	-0.03	0.032	-1.07	0.326
perceived fat	-0.52	0.645	-0.09	0.108	-0.81	0.451
visible fat	3.236	0.9	0.341	0.095	3.595	0.011
wavy shape	-2.27	0.807	-0.22	0.08	-2.81	0.031
rancid taste	-1.02	0.53	-0.2	0.106	-1.93	0.102

A PCA showed that four principal components could describe the ***X*** data without appreciable loss of information. Therefore, a PCR was carried out by doing the regression of the acceptability data on the four PC. Only PC1 was found significant for the regression, and thus no validation of the number of principal components is necessary. The main regression results are presented in Table 6. It must be noted that this PC1 is highly significant ($p \approx 0.003$). Following this method, sensory results can explain slightly less than 60% of the observed acceptability (*adjusted R^2=0.57*).

Table 6. Results from a PCR on the potato chip data with only one PC found to be significant.

Regression Summary for Dependent Variable Acceptability R=0.77987792 R²=0.60820956 Adjusted R²=0.56903052 F(1.10)=15.524 p<0.00277 SE of estimate: 0.96494						
	Beta	SE of Beta	B	SE of B	t(10)	p-level
Intercept	---	---	4.562500	0.278555	16.37917	0.000000
PC1	0.779878	0.197937	1.146317	0.290941	3.94003	0.002775

Comparing both results, it can be seen that both methods give a close result in terms of sensory analysis, because many of the descriptors chosen by SMLR (Table 5) are the same as the descriptors correlated with PC1 (Table 4), i.e., *rancid odour*, *rancid taste* and *perceived fat*. The R^2 indicates that SMLR provides a better result, explaining around 25% more of the variance in ***y***. But the significance of descriptors for SMLR is almost always superior to *0.05* (Table 5), while PC1 is highly significant with *$p \approx 0.003$*. These last results show that although the model favours the regression equation obtained by SMLR, it seems to be overoptimistic, and PCR will provide more reliable results if the model is put into practice. Nevertheless, validation tests would be required to evaluate both models.

Other multivariate and multi-way techniques

Many other methods are available and can be used for modelling, classification or calibration purposes. However, only some were found as being popular among food scientists dedicated to authenticity studies.

Canonical correlation analysis and other multi-way analysis

In many circumstances, data can be conveniently arranged in more than one multivariate matrix. Canonical correlation analysis (CCA) is an old technique that handles two matrices simultaneously. It tries to find a first dimension (latent variable) for one matrix, and a first dimension for the other matrix, such that both dimensions are correlated between matrices. Then, it tries to find second dimensions for both matrices, orthogonal to the first dimensions, and correlated between matrices, and so on. These canonical dimensions describe the correlations between two data sets and may be used to relate and simplify information [23,24].

In a study with green and black table olives, two data matrices were obtained: one relative to the sensory evaluation of all sample products; the other relative to the analysis of texture (force in two consecutive compression cycles). The texture profile was divided in several slices (variables) registering the force at each period of 0.5 seconds. Figure 14 presents a PCA of texture data. Figure 15 shows a PCA of sensory data, where the main descriptors used by sensory experts can be seen. For both PCA, predictive biplots were used.

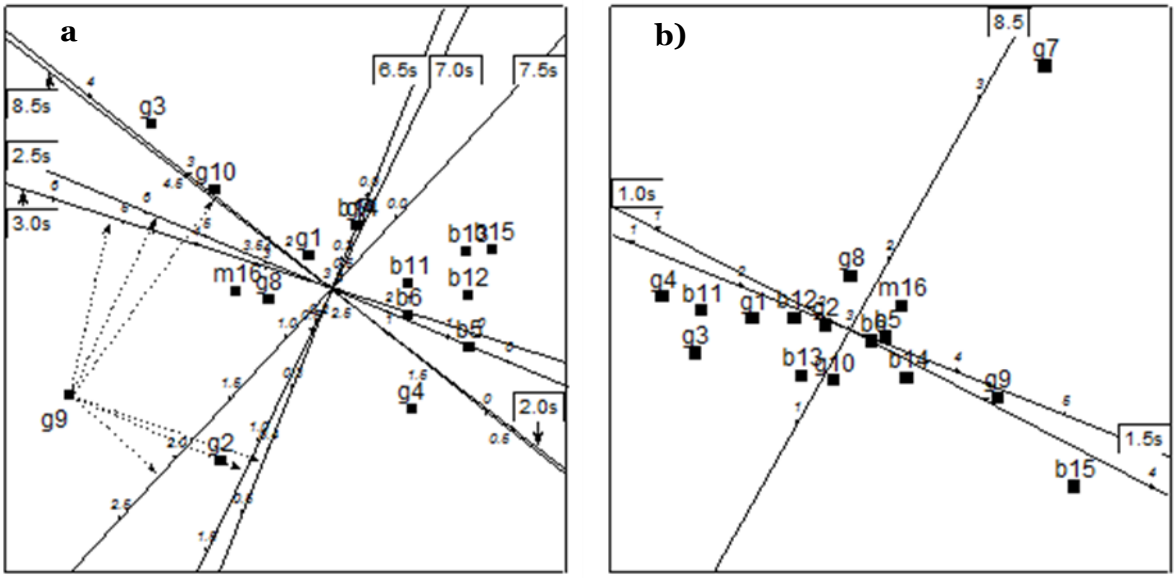

Figure 14. Predictive biplots of the texture of table olives, based on the planes of PC1 vs PC2 (a) and PC3 vs PC4 (b). Variables are time in half seconds. Values are force per half second. Letters identify olive type: g = green, b = black and m = intermediate.

Comparing both figures, it can be seen that the sample configurations produced by sensory experts and a texture analyser instrument are different. A CCR was then carried out on the principal components from both sets, in order to check if there was any hidden relationship between the two types of analysis. The results show that there are two important (significative) canonical correlations, which are the eigen values derived in CCA (Table 7). Therefore, two plots of the corresponding canonical dimensions for texture (Figure 16a) and sensory analysis (Figure 16b) were made.

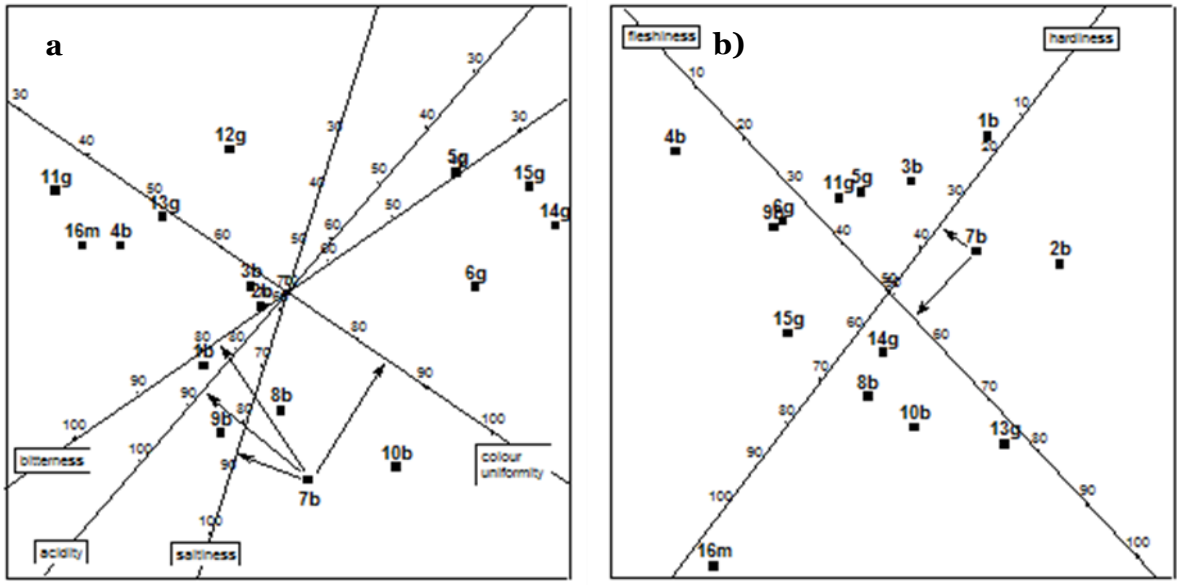

Figure 15. Predictive biplots of the sensory analysis of table olives, based on the planes of PC1 vs PC2 (a) and PC3 vs PC4 (b). Variables are sensory descriptors. Values are intensities in percentage of the full scale range. Letters identify olive type: g = green, b = black and m = intermediate.

Table 7. Evaluation of the significance of canonical correlations between two data sets (sensory and texture) relative to the analysis of table olives, black, green, and intermediate (Chi-Square Tests with Successive Roots Removed)

Root	Canonical R	Canonical R^2	Chi-square	d.f.	p	Lambda
0	0.975860	0.952303	52.97344	16	0.000008	0.006441
1	0.883810	0.781120	21.02306	9	0.012569	0.135038
2	0.618827	0.382947	5.07113	4	0.280101	0.616952
3	0.012817	0.000164	0.00173	1	0.966870	0.999836

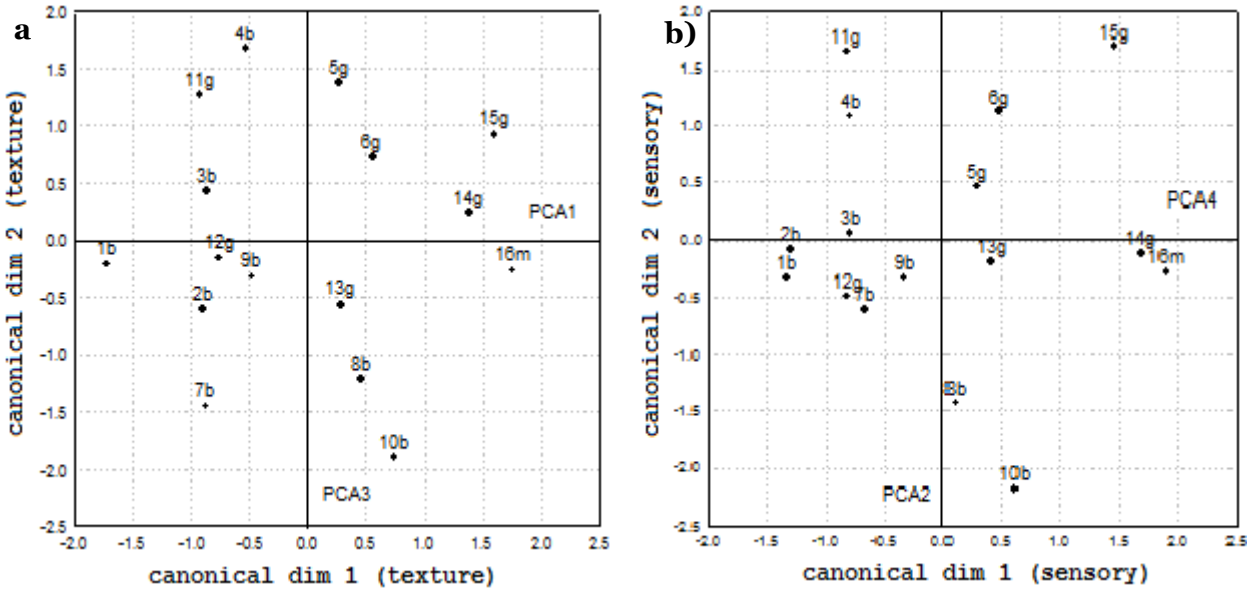

Figure 16. Scatter plots of canonical dimensions 1 (R^2=0.95) vs 2 (R^2=0.78) for the texture data (a) and sensory data (b). Letters identify olive type: g = green, b = black and m = intermediate.

In Figure 16, the projection of samples can be seen in the canonical dimensions 1 and 2, which is similar to the projection of samples in PCA or CVA. It can be observed that there is a close relationship between the sensory and physical data, mainly along the horizontal axis corresponding to the first canonical dimension. PC1 and PC3 derived from the texture studies are correlated, respectively, with PC4 and PC2 derived from sensory data. These relationships that could not be observed in Figures 14 and 15, could only be observed through an analysis specifically designed for the comparison of two data matrices.

Generalised procrusts analysis and 3-way Tucker-1 common loadings analysis [34] are examples of more flexible techniques than CCA, and have been used successfully applied to the simultaneous comparison of several data matrices. Many other multi-way analyses have been developed [9]. It is believed that in the near future many multi-way analyses will find their way in authenticity studies as soon as they become available in commercial statistical software packages.

K-means-clustering

K-means-clustering [35,36,37] is an interesting clustering methodology that can be used for inspection of data and to aid in model building when the scientist knows that *N* samples should be classified in *K* possible groups, but does not know the actual sample

membership. To our knowledge, this technique has not yet been applied in authenticity studies.

K-nearest-neighbour method

The KNN (K-nearest-neighbour method) is a classification methodology that does not impose any assumptions about data distributions. It is a methodology that found is way in the early eighties [38] and has been finding many and recent applications [25,39,40,41]. To classify a new sample, the algorithm calculates its distance to a set of other existing samples, looking for the K nearest ones. The number K must be tuned. The new sample is classified to the group that has the biggest number of samples within the K neighbours. The method can be easily cross-validated.

Neural networks

Neural networks, also known as artificial neural networks (ANN), are based on mathematical models that are non-linear in nature. They are close to KNN and more flexible. They have found some use in authenticity studies [40,42,43,44,45] but its application in food is limited due to the size of databases. As a matter of fact, any non-linear methodology requires a large number of samples to enable the development of stable models, a condition that is increasingly rare to meet. Nevertheless, it should be considered when there is enough information, and linear models do not provide satisfactory solutions due to overlapping class models.

Soft independent modelling of class analogies

Soft independent modelling of class analogies (SIMCA) is an important methodology based on discrimination and principal component analysis to separate modelling of each class [46,47,48,49]. The method needs only 5 sample units per class to yield stable models and is applicable with many variables. It is a supervised technique, since all units must be clearly allocated to a given class and no unknown units can be included in the model. Each class model can be univariate, bivariate or multivariate, according to the dimensionality reduction achieved. Boundaries, usually hyper-ellipsoids, are set for each class. Special

diagrams can be produced to enable the visualisation of distances between classes, possible outliers, etc. Classification of units is based on the standard deviation of their residuals.

CONCLUSION

Scientific and technological developments generate very powerful instruments that produce large amounts of data that needs to be handled properly. Global markets have raised a series of problems related to the authenticity of food products that need combinations of several sensory, chemical and physical techniques to provide clear and unquestionable solutions.

The complexity of data coming from all these analytical sources will require complex chemometric tools to handle many large data matrices simultaneously, providing answers with a high degree of precision. Many theoretical mathematical and statistical aspects have already been developed and an effort must be carried out in order to make those developments available to the scientific community.

Scientists in different fields also need to have a basic understanding of statistical procedures, or work in teams with the cooperation of statisticians, in order to design experiments carefully so that the best answers can be provided to well formulated questions.

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2. A comparison of several methods to present the displays of principal component analysis, with a special reference to the use of predictive biplots and the automatic selection of biplot axes

Carla Barbosa, M. Rui Alves e M. Beatriz P.P. Oliveira

A comparison of several methods to present the displays of principal component analysis, with a special reference to the use of predictive biplots and the automatic selection of biplot axes é um capítulo do livro ***Handbook of Research on Computational Simulation and Modeling in Engineering***. Neste livro é feita uma abordagem de temas como a modelação e simulação computacional orientada para assuntos tecnológicos e científicos, destinando-se a estudantes, investigadores e técnicos. Estes temas são relevantes na medida em que estes sustentam as estruturas das decisões tecnológicas e de engenharia. Os temas são abordados de forma a capacitar os estudantes e técnicos de competências nesta área proporcionando uma visão sobre a modelação em engenharia e *software* numérico. Estas metodologias são apresentadas de forma a demonstrar como podem ser eficazes na resolução de problemas no mundo real. No desenvolvimento do trabalho apresentado nesta tese, estas dificuldades foram sentidas, nomeadamente ao nível do volume variedade/variabilidade de informação a tratar.

Nesta contribuição pretende-se demonstrar a utilidade de *biplots preditivos* no tratamento e interpretação da análise de componentes principais de dados tão variados quantos os obtidos no trabalho desta tese. Para além disso, é feita uma comparação entre esta ferramenta e a análise de componentes principais clássica e ainda com os *biplots,* tal como desenvolvidos por outros autores, usando os mesmos dados.

Apresenta-se ainda a metodologia estatística que permite a construção automática de *biplots* preditivos, bem como a função do R, AutoBiplots.PCA( ), que tem por base uma análise de componentes principais. Esta metodologia foi desenvolvida a partir da definição do erro preditivo padrão médio das variáveis (*mspe*) como uma medida da precisão de leitura de valores originais a partir dos *biplots*. Para tal, é definido também um valor de tolerância (T_{axis}) que, quando comparado com o *mspe* da variável, permitirá, ou não, que o eixo desta seja incluído no gráfico. Esta função calcula, também, o erro preditivo padrão (*spe*) para cada unidade (amostra) em relação ao respetivo eixo no gráfico das componentes principais. Para este valor é também definida uma tolerância (T_{units}) que,

quando comparada com o *spe* informa acerca da possibilidade de existência (ou não) de *outliers*.

Assim, de forma automática, esta função do R auxilia o investigador a decidir o grau de precisão com que a abordagem aos dados experimentais pode ser feita, e ainda reduzir, com segurança, o volume de informação. Permite de forma lógica e automática a seleção de variáveis que explicam as dimensões latentes da análise.

A comparison of several methods to present the displays of principal component analysis, with a special reference to the use of predictive biplots and the automatic selection of biplot axes

Carla Barbosa, M. Rui Alves, M. Beatriz P.P. Oliveira

Abstract: Predictive biplots, as developed by J.C. Gower and coworkers, can be a very useful tool to aid the interpretation of the outcomes of multivariate analyses. This paper covers a statistical methodology that enables the automation of the construction of predictive biplots, as well as an R function, AutoBiplots.PCA( ), which applies the methodology to principal components analysis. A case study based on the sensory analysis of vegetables is used to illustrate the methodology as well as the outputs of the R function. The method relies on the definition of a variable's mean standard predictive error, mspe, as the degree of accuracy in the process of predicting the original values from the biplots, which is compared with a predefined tolerance value (T_{axis}) to decide if the correspondent biplot axis is drawn in the biplot. Standard predictive errors, spe, are calculated for each unit in relation to each biplot axis in each two-dimensional plot and are compared with a predefined tolerance value (T_{units}) to decide which units shall be faced as outliers. The R function automates the process, enabling the user to decide on the degree of precision of the actual analysis. Besides providing a solution for the automatic production of predictive biplots, the methodology offers new insights for the interpretation of multivariate analyses outputs on the basis of a sound principle, the degree of precision of the analysis. This provides an automatic way for the selection of variables that explain latent dimensions and also helps in deciding on the number of important latent dimensions for model developments. A case study consisting on a typical rectangular matrix with data from the analysis of vegetables by several chemical and physical parameters is used in order to carry out a quick overview of several PCA possibilities, comparing traditional PCA, Gabriel's PCA biplots, Gower's PCA biplots and biplots produced with the measure of fit defined in this paper.

Keywords: predictive biplots; predictive power; biplot axis; principal component analysis; R project

1. Introduction

Principal components analysis, which will be referred to as PCA, is one of the most important multivariate analysis, being used in profusion by researchers throughout the world and in almost all fields of science. In this chapter, a few algorithms will be presented (e.g., Mardia et al, 1979, Dunteman, 1989, Jolife, 2002), attempting to show the simplicity of the analysis in terms of algebraic procedures. In all algorithms, the terminology proposed by Mardia et al (1979) will be closely followed. Namely, capital bold letters will refer to matrices and small bold letters will refer to vectors (e.g., **X** is a data matrix and **x** is a column vector). Italic letters will be used to identify scalars, and these will be capital when referring to totals (e.g., *n* refers to a sample unit, while *N* indicates the total number of units). Symbol "*t*" used as a superscript indicates a transposed matrix. A **1** is a column vector with ones and with the appropriate dimension. **I** is the identity matrix.

After discussing the advantages, problems and pitfalls of PCA, biplots will be presented, with a special reference to Gabriel's biplots (1971, 1981) and Gower's predictive biplots (Gower and Hand, 1996, Gower et al., 2011). Problems associated with these biplots will be discussed, which will be followed by the discussion of autobiplots (Alves, 2012), i.e., the automatic construction of predictive biplots with measures of fit controlling the analysis.

All analysis presented in this chapter, as well as figures and tables were produced within R (R project for statistical computing, 2012). Function AutoBiplot.PCA, that carries out the automatic production of biplots, was also developed using R. Its version 6 can be obtained at the Wiley site, in Alves (2012). The version used in this chapter (version 8) is an upgrade of the function already published and is available to interested readers on request.

All algorithms will be applied to a physic-chemical data basis (Barbosa, 2014) in order to facilitate the discussion of pros and cons of the different approaches to PCA. It will be shown that predictive biplots automatically produced with function AutoBiplot.PCA are a very interesting way of dealing with PCA outputs, enabling the selection of the number of components, selection of variables to interpret principal components and identification of outliers. All this is accomplished through the definition of the mean standard predictive error, which is a measure of how accurate are the readings carried out in any PCA display. These biplots also have the advantage of keeping the modulation effect of PCA while simultaneously reasoning exclusively in terms of explicit, original data variables.

2. An algorithm for PCA

Although many papers have been published on PCA, the work of Dunteman (1989) contains a very easy, yet comprehensive approach and is recommended. In this work, a perspective of the evolution of PCA is also presented. It is important to realize that PCA was first mentioned by Pearson (1901) and was developed to its common structure by Hotelling (1933, 1936).

Consider **X** as the initial data matrix with *N* rows and *P* columns. Rows represent the observed units, each unit being referred to as the row vector $\mathbf{u}_n$ [n=1...N]. Columns represent measurement variables, each variable being referred to as $\mathbf{x}_p$ [p=1...P]. Before any standardization, it is important to calculate a row vector $\bar{\mathbf{x}}$ with variable means and another row vector **s** with variable standard deviations:

$\bar{\mathbf{x}} = N^{-1}\mathbf{1}^{\mathrm{t}}\mathbf{X}$ is a row vector with variable means $\bar{x}_1, \bar{x}_2, \ldots, \bar{x}_P$

$\mathbf{X} = \mathbf{X} - \mathbf{1}\bar{\mathbf{x}}$ is now the initial matrix standardized to mean zero

$\mathbf{s} = \mathrm{sqrt}((N-1)^{-1}\mathrm{diag}(\mathbf{X}^{\mathrm{t}}\mathbf{X}))$ is a row vector with standard deviations $s_1, s_2, \ldots, s_P$

$\mathbf{X} = \mathbf{X} \times \mathbf{1}\mathbf{s}^{-1}$ is now the initial matrix standardized to unit standard deviation

Starting with standardized matrix **X**, through the spectral decomposition of the correlation matrix, or SD(**R**), a complete algorithm for PCA can be written as follows:

$\mathbf{R} = (N-1)^{-1}\mathbf{X}^{\mathrm{t}}\mathbf{X}$ is the correlation matrix

$\mathrm{SD}(\mathbf{R}) = \mathbf{V}\mathbf{T}\mathbf{V}^{\mathrm{t}}$ is the spectral decomposition of **R**

$\mathbf{Y} = \mathbf{X}\mathbf{V}$ is the matrix of principal components

Alternatively, instead of carrying out the diagonalization of **R** through its spectral decomposition, one can obtain latent vectors and values through the singular value decomposition of **X**, or SVD(**X**), noting that singular values **S** are the square roots of latent values **T**:

$\mathrm{SVD}(\mathbf{X}) = \mathbf{U}\mathbf{S}\mathbf{V}^{\mathrm{t}}$ is the singular value decomposition of **X**

$\mathbf{Y} = \mathbf{X}\mathbf{V}$ is the matrix of principal components

$\mathbf{R} = \mathbf{V}\mathbf{S}^2\mathbf{V}^{\mathrm{t}}$ is the correlation matrix

Therefore, with these simple algorithms, the following three important structures are obtained: (i) a matrix **V**, called latent vectors matrix, whose columns are the latent vectors, or eigen vectors of **R**, indicated as $\mathbf{v}_q$ [q=1...Q_{max}], and whose rows refer to the data

variables $\mathbf{x}_p$; (ii) a diagonal matrix **T**, called latent values matrix, whose values are the latent values, or eigen values of **R**, referred to as t_q [q=1…Q_{max}]; (iii) a matrix **Y**, called principal components matrix, whose column vectors $\mathbf{y}_q$ [q=1…Q_{max}] are the principal components, and whose rows refer to the data units $\mathbf{u}_n$. In all these matrices, Q_{max} equals N-1 or P, whichever is smaller.

It becomes obvious that the central point in PCA is the correlation matrix and therefore the interplay of original data variables is the key issue. One can say, in very simple terms, that the information contained in the initial data matrix **X** is transported in terms of correlation coefficients into matrix **R**. The latent values in **T**, organized in such a way that $t_1 \geq t_2 \geq \ldots \geq t_{Qmax}$, are the successive maxima of **R**, and therefore the higher the absolute value of a given latent value t_q, the higher the number of the corresponding inter-correlated initial variables it represents, or, in other words, the higher the amount of information it represents.

Following the interpretation of latent values given in the previous paragraph, any latent vector $\mathbf{v}_q$ can be seen as defining a direction passing through a bunch of inter-correlated variables (Alves, 2006) and thus summarizing the information contained in those variables. The corresponding principal component $\mathbf{y}_q$ is therefore seen as the set of coordinates of the data units in that direction. As a consequence, a principal component shall be interpreted in terms of a set of inter-correlated variables and gives the position of data units in relation to them. So, in PCA one starts with a data matrix **X** relating units (a sample of the objects of the study) to explicit, analytical parameters (the parameters used by the researcher to study a given problem), and finishes with a matrix **Y** of principal components, relating units to groups of inter-correlated variables.

As said before, latent values are organized in **T** by decreasing order of importance, and the same happens with latent vectors in **V** and principal components in **Y**. In this way, the most important information is condensed in the first Q components, and irrelevant or spurious information, including errors, are relegated to the last dimensions, which can then be discarded. In this way one can reduce the dimensionality of a problem from the initial P dimensions (the total set of initial data variables $\mathbf{x}_p$) to the final small set of Q important dimensions, i.e., the most important principal components $\mathbf{y}_q$ [q=1…Q]. This information can then be "seen" by plotting and interpreting pairs of principal components.

Although being a simple, widely used multivariate statistical technique, PCA encloses a set of problems that are quite often misunderstood, mainly when applied by researchers lacking a strong statistical background. Some of these problems will be addressed in the following sections, with a particular emphasis on biplots.

3. A case study

What is the data matrix?

In order to help discussing PCA, PCA biplots and respective mathematical approaches, a matrix to be used as a case study (Barbosa, 2014) is presented in Table 1, reporting results of the analysis of minerals, bioactive compounds, texture and water content (in a total of $P = 14$ variables) in raw and cooked vegetables (in a total of $N = 22$ units) preserved with modified atmospheres. A comprehensive description of all units, variables and preservation methods is provided in the legend of Table 1.

Table 1: Initial data values - matrix **X**

	Ca	P	Mg	Na	K	Fe	Cl	S	Tx	W	TC	TP	An	TF
Cr0	2.53	0.16	0.05	0.60	2.33	0.00	0.77	0.58	12.28	95.78	3.00	193.65	107.29	267.19
Cr1	6.21	0.41	0.00	0.00	7.89	0.22	1.73	1.06	19.64	94.90	0.45	174.38	102.70	81.25
Cr2	1.58	0.11	0.00	0.00	3.58	0.30	0.38	0.62	12.24	95.21	0.12	90.00	107.71	43.75
Cr3	2.66	0.32	0.00	0.06	3.75	0.16	0.44	0.74	11.14	96.23	0.17	94.17	115.22	75.00
Cr4	4.29	0.28	0.00	0.00	3.70	0.00	0.88	0.62	8.59	95.65	0.21	98.33	106.87	43.75
Cc0	1.26	0.34	0.11	2.85	2.28	0.87	5.01	0.66	0.88	94.09	1.48	183.33	91.01	160.94
Cc1	0.77	0.17	0.14	2.42	1.75	0.66	3.66	0.51	0.25	95.16	0.89	186.46	101.86	228.13
Cc2	0.81	0.20	0.04	2.55	1.53	0.32	3.32	0.53	0.39	94.50	3.03	165.63	93.51	253.13
Cc3	0.80	0.29	0.09	2.95	1.73	0.25	3.62	0.54	0.89	94.35	3.95	177.08	103.53	253.13
Cc4	0.88	0.14	0.00	3.09	1.26	0.00	4.27	0.27	0.57	95.14	1.90	132.29	91.84	212.50
Cc5	0.61	0.20	0.12	3.23	1.51	0.48	4.03	0.68	0.55	94.48	2.65	132.29	91.84	240.63
Gr0	0.52	0.54	0.17	0.14	2.67	0.17	0.16	0.16	38.43	94.93	5.30	81.24	109.38	84.38
Gr1	1.03	0.13	0.32	0.14	1.96	0.17	0.23	0.11	64.44	95.05	1.87	94.00	99.36	68.75
Gr2	0.70	0.28	0.30	0.01	4.10	1.00	0.51	0.26	40.73	93.79	4.05	115.35	101.86	103.13
Gr3	1.22	0.37	0.25	0.15	2.72	0.40	0.37	0.15	33.26	95.06	2.23	88.06	107.71	59.38
Gr4	1.19	0.22	0.24	0.14	1.59	0.00	0.09	0.16	27.34	93.87	3.38	117.23	110.21	184.38
Gc0	0.19	0.05	0.00	1.85	0.40	0.27	3.47	0.06	8.90	93.00	31.91	66.09	92.26	134.38
Gc1	0.39	0.12	0.19	3.17	0.92	0.31	4.89	0.15	5.69	94.35	7.48	57.29	96.02	90.63
Gc2	0.30	0.35	0.12	2.83	0.76	0.09	4.08	0.15	4.34	93.87	11.00	62.40	91.84	153.13
Gc3	0.24	0.25	0.25	3.65	1.02	0.43	4.67	0.08	4.08	94.20	6.10	53.44	91.84	93.75
Gc4	0.32	0.05	0.08	3.04	0.71	0.00	4.50	0.04	4.27	95.41	6.83	57.60	92.68	93.75
Gc5	0.30	0.16	0.18	3.66	0.98	0.07	4.90	0.06	5.08	93.85	11.44	58.33	95.18	81.25

Units (rows):

a) C = cabbage, G = green bean, r = raw; c = cooked.
b) For raw samples: 0, 1, 2, 3 and 4 correspond to 0, 5, 10, 14 and 17 days packed in an atmosphere of 10% O_2 and 45% CO_2.
c) For cooked samples: 0, 1, 2, 3, 4 and 5 correspond to 0, 7, 14, 24 and 28 days packed in an atmosphere of 60% N_2 and 40% CO_2.

Variables (columns):

a) Minerals (compositional weight percent): Ca = calcium, P = phosphorous, Mg = magnesium, Na = sodium, K = potassium, Fe = iron, Cl = chlorine and S = sulfur
b) Bioactive compounds: TC = total carotenoids (μg/g fresh weigt), TP = total phenolic (mg gallic acid equivalent), Ant = anthocyanins (mg cyanidin-3-glucoside equivalent), TF = total flavanoids (mg catechine equivalent)
c) Other parameters: Tx = texture (N), W = moisture (compositional weight percent)

4. The correlation matrix

Are there groups of inter-correlated variables?

As PCA is based on the diagonalisation of the correlation matrix, it is always important to check matrix **R**, shown in Table 2, and evaluate the magnitude of correlation coefficients and the number of inter-correlated variables. In general terms, a high number of inter-correlated variables and high absolute values of correlation coefficients lead to a good a PCA solution, with clear, easily interpretable results.

Taking into consideration that the value of the student's t distribution corresponding to a 95% confidence interval and 20 degrees of freedom is $t_{(0.975,\ v=20)} = 2.086$, and also that

$$t_{obs} = |r|\sqrt{N-2}/\sqrt{1-r^2}$$

where r is the correlation coefficient, it follows that any correlation coefficient lower than 0.423 (a value obtained in any table with the student´s t distribution) cannot be considered as significantly different from zero. Therefore, in this case study, observing Table 2, it is seen that correlations between variables are not very high, and PCA will pose some difficulties. Nevertheless, some groups of more or less inter-correlated variables exist. To help this discussion, in Table 2 all correlation coefficients are shown in the lower matrix triangle, while only significant correlation coefficients are shown in the upper triangle.

Table 2: Correlation matrix **R**

	Ca	P	Mg	Na	K	Fe	Cl	S	Tx	W	TC	TP	An	TF
Ca	1.00		*-0.45*	*-0.56*	**0.86**			**0.74**		*0.48*			*0.46*	
P	0.33	1.00			*0.51*									
Mg	-0.45	0.11	1.00					*-0.57*	*0.60*					
Na	-0.56	-0.34	-0.02	1.00	*-0.67*		***0.97***		***-0.70***				***-0.81***	
K	0.86	0.51	-0.21	-0.67	1.00		*-0.55*	**0.70**			*-0.48*		*0.52*	
Fe	-0.20	0.16	0.35	0.07	0.14	1.00								
Cl	-0.42	-0.32	-0.12	0.97	-0.55	0.13	1.00		*-0.73*				***-0.85***	
S	0.74	0.28	-0.57	-0.27	0.70	0.16	-0.17	1.00		*0.43*	*-0.52*	*0.70*		
Tx	0.08	0.25	0.60	-0.70	0.31	0.04	-0.73	-0.26	1.00				***0.42***	*-0.44*
W	0.48	0.08	-0.33	-0.38	0.36	-0.33	-0.40	0.43	0.05	1.00	*-0.66*		*0.53*	
TC	-0.41	-0.33	-0.07	0.29	-0.48	-0.10	0.33	-0.52	-0.13	-0.66	1.00	*-0.45*		
TP	0.38	0.15	-0.27	-0.08	0.35	0.28	-0.06	0.70	-0.17	0.20	-0.45	1.00		*0.68*
An	0.46	0.38	-0.02	-0.81	0.52	-0.19	-0.85	0.30	0.42	0.53	-0.41	0.13	1.00	
TF	-0.22	-0.16	-0.18	0.41	-0.34	0.06	0.32	0.20	-0.44	-0.13	-0.03	0.68	-0.25	1.00

Variables as shown in legend for Table 1. All correlations are shown in lower triangle. Significant correlations (greater than 0.423) shown in upper triangle.

For example, taking into consideration correlation coefficients ≥ 0.700, the correlation coefficients between *Ca*, *K* and *S* (highlighted in bold in Table 2) are high. Although with lower correlation coefficients, *Ca* is also positively correlated with *W* and *An* and negatively correlated with *Mg* and *Na*. However, although *S*, like *Ca*, is also correlated with *Mg* and *W*, it is not correlated with *Na* nor with *An*, and unlike *Ca*, is correlated with *TC* and *TP*. Observing the correlations of *K* with other analytical parameters, it is seen that like *Ca*, *K* is also correlated with *Na* and *An*, and like *S* is correlated with *TC*, but in contrast to both *Ca* and *S*, *K* is correlated with *P* and *Cl*. This type of situation tends to happen when correlation coefficients are relatively small.

Another group of highly correlated variables is *Na*, *Cl*, *Tx* and *An* (highlighted in bold italic in the same table). But for example, in this case, it can be seen that the correlation between *Tx* and *An* is small. And each of these parameters presents small correlations with other parameters establishing different inter-relationships.

As a consequence, since PCA is based on a correlation matrix, it will be seen that some problems will be faced due to the fact that in general correlation coefficients are small.

5. Main structures in PCA

What are the outcomes of a PCA?

Tables 3 and 4 present the matrices with latent vectors and values (**V** and **T**, respectively) resulting from the spectral decomposition of the correlation matrix **R**. Table 5 shows a special matrix, called the "loadings" matrix $\mathbf{L} = \mathbf{V}\mathbf{T}^{1/2}$, which will be discussed later, and Table 6 shows the principal components matrix (**Y**). It is important to observe that matrices **X** and **Y** are similar, but while in the former all units are referred to explicit (initial) variables, well known by the researcher, in the latter all units are referred to the directions defined by the latent vectors shown in Table 3 and must be interpreted. The importance of each direction, and hence of each principal component is given by the latent values.

Table 3: Latent (eigen) vectors – matrix **V**

	v_1	v_2	v_3	v_4	v_5	v_6	v_7	v_8	v_9	v_{10}	v_{11}	v_{12}	v_{13}	v_{14}
Ca	**-0.35**	-0.18	0.15	**0.29**	0.04	-0.07	-0.41	0.22	-0.31	-0.05	-0.49	-0.10	-0.21	0.36
P	-0.22	0.04	**-0.28**	0.26	-0.15	0.83	0.07	-0.27	-0.02	-0.01	-0.08	-0.06	0.02	-0.02
Mg	0.07	**0.39**	**-0.43**	-0.19	-0.21	-0.01	-0.28	0.39	-0.28	0.28	-0.22	0.05	0.23	-0.29
Na	**0.37**	-0.22	-0.03	0.03	-0.30	0.12	-0.10	0.11	-0.15	0.21	0.32	0.08	0.30	0.65
K	**-0.38**	-0.07	-0.09	**0.36**	0.00	-0.13	-0.18	0.07	-0.01	-0.07	0.38	0.69	0.13	-0.15
Fe	0.03	-0.03	**-0.63**	0.23	-0.07	-0.38	0.49	-0.15	-0.13	-0.05	-0.19	0.06	-0.16	0.23
Cl	**0.35**	-0.25	-0.02	0.20	-0.28	0.03	-0.14	0.06	-0.31	-0.03	0.27	-0.10	-0.60	-0.36
S	**-0.27**	**-0.40**	-0.08	0.16	0.02	-0.11	0.07	0.07	0.24	0.72	0.08	-0.31	0.08	-0.18
Tx	-0.19	**0.44**	-0.17	-0.13	0.13	-0.13	-0.38	-0.40	0.00	0.23	0.37	-0.17	-0.33	0.27
W	**-0.27**	-0.12	**0.28**	**-0.35**	-0.44	-0.11	0.15	-0.47	-0.44	0.16	-0.11	0.16	0.08	-0.09
TC	**0.27**	0.14	0.19	**0.32**	0.58	0.04	0.13	-0.19	-0.51	0.28	-0.01	0.05	0.19	-0.09
TP	-0.15	**-0.40**	**-0.33**	-0.24	0.26	-0.04	-0.18	-0.08	-0.30	-0.39	0.23	-0.35	0.33	-0.11
An	**-0.35**	0.13	0.09	-0.23	0.12	0.18	0.47	0.51	-0.30	0.04	0.32	-0.04	-0.24	0.15
TF	0.12	**-0.36**	-0.21	**-0.46**	0.36	0.21	-0.10	-0.04	0.07	0.21	-0.20	0.47	-0.31	0.06

$\mathbf{v}_{[1...14]}$ = latent vectors; Variables (rows) as shown in legend for Table 1.

Highlighted in bold are values of the two first vectors greater than $1/P^{½} = 0.267$

Table 4: Latent (eigen) values – matrix **T**

	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9	t_{10}	t_{11}	t_{12}	t_{13}	t_{14}
Abs	**5.30**	**3.08**	**1.78**	**1.21**	0.93	0.69	0.46	0.22	0.13	0.07	0.06	0.05	0.01	0.00
Cum%	37.9	59.9	72.6	81.2	87.9	92.8	96.1	97.7	98.6	99.1	99.6	99.9	100.0	100

Abs = absolute value; Cum% = latent values in cumulative percentage; $t_{[1...14]}$ = latent values

Highlighted in bold latent values greater than 1.

Table 5: Correlations between components and variables – loadings matrix L = VT½

	l_1	l_2	l_3	l_4	l_5	l_6	l_7	l_8	l_9	l_{10}	l_{11}	l_{12}	l_{13}	l_{14}
Ca	**-0.81**	-0.32	0.20	0.32	0.04	-0.06	-0.28	0.10	-0.11	-0.01	-0.12	-0.02	-0.02	0.00
P	-0.51	0.07	-0.37	0.29	-0.14	0.69	0.05	-0.13	-0.01	0.00	-0.02	-0.01	0.00	0.00
Mg	0.16	0.68	-0.57	-0.21	-0.20	-0.01	-0.19	0.18	-0.10	0.07	-0.05	0.01	0.02	0.00
Na	**0.85**	-0.39	-0.04	0.03	-0.29	0.10	-0.07	0.05	-0.05	0.06	0.08	0.02	0.03	0.00
K	**-0.87**	-0.12	-0.12	0.40	0.00	-0.11	-0.12	0.03	0.00	-0.02	0.09	0.15	0.01	0.00
Fe	0.07	-0.05	**-0.84**	0.25	-0.07	-0.32	0.33	-0.07	-0.05	-0.01	-0.05	0.01	-0.02	0.00
Cl	**0.81**	-0.44	-0.03	0.22	-0.27	0.02	-0.09	0.03	-0.11	-0.01	0.07	-0.02	-0.06	0.00
S	-0.62	**-0.70**	-0.11	0.18	0.02	-0.09	0.05	0.03	0.09	0.19	0.02	-0.07	0.01	0.00
Tx	-0.44	**0.77**	-0.23	-0.14	0.13	-0.11	-0.26	-0.19	0.00	0.06	0.09	-0.04	-0.03	0.00
W	-0.62	-0.21	0.37	-0.39	-0.42	-0.09	0.10	-0.22	-0.16	0.04	-0.03	0.04	0.01	0.00
TC	0.62	0.25	0.25	0.35	0.56	0.03	0.09	-0.09	-0.18	0.07	0.00	0.01	0.02	0.00
TP	-0.35	**-0.70**	-0.44	-0.26	0.25	-0.03	-0.12	-0.04	-0.11	-0.10	0.06	-0.08	0.03	0.00
An	**-0.81**	0.23	0.12	-0.25	0.12	0.15	0.32	0.24	-0.11	0.01	0.08	-0.01	-0.02	0.00
TF	0.28	-0.63	-0.28	-0.51	0.35	0.17	-0.07	-0.02	0.03	0.06	-0.05	0.11	-0.03	0.00

$\mathbf{l}_{[1...14]}$ = loadings; Variables (rows) as shown in legend for Table 1.
Highlighted in bold are values of the two first vectors greater than 0.700.

5.1 Reduction in dimensionality

How many components should be retained for further analysis?

Looking at matrix **T**, or at the scree plot in Figure 1 (which is just a barplot of latent values), it is seen that from the 5th latent value (equal to 0.928) onwards, all values are lower than 1, and therefore all contain less than the information contained in any initial variable. Thus, the obvious decision is to keep the first four principal components, passing from 14 initial variables to 4 components, in a process known as reduction in dimensionality. In the process, slightly over 81% of the total information is kept for further analysis, as it is shown in the cumulative percentages in the last row of Table 4, and it is assumed that only less important information and errors contained in the last 10 components are lost in the process.

But should principal components 3 and 4 (to which correspond latent values close to 1) be included? Why not keep just the first two components? Or the first three? As a matter of fact, as seen in Table 4, retaining the first two principal components, one keeps around 60% of the total information, with three components 73% of the information is kept, while retaining 4 components one keeps close to 81% of the total information in **X**. As a general rule, it is important to keep a good balance between the minimum number of components and the maximum amount of information. And it is also important that all components kept are easily interpretable so that the the corresponding information can be well understood.

When dealing with matrices with a very large number of variables, as those obtained with the use of modern equipments (e.g., infrared spectroscopy, nuclear

magnetic resonance, etc), the decision on the number of components to retain may be difficult. In those circumstances Wold (1978) proposed the cross validation of the number of components, a process that is now-a-days implemented in most statistical packages. These methodology will not be dealt with in this chapter, but its reference is important for further studies on PCA.

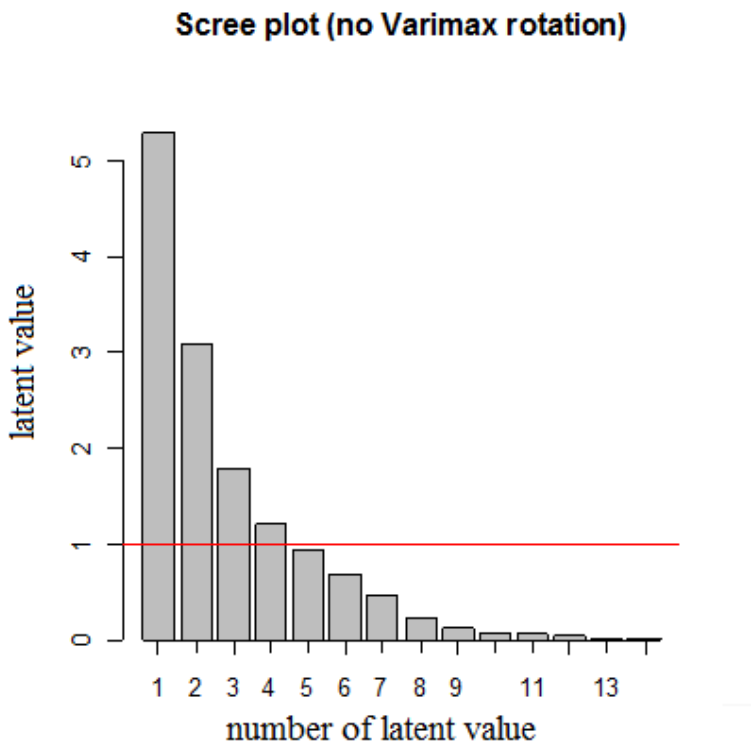


Figure 1: Scree plot (graphical display of latent values, produced by AutoBiplot.PCA function.

5.2 Principal component plots

How are principal components displayed?

Keeping 4 components slightly over 81% of total information is kept for analysis. The conventional way of displaying PCA results is to draw two-dimensional plots. With 4 components, one can produce six different plots ($\mathbf{y}_1$ *vs* $\mathbf{y}_2$, $\mathbf{y}_1$ *vs* $\mathbf{y}_3$, ..., $\mathbf{y}_3$ *vs* $\mathbf{y}_4$). In order to restrict the number of plots and to avoid repeated information, graphs of $\mathbf{y}_1$ *vs* $\mathbf{y}_2$ and $\mathbf{y}_3$ *vs* $\mathbf{y}_4$, are a logic choice. Figure 2 shows the $\mathbf{y}_1$ *vs* $\mathbf{y}_2$ plot. It is important to note that this plot is obtained by just plotting the two first columns of matrix $\mathbf{Y}$, shown in Table 6. Typically the most important component ($\mathbf{y}_1$) is drawn horizontally, the other, less important component ($\mathbf{y}_2$) is shown vertically.

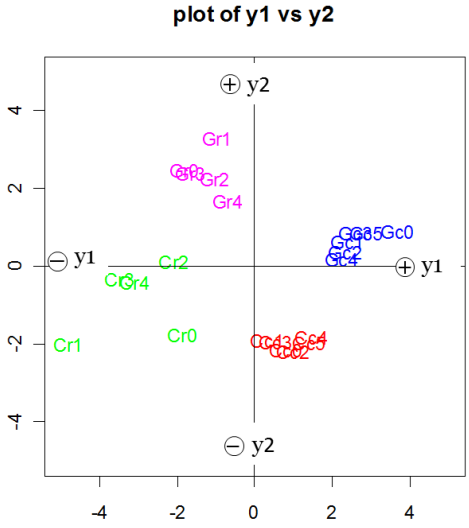


Figure 2: First step in the production of a principal component plot: the horizontal axis is component $\mathbf{y}_1$, the vertical axis is component $\mathbf{y}_2$, sample units are displayed by their symbols (see Table 1). Colours are used to facilitate interpretation.

Some authors draw three-dimensional graphs, plotting three components together, but examples in the literature are scarce, and the reason is obvious: in day-to-day work, PCA is important in cases where initial dimensionality is high, and in these cases, interpretations and conclusions using three dimensional plots are difficult.

Once a principal component plot is produced, as shown in Figure 2, it is necessary to carry out its interpretation, a process that will be dealt within the next section.

5.3 Interpreting principal components

How are principal components interpreted?

The meaning of each principal component is given by the relationships between initial variables and latent vectors. $\mathbf{V}$ is as a rotation matrix, and each latent vector, $\mathbf{v}_q$, is a new direction through a group of inter-correlated variables, or along which the variance of $\mathbf{X}$ is maximized. Hence the closeness of the meanings of variance and information. As $\mathbf{V}^t\mathbf{V} = \mathbf{V}\mathbf{V}^t = \mathbf{I}$ (where $\mathbf{I}$ is the identity matrix), all vectors are orthogonal, thus uncorrelated. Being orthogonal to each other, so are the principal components, and thus each principal component must be interpreted by itself. This is done by analysis of each latent vector $\mathbf{v}_q$.

As $\mathbf{V}$ is a rotation matrix, each value of a latent vector is the cosine of the angle subtended between the vector and an initial variable. The squared cosines of a latent

vector add up to 1. Consequently, a latent vector subtending an equal angle with all initial variables has all cosines equal to $1/P^{1/2}$, which, in this case study, is $1/14^{1/2}$ = 0.267. It follows that a cosine between a vector $\mathbf{v}_q$ and a variable $\mathbf{x}_p$ greater than 0.267 means that the latent vector is closer to that variable and therefore more related to it. Thus, that variable will be important to interpret the corresponding principal component. Increasing the angle between the latent vector and the initial variable, lowers the cosine to a value lower than 0.267, and the variable will not be important to explain that component.

Following this criterion, by observation of the first column of Table 3, it becomes evident that variables *Ca*, *K*, *S*, *W* and *An* are negatively correlated with $\mathbf{y}_1$, while variables *Na*, *Cl* and *TC* are positively correlated with the same component. These facts can be transported to Figure 2, producing Figure 3. In the horizontal axis representing $\mathbf{y}_1$, variables *Ca*, *K*, *S*, *W* and *Ant* are written on the left, negative part of the component, while *Na*, *Cl* and *TC* are written on the opposite axis' edge. Units lying towards the right hand-side of the plot (cooked green beans – Gc) have high values in variables *Na*, *Cl* and *TC* and low values in variables *Ca*, *K*, *S*, *W* and *Ant*. Raw cabbages (Cr) lye on the opposite side of the graph, having high values in variables *Ca*, *K*, *S*, *W* and *An* and low values in *Na*, *Cl* and *TC*. Raw green beans (Gr) and cooked cabbages (Cc) project to the center of component $\mathbf{y}_1$, and are not well defined in this component.

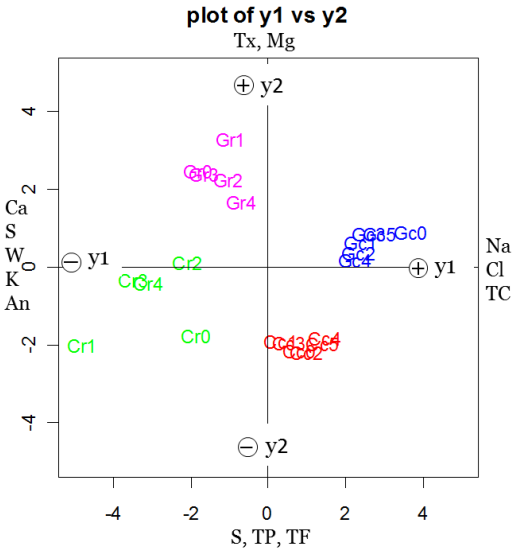


Figure 3: Second step in the production of a principal component plot: variables' names are written at axes edges, according to their relevance in the interpretation of each component axis, based on latent vectors.

Looking at latent vector $\mathbf{v}_2$ (the second column of Table 3), it is seen that *Mg* and *Tx* are positively correlated with component $\mathbf{y}_2$, in opposition to variables *S*, *TP* and *TF*,

which are negatively correlated with it. These facts are also shown in Figure 3, with these variable´s names written at the respective edges of component $\mathbf{y}_2$. It is now concluded that raw green beans (Gr), being related to the positive part of $\mathbf{y}_2$, have high values of Mg and Tx and low values of S, TP and TF, the opposite being true for cooked cabbages (Cc) and a few raw cabbages (Cr0 and Cr1). The other sample units, projecting to the center of component $\mathbf{y}_2$, are not represented by this component.

It is important to see that because principal components are uncorrelated, each principal component is being interpreted by itself on the basis of a set of inter-correlated variables: $\mathbf{y}_1$ was interpreted based on a set of 8 inter-correlated variables and component $\mathbf{y}_2$ on the basis of 5 inter-correlated variables.

Using latent vectors to interpret principal components has problems. For example, checking Table 4, should we consider that water content (*W*) is important to explain principal component 1, 3 or 4? And sulfur (*S*), is it important to explain component 1, or 2, or both? And these are just two examples showing uncertainties that quite often arise in PCA.

Another problem is related to the actual conclusions about the values of sample units. For example, it was concluded that cooked green bean samples, being highly loaded on the positive side of component 1, have a high sodium (*Na*) content, and that cooked cabbages (Cc) were not well represented in component 1, and therefore had intermediate *Na* content. The same reasoning was true for the chlorine (Cl) contents of cooked samples. But inspecting Table 1 it is seen that both cooked samples (Gc and Cc) have high *Na* and *Cl* contents, and not only Gb as previously concluded. The same effect is seen for raw cabbages (Cr) and green beans (Gr), both having very low contents in *Na* and *Cl* (Table 1) in contrast to what is seen in Figure 3 where the Cr samples, with extreme loadings on the negative part of $\mathbf{y}_1$, seem to be the only ones with low *Na* and *Cl* contents. These are just examples of some erroneous conclusions that can easily be withdrawn from a PCA.

Some of these problems can be solved by the use of "loadings" instead of latent vectors. "Loadings" is the term used to refer to exact correlations between initial variables ($\mathbf{x}_p$) and principal components ($\mathbf{y}_q$), which can be easily obtained in matrix $\mathbf{L} = \mathbf{V}\mathbf{T}^{½}$. This has been advocated by several researchers, mainly Jolife (1972, 2002). In matrix $\mathbf{L}$, columns are referred to as $\mathbf{l}_q$ [q=1...Q_{max}]. The sum of the squared loadings along a column adds up to the respective latent value, while summing squared loadings across a row adds up to 1. Therefore, loadings will always be decreasing with increasing number of the principal component, a fact that usually makes it easier to carry out interpretations because there will be less doubts along rows. The loadings matrix applied to the case study was shown in Table 5. According to Jolife, principal components should be interpreted based on variables with loadings superior to 0.700.

Following Jolife's recommendation, as it is shown in Table 5, variables *Ca*, *Na*, *K*, *Cl* and *An* are important to explain component $\mathbf{y}_1$, while *S*, *Tx* and *TP* are important for component $\mathbf{y}_2$. Only *Fe* is important for component $\mathbf{y}_3$ and no variable seems to be explaining component $\mathbf{y}_4$. Therefore, as $\mathbf{y}_3$ is explained just by one variable and $\mathbf{y}_4$ has no plausible interpretation, probably only the two first components are important, and all the others should be discarded. This methodology is therefore more restrictive, leading to less variables being used to interpret principal components. Figure 3 corresponds to an adjustment of Figure 2 to the use of loadings and Jolife's recommendations for interpretation for interpretation of principal components.

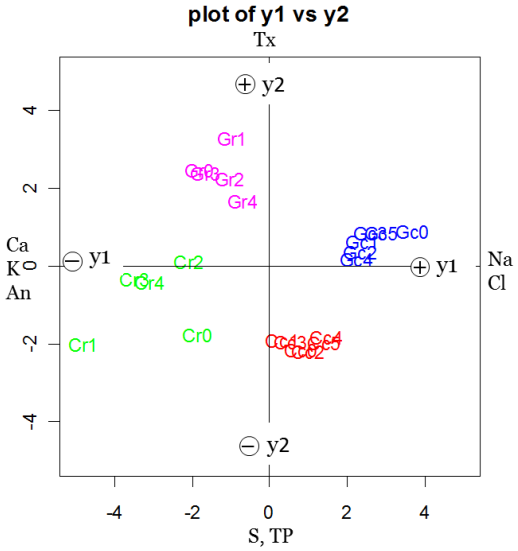


Figure 4: Alternative step in the production of a principal component plot: variables' names are written at axes edges, according to their relevance in the interpretation of each component axis, based on loadings (correlations between initial variables and principal components).

Again some problems are encountered. The position of sample points in relation to principal components is not changed. Only, the variables used for component's interpretation were reduced. But as a consequence, some variables are not accounted for in the analysis. For example, looking at Table 5, it is immediate to see that *P*, *Mg*, *W*, *CT* and *TF* will not be used to interpret any of the first four components, and Fe will be taken into consideration only if the third component $\mathbf{y}_3$ is used. Thus, using loadings instead of latent vectors, with the strategies discussed before, a more restrictive solution is found, making the analysis easier, but losing a higher proportion of the available information.

Also, the analysis of samples may also be affected, in the same way as seen with latent vectors. For example, sulfur (*S*), loaded on the negative part of component $\mathbf{y}_2$, means that samples on the upper part of the plot are low in *S*, while those at the bottom are high in *S*. If values are checked in Table 1, it is seen that cabbage sample units (Cc and Cr) are high in *S* content, while all green bean samples (Gr and Gc) have low values. Reading the plot of Figure 4 one is lead to the conclusion that Gr samples are low in *S*, Gc and Cr have intermediate *S* contents, and Cc have the highest *S* amounts. Therefore, as before, this example also shows that it is very easy to withdraw wrong conclusions and that the modulation effect of PCA may sometimes be misleading.

Interpretations of principal components on the basis of latent vectors can be eased by a plot of latent vectors (Alves, 2006), as it is shown in Figure 5. This figure is obtained just plotting the two first columns of matrix **V** in Table 3 against each other. As the maximum absolute value of a cosine is 1, and a cosine equal for all variables is, in the present example, equal to 0.267, two circles of radii 1 and 0.267 (Alves, 2006) were superimposed in Figure 5. Variables represented by vectors with edges lying between the two circles are important for principal component interpretation. As it is seen in Figure 5, almost all variables do not seem to be very important, but just slightly important (with edges closer to the inner circle than to outer one) and the display is not very useful in this case study. This always happens when correlations between initial variables are small, as discussed on the analysis of matrix **R** (Table 2).

As it was done with the plot of latent vectors, many of the already cited authors, as well as some software packages, present a plot of loadings in what is known as the "correlation circle". In Figure 6, a correlation circle corresponding to loadings $\mathbf{l}_1$ *vs* $\mathbf{l}_2$ is presented: it is obtained by just plotting the two first columns of matrix **L** (Table 5), and superimposing two circles: one with radius 1 and another with radius 0.7 to follow Jolife's recommendations (Alves, 2006). Variables important for explaining components have edges between the two circles, while variables represented by short vectors with edges inside the inner circle are not important to explain that principal components plane.

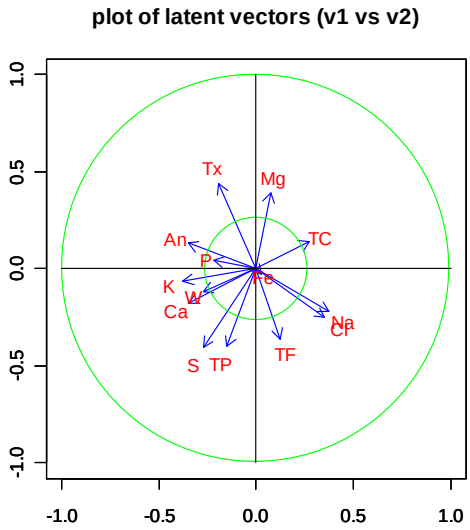


Figure 5: Plot of latent vectors $\mathbf{v}_1$ *vs* $\mathbf{v}_2$ with an outer circle of radius 1 and an inner circle of radius $1/P^{½}$

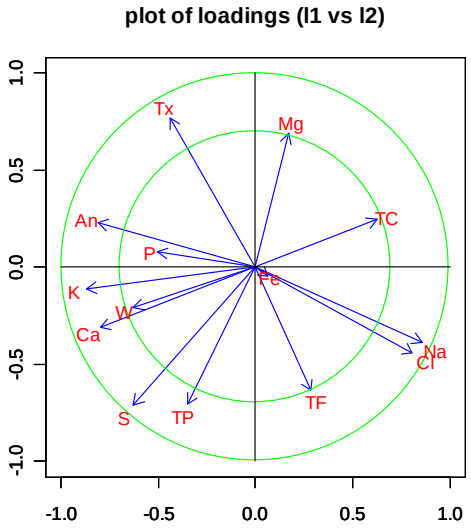


Figure 6: Plot of loadings $\mathbf{l}_1$ *vs* $\mathbf{l}_2$ with an outer circle of radius 1 and an inner circle of radius 0.700.

Following the latest paragraphs, instead of recurring to matrices **T**, **V** or **L** and **Y**, PCA can be studied just by looking at plots of latent values (Figure 1), plots of principal components (Figures 2, 3 or 4) and plots of latent vectors or loadings (Figures 5 or 6), which may be more comfortable for users of PCA.

6. Some solutions to PCA problems

Whatever the methodology used to display and interpret principal components, it is important to realize that it is up to the researcher, regardless of his/her statistical background, to decide on how many components to retain, and on how many and which original variables are used to explain each principal component. Also, depending on the type of data one is dealing with, it is sometimes very difficult to find the best solution to a PCA output.

Some methods have been devised in order to facilitate interpretation of principal components, such as the Varimax (Kaiser, 1958) and Quartimax (Child, 2006) rotations. Other methods were developed to help deciding on the correct number of components to keep, especially when the number of initial variables is very large, as is the

case of the cross validation of the number of components proposed by Wold (1978) and available in the majority of statistical software packages. All these methods are very helpful but, to produce a good result, require that the user has a deep knowledge of the particularities of PCA and of the methods developed to solve specific problems.

Biplots can be seen as another tool to help the analysis of multivariate data through principal components. A very short approach devised by Gabriel will be discussed, which will be followed by Gower's biplots and autobiplots.

7. Gabriel's biplots

The term "biplot" was coined by Gabriel (1971) referring to a method of plotting together sample units and variables in the same principal component plot. Innumerous developments and variants of Gabriel's biplots (e.g., Gabriel, 1971, 1972, 1981; Gabriel and Zamir, 1978; Kempton, 1984), applied to PCA and to other multivariate analysis can be found in the literature (e.g., Benzecri, 1978; Greenacre, 1993). Many common statistical software packages also provide the possibility to use these techniques (SSPS, Statistica for Windows, R, etc).

Basically, Gabriel's biplots are a simple method of adjusting the scales of latent vector plots (as the one presented in Figure 5) and principal component plots (as those presented in Figures 2 to 4) in such a way that they can be plotted together, maximizing some aspect inherent to the data. An example of such a biplot is shown in Figure 7.

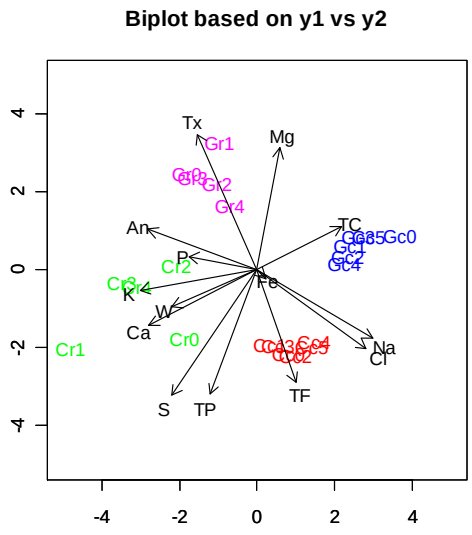


Figure 7: A type of Gabriel's biplot based on principal component $\mathbf{y}_1$ *vs* $\mathbf{y}_2$, with superimposed latent vectors $\mathbf{v}_1$ *vs* $\mathbf{v}_2$.

The major problem with this type of biplot is the fact that there is a tendency to carry out interpretation of PCA based on proximities between sample points and the edges of variable's vectors. For example, looking at the biplot of Figure 7, it is easy to conclude that cooked green beans (Gc) have high contents in total carotenoids (*TC*). Although this seems to be true, it is also true that they are poor in calcium, water and potassium (*Ca*, *W* and *K*), a fact that is not easily deduced. These problems are solved by predictive biplots, as will be discussed in the next section.

8. Gower's bipots

Gower's predictive biplots (Gower and Hand, 1996; Gower et al., 2011) attain a double objective: on one hand, through PCA, a matrix **X** is approached through a lower dimensional space, obtaining a modulation effect and a reduction in dimensionality. On the other hand, there is no attempt to explain principal components and relationships between variables and components, but the aim is to judge distances between sample units in terms of their original exact values. It is assumed that PCA has an important modulation effect, but the interest is to use original values for discussions.

In predictive biplots, for each initial variable, instead of drawing an arrow corresponding to each initial variable, a complete axis is drawn in the principal component two-dimensional space, which is called a "biplot axis". The axis is equipped with an appropriate scale for measurement. The scale is such that carrying out the orthogonal projection from a given sample point onto a biplot axis, will read the true value of the sample point in that variable, as it is illustrated in Figure 8a. In this figure, a simulation of a two-dimensional principal component plane is presented, with three biplot axes corresponding to idealized initial variables $\mathbf{x}_1$, $\mathbf{x}_2$ and $\mathbf{x}_3$, with appropriate scales, and a sample $\mathbf{u}_n$ to exemplify the predictive process: carrying out orthogonal projections from the sample point onto the biplot axis of interest, the actual sample values are read off directly from the graph. In the example, as it is read from the graph, the values of $\mathbf{u}_n$ in the three variables are: $\mathbf{x}_1 \simeq 0.5$, $\mathbf{x}_2 \simeq 1.1$ and $\mathbf{x}_3 \simeq 5.3$. Therefore, as it can be seen in this example, the great advantage of this approach is that the researcher is always thinking in terms of original variables and true sample values, without losing the PCA modulation effect.

Gower and coworkers (Gower and Hand, 1996) also developed the idea of interpolative biplots. As with predictive biplots, once a PCA is carried out, two-

dimensional principal component displays are produced and interpolative axes with measurement scales are superimposed in the graph. The scales are calculated in such a way that new sample points can be correctly positioned in the graph. The process of interpolation is exemplified in Figure 8b. In this example it is shown how a sample point $\mathbf{u}_n$ with values $\mathbf{x}_1 = 3.5$, $\mathbf{x}_2 = 2$ and $\mathbf{x}_3 = 1.4$ is interpolated to its right position in the graph.

In PCA, the directions of predictive and interpolative biplot axes are the same, but the scales are different because the processes are also different. While in interpolative biplots there is a direct positioning of a sample point in a graph that already exists, in predictive biplots the process is reversed, and a sample point in the graph, referred to principal components, is back-projected to the original variables.

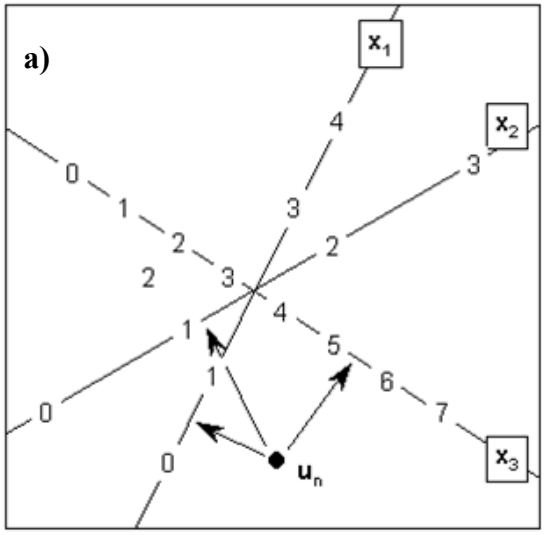


Figure 8a: Illustration of the way predictive biplots work: carrying out orthogonal projections from a sample point to an axis and reading directly the of the sample unit in the corresponding variable.

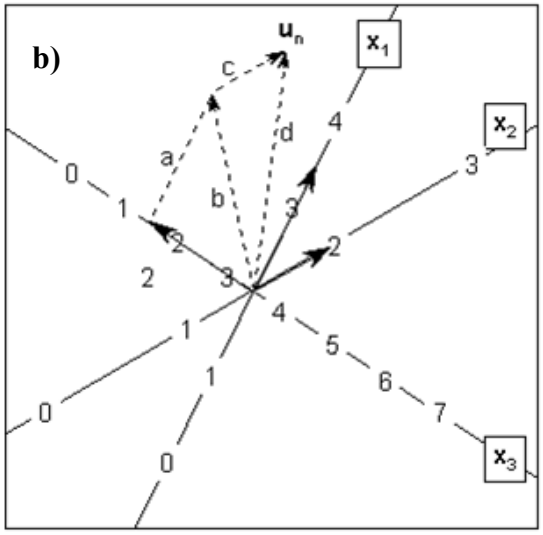


Figure 8b: Illustration of the way interpolative biplots work: a new sample unit, not used for PCA development, is interpolated (positioned in the plot) by the vector sum method.

Concentrating on predictive biplots, it is necessary to create a convenient scale for each variable. For example, returning to the case study that has been used along this chapter (Table 1) and looking at variable $\mathbf{x}_8$ that represents the sulfur (*S*) content of vegetables, it is seen that the minimum and maximum values observed are 0.04 and 1.06, respectively. Therefore, a convenient scale for a biplot axis could be the set of values 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0. In order to produce biplots, a column vector $\boldsymbol{\mu}_8$ with these scale values can be defined, and standardized in the same way as the original variables, and then projected in the space defined by the first two principal component directions:

$\boldsymbol{\mu}_8^t = [0.0\ ,0.2, 0.4, 0.6, 0.8, 1.0]$ is a column vector with convenient scale values

$\mathbf{e}_8^t = [0,0,0,0,0,0,0,1,0,0,0,0,0,0]$ is the canonical vector for variable $\mathbf{x}_8$

$\boldsymbol{\mu}_8 = (\boldsymbol{\mu}_8 - \mathbf{1}\bar{x}_8) \times s_8$ $\boldsymbol{\mu}_8$ is now a column vector with standardized scale markers

$\mathbf{V}_{[1,2]}$ is a reduced latent vector matrix (with the first two latent vectors)

$\mathbf{E}_8 = \boldsymbol{\mu}_8\mathbf{e}_8^t\mathbf{V}_{[1,2]}\left(\mathbf{e}_8^t\mathbf{V}_{[1,2]}\mathbf{V}_{[1,2]}^t\mathbf{e}_8\right)^{-1}$ is a matrix with coordinates

Vector $\mathbf{e}_8$, the canonical vector, is used to ensure that the scale values will refer to for variable $\mathbf{x}_8$. Also, the standardization of vector $\boldsymbol{\mu}_8$ by subtracting the $\bar{x}_8$ and dividing by s_8 is necessary to make sure that variable and scale values are in correspondence. $\mathbf{V}_{[1,2]}$ is used to refer to the plane defined by the two first principal components. Then

$$\mathbf{E}_8 = \boldsymbol{\mu}_8\mathbf{e}_8^t\mathbf{V}_{[1,2]}\left(\mathbf{e}_8^t\mathbf{V}_{[1,2]}\mathbf{V}_{[1,2]}^t\mathbf{e}_8\right)^{-1}$$

is a matrix with coordinates of scale markers on the plane of the two first principal components. A scale marker is therefore a point indicating the position of a scale value, and the complete set of scale markers in matrix E8 gives the position of all scale values chosen for variable $\mathbf{x}_8$. In Figure 9a a plot of principal components $\mathbf{y}_1$ *vs* $\mathbf{y}_2$ is shown, highlighting the position of sample Cr1 to be used as an example. Figure 9b shows the scale markers, i.e., the projection of standardized scale values, occupying the positions defined in matrix $\mathbf{E}_8$. Figure 9c presents the final work: scale markers are substituted by scale values and a straight line (the sulfur biplot axis) is drawn linking all values together. The name of the axis is then written at the edge where scale values are higher.

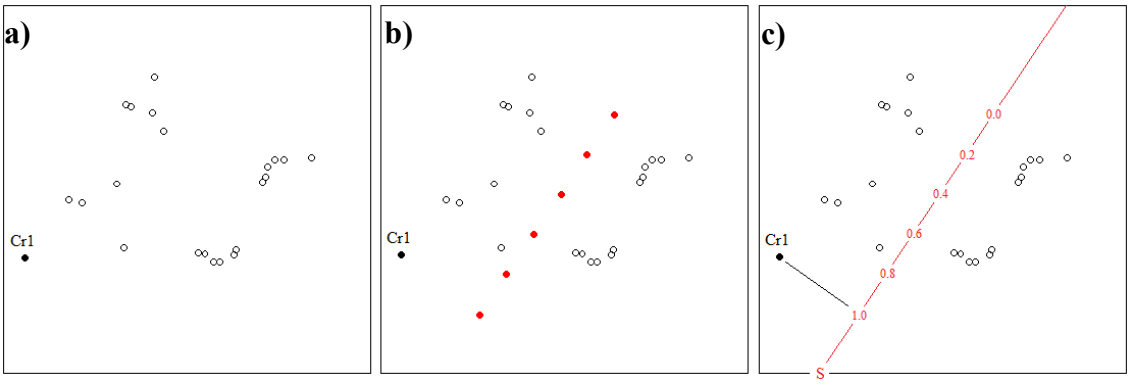


Figure 9a: a biplot is constructed by first drawing a principal component plot.In this figure the plot is simplified for clarity purposes.

Figure 9b: scale markers are projected onto the principal component plot.

Figure 9c: scale markers are substituted by scale values and linked by a straight line, making orthogonal projections possible.

Figure 9c also shows how original values are predicted: using sample unit Cr1 as an example, a straight line is drawn orthogonally from the sample point onto the variable's axis, and the original value of the sample is read. In this example, Cr1 projects roughly to 1. Checking Table 1 it is seen that the value of sample Cr1 for sulfur is 1.06, which is a very accurate prediction.

In order to finish the biplot, calculations presented above for variable $\mathbf{x}_8$ must be repeated to the remaining thirteen variables. Figure 10 shows the final predictive biplot. If other principal component planes are of interest, then other predictive biplots can be constructed by using the corresponding matrix $\mathbf{V}_{[i,j]}$ (where i and j refer to any latent vectors of interest).

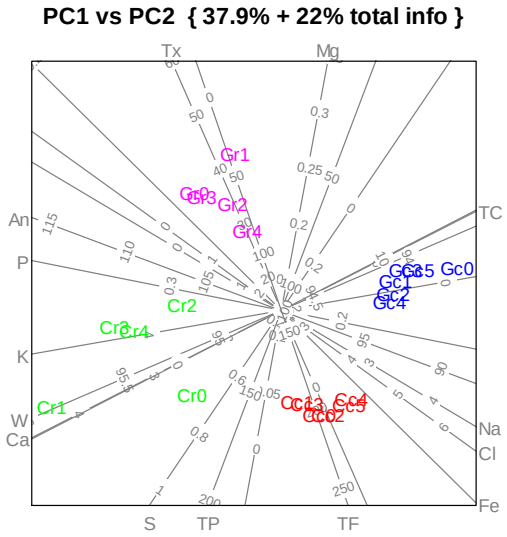


Figure 10: Example of a predictive biplot based on the plane of principal components $\mathbf{y}_1$ *vs* $\mathbf{y}_2$, applied to the case study. The biplot was obtained with function AutoBiplot.PCA.

Gower's predictive biplots have several advantages (Gower et al., 2011, Alves, 2012). The first, obvious advantage is that there is no need to concentrate and try to interpret principal components, all interpretations being carried out in relation to original variables. This is very comfortable for any PCA user because each original variable has a specific meaning of itself. A second advantage is that interpretations can be carried out on the basis of sample true values, which is also very comfortable for any researcher who has dealt with the data since the

beginning. It is important to understand that the one who produced the initial data matrix can now carry out interpretations based on field work knowledge.

Another very important advantage of predictive biplots is that interpretations are more accurate in comparison to traditional principal component methods. Observing Figure 10 and concentrating on the sodium (*Na*) and chlorine (*Cl*) axes, it is now evident that Gc samples project from 2% to slightly above 3.5% *Na* and from slightly above 3% to around 5% *Cl*. It is also evident that raw samples (Cr and Gr) project to similar low values in these two variables. These interpretations are in close agreement with Table 1 and contrast to the wrong conclusions that can be withdrawn from conventional PCA analysis, as discussed in previous sections.

Another example previously discussed (section 5.3) relates to the samples' sulfur (*S*) contents. It is now obvious that green bean sample units, whether cooked (*c*) or raw (*r*), display similar low *S* contents, from 0 to slightly above 0.2%, while cabbage samples (Cc and Cr) show S levels ranging from 0.4% to 1%, again in agreement with Table 1 and in contrast to erroneous conclusions withdrawn from conventional PCA.

These biplots also have disadvantages. A first obvious disadvantage is that as the dimension of the **X** matrix increases (increasing the number of units, of variables, or both), the biplot tends to become very difficult to read. This aspect is evident in Figure 10. Gower and coworkers developed ways to mitigate this problem. Whenever the number of units is too high, they suggested the substitution of the full set of sample points by convex hulls, confidence circles, etc. When the number of variables is too high, they suggested interactive ways of highlighting groups of variables while fading others, in order to enable easier readings and interpretations.

A second disadvantage is that there is no selection of important variables. As all variables are projected in the principal component displays, there will be a natural tendency to face all variables as equally important, which is not the case in PCA. As a consequence, while important variables will show accurate readings, less important variables are likely to induce erroneous interpretations. A third disadvantage is that even important variables providing accurate readings may, in relation to some units, lead to inaccurate predictions, and these aspects are not controlled in these biplots.

Taking these disadvantages into consideration, Gower and coworkers proposed some measures of fit, namely: the "quality index" of a display and the "adequacy" of representation of each variable in the important PCA dimensions (Aldrich et al., 2004), as well as the "axis predictivities" and "sample predictivities" (Gardner and Le Roux, 2008). To understand these measures it is important to realize that starting with matrix **X**, principal components and latent vectors are obtained (respectively **Y** and **V**) and that, using the whole set of principal components and latent vectors matrices, the initial data matrix can be fully recovered. However, if a reduction in dimension is operated, obtaining reduced matrices $\mathbf{Y}_Q$ and $\mathbf{V}_Q$, it is not possible to recover the initial data matrix X, but only an approximation $\widehat{\mathbf{X}}$ in Q dimensions. This is illustrated as follows:

$\mathbf{Y} = \mathbf{XV}$ is the complete set of P principal components
$\mathbf{X} = \mathbf{YV}^{\mathrm{t}}$ given **Y** and **V**, the initial matrix **X** can be completely reconstituted
$\widehat{\mathbf{X}} = \mathbf{Y}_Q\mathbf{V}_Q^{\mathrm{t}} = \mathbf{XV}_Q\mathbf{V}_Q^{\mathrm{t}}$ is the approximation of **X** based in just Q dimensions

Based on these facts several measures of fit were developed (Aldrich et al., 2004;, Le Roux and Gardner, 2005; Gardner et al., 2008; Gardner and Le Roux, 2003) of which some are here highlighted:

$\sum_{q=1}^{Q} \mathrm{t}_q / \sum_{q=1}^{Q_{max}} \mathrm{t}_q$ overall quality index
$\mathrm{diag}(\mathbf{V}_Q\mathbf{V}_Q^t$ variable adequacies
$\mathrm{diag}\left(\widehat{\mathbf{X}}^{\mathrm{t}}\widehat{\mathbf{X}}\right)\{\mathrm{diag}(\mathbf{X}^{\mathrm{t}}\mathbf{X})\}^{-1}$ axis predictivities
$\mathrm{diag}\left(\widehat{\mathbf{X}}\,\widehat{\mathbf{X}}^{\mathrm{t}}\right)\{\mathrm{diag}(\mathbf{XX}^{\mathrm{t}})\}^{-1}$ sample predictivities

Detailed demonstrations and specific software, with a special reference to an R function that calculates these measures of fit called PCA.predictivities( ), can be found in Gower et al. (2011). These measures enable the practitioner to evaluate the predictive power of variables, as well as how well any given sample is accounted for in the approximation of **X** in the Q dimensions. The disadvantage of these measures of fit is that take into consideration the whole set of variables, axes, sample and global information, although Gower and coworkers also suggested the

reduction in the number of biplot axes visible in a display on the basis of their respective axis predictivities.

9. Autobiplots

Following the works of Gower and coworkers, Alves (2012) defined the concept of a sample's standardized predictive error, indicated as *spe* or $\varepsilon_{(n,p)}$, as the absolute difference between the real value of sample $\mathbf{u}_n$ in variable $\mathbf{x}_p$ and the predicted value, as illustrated in Figure 11a and b. Figure 11a shows the orthogonal projection of sample $\mathbf{u}_n$ onto the $\mathbf{x}_p$ predictive axis, obtaining a reading identified as $u_{pr(n,p)}$, which is ≃27. In this figure it is also shown that the true value of sample $\mathbf{u}_n$ in $\mathbf{x}_p$ variable is 40. Figure 11b illustrates the meaning of the predictive error, $\varepsilon_{(n,p)} = |27-40| = 13$. It is important to note that this is a standardized predictive error because the initial data matrix X is standardized to mean zero end unit variance. This error $\varepsilon_{(n,p)}$ can be converted to an absolute error by multiplying it by the variable's standard deviation. These measures are outlined as follows:

$u_{(n,p)}$ is the real value of sample $\mathbf{u}_n$ in variable $\mathbf{x}_p$
$u_{pr(n,p)}$ is the predicted value of sample $\mathbf{u}_n$ in variable $\mathbf{x}_p$ as read in the biplot
$\varepsilon_{(n,p)} = \left|u_{pr(n,p)} - u_{(n,p)}\right|$ is the standard predictive error

In order to automate the process of calculating the standard predictive errors it is enough to see that the reading that is done by the user of the biplot, if done accurately, can be calculated as:

$\mathbf{V}_{[i,j]}$ is a matrix with two latent vectors $\mathbf{v}_i$ and $\mathbf{v}_j$ of interest
$\mathbf{v}_{(p)}$ is a row of matrix $\mathbf{V}_{[i,j]}$ corresponding to variable $\mathbf{x}_p$
$u_{pr(n,p)} = \mathbf{u}_n\mathbf{V}_{[i,j]}\mathbf{v}_{(p)}^t$ is the exact value of the prediction (orthogonal projection)
$\varepsilon_{(n,p)} = \left|u_{pr(n,p)} - u_{(n,p)}\right| = \left|u_{(n,p)} - \mathbf{u}_n\mathbf{V}_{[i,j]}\mathbf{v}_{(p)}^t\right|$ is the unit's *spe*

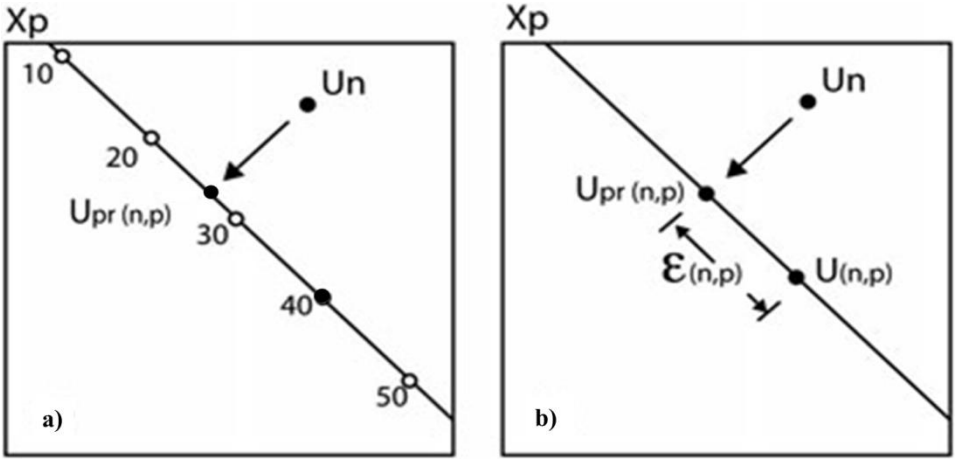


Figure 11: a) Example of a predictive biplot, showing the meaning of predicted value, $u_{pr(n,p)}$, and true value, $u_{(n,p)}$; b) Illustration of the concept of standard predictive error, $\varepsilon_{(n,p)}$.

Once the predicted value of any unit in relation to any given variable can be calculated in relation to any pair of components $\mathbf{y}_i$ and $\mathbf{y}_j$, a mean standard predictive error (*mspe* or $\bar{\varepsilon}_p$) for variable $\mathbf{x}_p$ in the plane of components $\mathbf{y}_i$ and $\mathbf{y}_j$, can be calculated as:

$$\bar{\varepsilon}_p = N^{-1} \times \mathbf{1}^t \left| \mathbf{x}_p - \left(\mathbf{X}\mathbf{V}_{[i,j]} \mathbf{v}_{(p)}^t \right) \right|$$

It is important to emphasize that this mean error, $\bar{\varepsilon}_p$, is the average error of prediction of all N units in relation to variable $\mathbf{x}_p$ in just a given two-dimensional principal component biplot. Therefore it can be used to decide whether the $\mathbf{x}_p$ biplot axis shall, or shall not, be drawn in the plot. For that, if a tolerance value, represented as T_{axis}, is defined, it is easy to automate the process of biplot construction:

$$\text{if } \bar{\varepsilon}_p \leq T_{axis} \rightarrow \text{ biplot axis for } \mathbf{x}_p \text{ is drawn in the biplot}$$
$$\text{if } \bar{\varepsilon}_p > T_{axis} \rightarrow \text{ biplot axis for } \mathbf{x}_p \text{ is not drawn in the biplot}$$

Using the same T_{axis} for all variables and for all $\mathbf{y}_i$ *vs* $\mathbf{y}_j$ biplots, the decision on which variables shall be incorporated in a biplot is carried out automatically, and the biplot user knows that all variables appearing in the biplot will provide readings whose accuracy is controlled through a pre-specified tolerance value. For example, if T_{axis} is set to 0.5, one knows that standard prediction errors, in average, will be at the most equal to 0.5. If the user finds out that the tolerance value is too low, then increasing T_{axis} will enable

more biplot axes to be introduced in the biplot at the cost of a decrease in accuracy. Therefore, it is up to the user to decide on the degree of precision, but once decided, the rest of the analysis is automatic.

Of course, using a mean standard predictive error, $\bar{\varepsilon}_p$, to control a biplot axis has one problem: it may happen that for the majority of units the standard predictive errors, $\varepsilon_{(n,p)}$, in relation to variable $\mathbf{x}_p$ are quite low, but one or just a few units may have unacceptable errors which are hidden by the mean value. Therefore, it is very helpful to decide upon a unit's tolerance value, T_{units}. Any $\varepsilon_{(n,p)}$ higher than T_{units} is faced as an outlier (in relation to the biplot objectives) a warning can be produced:

$$\text{if} \quad \varepsilon_{(n,p)} > T_{units} \rightarrow \quad \text{warning of outlier unit}$$

$$\text{if} \quad \varepsilon_{(n,p)} \leq T_{units} \rightarrow \quad \text{no warning is produced}$$

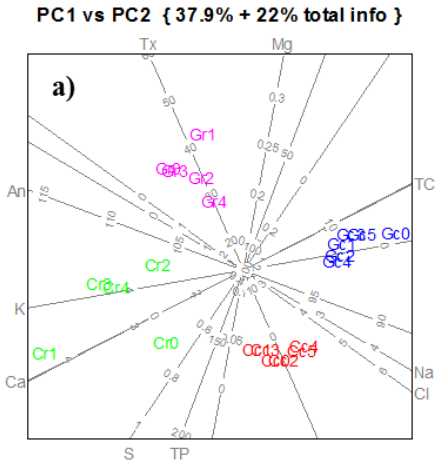
In this way not only biplots are automatically produced, as well as a list of outlier warnings can also be produced highlighting all possible units for which predictions are exceeding a predefined value.

10. AutoBiplot.PCA function

A function was written using the R language (Alves, 2012) and is available in version 6 from the Wiley site (http://onlinelibrary.wiley.com/doi/10.1002/cem.2433). The version used in this chapter is version 8 and includes improvements in sample units display, with the possibility to define groups that, although not taking part in the biplot algorithm, can be used to control the appearance of sample units by changing colours, constructing ellipsoids, confidence circles or convex hulls, or just controlling if points or labels are used to indicate units in the biplot. In many circumstances, these improvements followed the suggestions of Gower and coworkers implemented in their UBbipl function (available from www.wiley.com/go/biplots), which can be quite helpful for interpretation purposes.

Figures 12 (a and b) and 13 (a and b) show the result of using the AutoBiplot.PCA function applied to the case study. In Figures 12 a T_{axis} value of 0.6 was used, while in Figures 13 the T_{axis} value was decreased to 0.4. Concentrating on Figure 12a, the biplot based on principal components $\mathbf{y}_1$ *vs* $\mathbf{y}_2$ is shown. The amount of information corresponds to the latent values t_1 and t_2 expressed in percentage. Two tables come together with the

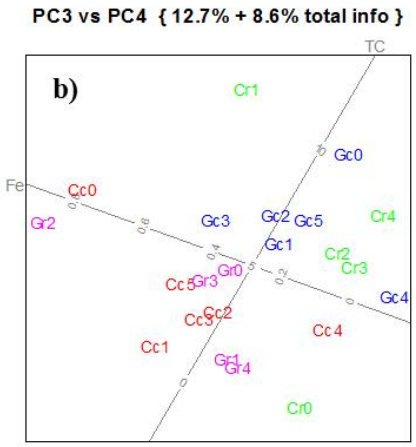
biplot: (i) an upper table showing standard deviations (*SDevs*) and mean standard predictive errors (*Axis.mspe*) for all variables; (ii) a lower table listing what units are faced as outliers and the respective biplot axis, based on a T_{units} = 1. For example, variable *Na* has a low mean standard predictive error ($\bar{\varepsilon}_4$= 0.244) and is thus displayed in the biplot. Because this error is very small, it is assumed that all readings are very accurate. However there is a warning for an outlier (unit Gc0), meaning that the value that is read off in the plot is inaccurate. Taking into consideration that this variable's standard deviation is 1.49, and because the standard predictive error (*Unit.spe*) is indicated as 1.028, the reading error is 1.028 × 1.49 = 1.53. Checking the biplot, drawing an orthogonal line from the Gc0 unit to the *Na* axis, a value of ≃3.3 is obtained, while in Table 1 the true value is seen to be 1.85. Hence, the 1.53% absolute error.




```
Evaluation of biplot axes: PC 1 vs PC 2

   SDevs Axis.mspe    action
Ca  1.47     0.3985 Draw axis
P   0.12     0.6546   Deleted
Mg  0.11     0.5672 Draw axis
Na  1.49     0.2440 Draw axis
K   1.66     0.3416 Draw axis
Fe  0.28     0.7245   Deleted
Cl  1.93     0.2962 Draw axis
S   0.29     0.2541 Draw axis
Tx 16.93     0.3556 Draw axis
W   0.78     0.6030   Deleted
tc  6.82     0.4661 Draw axis
TP 48.21     0.5307 Draw axis
An  7.60     0.4437 Draw axis
TF 75.50     0.6125   Deleted

Evaluation of outliers: PC 1 vs PC 2

  BiplAxis Outlier Unit.spe
1       Ca     Cr1 1.320593
2       Mg     Cr2 1.052151
3       Mg     Gc0 1.773512
4       Na     Gc0 1.027839
5        K     Cr1 1.472961
6       Tx     Gr1 1.361540
7       tc     Gc0 2.829120
8       TP     Cr3 1.041794
9       An     Cr1 1.083079
```

Figure 12a: Predictive biplot relative to the case study, based on components $\mathbf{y}_1$ *vs* $\mathbf{y}_2$, produced by function AutoBiplot.PCA, showing the biplot on the right hand-side and, on left hand-side, an upper table with information on mean standard predictive errors and a lower table with information on standard predictive errors and outlier warnings. The T_{axis} value was 0.6.

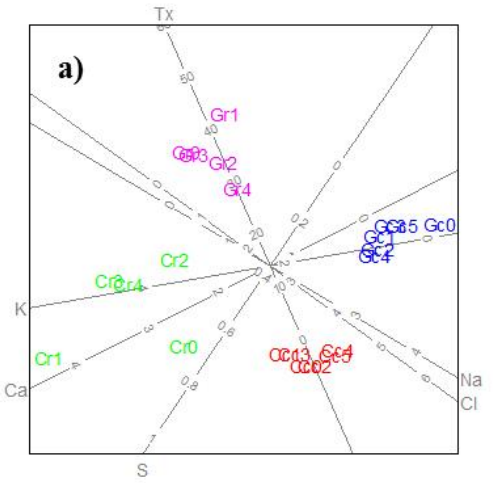
In Figure 12b, corresponding to components $\mathbf{y}_3$ *vs* $\mathbf{y}_4$, only two biplot axes are displayed, and one, *TC*, shows four outliers. Since the T_{units} value was set to a high value, this is not a good result, and probably the T_{axis} value is too high.




```
Evaluation of biplot axes: PC 3 vs PC 4

   SDevs Axis.mspe    action
Ca  1.47    0.6447   Deleted
P   0.12    0.6942   Deleted
Mg  0.11    0.7072   Deleted
Na  1.49    0.9356   Deleted
K   1.66    0.6942   Deleted
Fe  0.28    0.3887 Draw axis
Cl  1.93    0.9153   Deleted
S   0.29    0.8858   Deleted
Tx 16.93    0.7316   Deleted
W   0.78    0.6321   Deleted
tc  6.82    0.5266 Draw axis
TP 48.21    0.7102   Deleted
An  7.60    0.8556   Deleted
TF 75.50    0.7007   Deleted

Evaluation of outliers: PC 3 vs PC 4

  BiplAxis Outlier Unit.spe
1       tc     Cr1 1.476366
2       tc     Cr2 1.008580
3       tc     Cr4 1.297979
4       tc     Gc0 3.158246
```

Figure 12b: Predictive biplot relative to the case study, based on components $\mathbf{y}_3$ *vs* $\mathbf{y}_4$, produced by function AutoBiplot.PCA, showing the biplot on the right hand-side and, on left hand-side, an upper table with information on mean standard predictive errors and a lower table with information on standard predictive errors and outlier warnings. The T_{axis} value was 0.6.

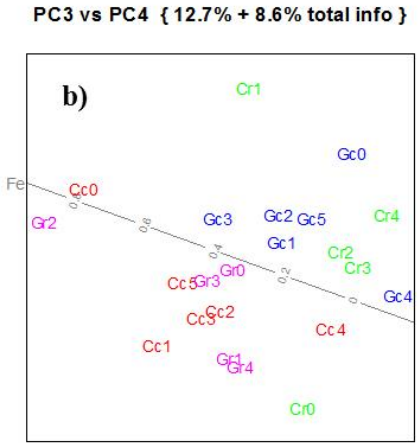
Observing Figures 13 (a and b) the effect of reducing the T_{axis} value from 0.6 to 0.4 can be observed. The number of biplot axis was reduced from 10 to 6 in the plane of $\mathbf{y}_1$ *vs* $\mathbf{y}_2$ and from 2 to 1 in the plane of components $\mathbf{y}_3$ *vs* $\mathbf{y}_4$. In the first biplot the number of outliers is much smaller, an effect that is expected because variables with high *mspe* were removed. In the second biplot only one variable is displayed with no outliers.




```
Evaluation of biplot axes: PC 1 vs PC 2

   SDevs Axis.mspe    action
Ca  1.47    0.3985 Draw axis
P   0.12    0.6546   Deleted
Mg  0.11    0.5672   Deleted
Na  1.49    0.2440 Draw axis
K   1.66    0.3416 Draw axis
Fe  0.28    0.7245   Deleted
Cl  1.93    0.2962 Draw axis
S   0.29    0.2541 Draw axis
Tx 16.93    0.3556 Draw axis
W   0.78    0.6030   Deleted
tc  6.82    0.4661   Deleted
TP 48.21    0.5307   Deleted
An  7.60    0.4437   Deleted
TF 75.50    0.6125   Deleted

Evaluation of outliers: PC 1 vs PC 2

  BiplAxis Outlier Unit.spe
1       Ca     Cr1 1.320593
2       Na     Gc0 1.027839
3        K     Cr1 1.472961
4       Tx     Gr1 1.361540
```

Figure 13a: Predictive biplot relative to the case study, based on components $\mathbf{y}_1$ *vs* $\mathbf{y}_2$, produced by function AutoBiplot.PCA, showing the biplot on the right hand-side and, on left hand-side, an

upper table with information on mean standard predictive errors and a lower table with information on standard predictive errors and outlier warnings. The T_{axis} value was 0.4.




```
Evaluation of biplot axes: PC 3 vs PC 4

   SDevs Axis.mspe    action
Ca  1.47    0.6447   Deleted
P   0.12    0.6942   Deleted
Mg  0.11    0.7072   Deleted
Na  1.49    0.9356   Deleted
K   1.66    0.6942   Deleted
Fe  0.28    0.3887 Draw axis
Cl  1.93    0.9153   Deleted
S   0.29    0.8858   Deleted
Tx 16.93    0.7316   Deleted
W   0.78    0.6321   Deleted
tc  6.82    0.5266   Deleted
TP 48.21    0.7102   Deleted
An  7.60    0.8556   Deleted
TF 75.50    0.7007   Deleted

Evaluation of outliers: PC 3 vs PC 4

data frame with 0 columns and 0 rows
```

Figure 13b: Predictive biplot relative to the case study, based on components $\mathbf{y}_3$ *vs* $\mathbf{y}_4$, produced by function AutoBiplot.PCA, showing the biplot on the right hand-side and, on left hand-side, an upper table with information on mean standard predictive errors and a lower table with information on standard predictive errors and outlier warnings. The T_{axis} value was 0.4.

Conjugating Figures 12 and 13, it is apparent that the second is a better solution, with a clear, easily interpretable biplot, with an expected low number of wrong assumptions. It is also seen that irrespective of using a Taxis of 0.6 or 0.4, the plane of $\mathbf{y}_3$ *vs* $\mathbf{y}_4$ has little interest, because interpreting a lane with one or two variables is always worse than interpreting the variables just by themselves. This shows that auto biplots can also be used to help deciding on how much reduction on the data can be done, i.e., on how many components to keep in the analysis.

11. Conclusions

Several problems related to PCA and attempts to solve them were discussed in this chapter. It was shown that although being widely used, PCA has some problems that may present difficulties for researchers lacking statistical or mathematical backgrounds. It was also shown that biplots are a very useful tool, that can be used to simplify the analysis and interpretation of PCA results. However, as it was demonstrated, if the data matrices have a high dimensionality, biplots will contain too much information and will become difficult to use. Some methodologies have been devised by several researchers in order to help

reducing variables or sample points in the PCA biplot displays, rendering them simpler, but many of these methodologies do not provide satisfactory results.

Function AutoBiplot.PCA was applied to the case study. The case study is a somewhat difficult case because correlations between data variables were relatively small. Nevertheless, the AutoBiplot.PCA function proved to be capable of dealing with the case study with no problem. Once a tolerance value for the mean standard predictive error is defined, the rest of the analysis is automatic. The user can change the tolerance value, but any change will affect the whole analysis, in such a way that the outputs are not subjected to the researcher's beliefs or wills.

As the function was written in the R environment, it is available to any interested user, since R is free and can be readily downloaded from the site of the R statistical project.

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IV Effect of High CO2 Modified Atmosphere Packaging on Quality, Safety and Acceptability of Fresh-cut Vegetables

Carla Barbosa, M. Rui Alves, S. Fonseca, L. Silva, J. Miranda e M. Beatriz P.P. Oliveira

Neste artigo, o principal objetivo foi avaliar a possibilidade de atribuir um prazo de validade mais alargado a legumes minimamente processados. Com esta informação, a indústria e os produtores poderão usufruir de mais alguns dias de armazenamento, o que facilitará as operações de logística, sem por em questão a qualidade sensorial, nutricional e a segurança dos produtos. Para além da logística, o facto de os produtos apresentarem mais algum tempo de validade, seguramente, levará à redução do desperdício. No caso específico das cantinas, permite que se disponibilize uma maior variedade de produtos prontos-a-consumir, minimizando perdas e otimizando recursos materiais e humanos.

Para tal, o trabalho desenvolvido experimentalmente consistiu em:

- seleção de atmosferas adequadas à conservação de uma variedade de legumes (couve coração, feijão verde, cenoura e pimentos verde, vermelho e amarelo) minimamente processados, embalados em AM e refrigerados;
- monitorização de parâmetros organoléticos, físico-químicos (atividade antioxidante, pH, acidez, humidade, cinzas, cor e textura) e crescimento microbiano ao longo do tempo de armazenamento, mais alargado que o habitualmente atribuído pela indústria (17 dias);
- avaliação da aceitabilidade;
- avaliação do comportamento dos legumes selecionados, MP e embalados em AM e dois tipos de embalagem (filmes com diferentes características de permeabilidade).

Procurou-se com este trabalho discutir a possibilidade de usar um mesmo sistema de embalagem para uma variada gama de produtos (diferentes cores e com taxas de respiração diferentes), simulando as práticas industriais. Os resultados obtidos permitem discutir a possibilidade de alargamento de prazo de validade, sem colocar em risco a qualidade e segurança dos produtos em estudo.


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Submitted

Effect of High CO_2 Modified Atmosphere Packaging on Quality, Safety and Acceptability of Fresh-cut Vegetables

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Abstract: The objective of the work was to extend the shelf life of fresh-cut vegetables, without safety concerns. For that the evolution of physicochemical parameters (antioxidant activity, pH, acidity, moisture, ash, colour, and texture), microbial growth, sensory analysis and acceptability were assessed. Samples (cabbage, carrots, green beans, and green, red and yellow peppers) stored at 5 ºC under modified atmosphere packaging conditions (10, 15 % O_2 and 40, 45 % CO_2), over 10 days in a first experiment, were monitored and compared with the same products in air. Results showed small changes over storage time without a consistent tendency. The best O_2/ CO_2 combination was 10/45 (assessed along 17 days in a second experiment) presenting negligible decrease in antioxidant activity, without decrease in organoleptic properties and acceptable microbial growth. At the end of the study, four days to vegetables shelf time was achieved which is considered advantageous for industry.

Keywords: modified atmosphere packaging, fresh-cut vegetables, sensory evaluation, antioxidant activity, consumer acceptability, shelf life extension

1. Introduction

The consumption of vegetables and fruits due to their protection properties against several chronic diseases like cancer, cataract and macular degeneration is recommended (Flood, et al., 2002; Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009; Murcia, Jiménez-Monreal, García-Diz, Carmona, Maggi, & Martínez-Tomé, 2009; Roy, Takenaka, Isobe, & Tsushida, 2007). They are important sources of micronutrients and bioactive compounds with antioxidant activity, being also important to maintain organoleptic characteristics during shelf life (Jiménez-Monreal, et al., 2009).
An important issue related to vegetable's quality is the demand for ready-to-eat products, usually known as minimally processed. They are cut in small pieces, leaving dilacerated surfaces exposed to the deleterious action of oxygen, with possible loss of nutritional value and bioactive compounds. These possible modifications need to be studied and evaluated.

Fresh and cooked vegetables are popular side dishes in Mediterranean and Southern European Atlantic diets (Guallar-Castillon, Oliveira, Lopes, Lopez-Garcia, & Rodriguez-Artalejo, 2013). In Portugal, as a consequence of its geographical situation, aspects of both Atlantic and Mediterranean diets are present, with fresh vegetables being consumed mainly in summer, while cooked vegetables are preferred in colder seasons. The small size of Portuguese producing areas and the variety of products in each area enhance quality, but make logistic operations complex and tend to rise prices. Thus, some work carried out in order to extend products' shelf lives, allowing for a better rationalization of harvesting and post harvesting procedures as well as packaging and distribution, while satisfying consumer's demand for freshness and high quality standards is of utmost importance.

Minimal processing (MP) involves careful selection, cutting and washing/disinfection of vegetables, followed by packing, in a more or less permeable package. Due to continuing metabolism reactions, such as respiration, it will promote alterations of the surrounding atmosphere then one are talking about modified atmosphere packaging (MAP) in a passive way. If packaging procedures involves substitution of the atmosphere by another with a gas composition different from air, then this is active MAP (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007).

In MAP, decreasing O_2 levels and increasing the CO_2 content lead to a decrease in respiration rates, delaying senescence and reducing enzymatic browning, softening and microorganism growth, while retaining vegetable freshness (Murcia, et al., 2009; Oms-Oliu, Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008). These beneficial effects will hold as long as the CO_2 concentration does not become excessively high. CO_2 in excess has been reported to solubilize very rapidly in the product, promoting undesirable

degradation reactions with off-odour production, and causing an increase in acidity, with a consequent loss of typical flavour, texture and colour. Therefore, as CO_2 diffuses through the plastic films 2 to 6 times faster than O_2, a selective permeability with an appropriate ratio CO_2/O_2 is needed to provide the right, harmless CO_2 concentrations without promoting injury (Devlieghere, Gil, & Debevere, 2002; Montanez, Rodríguez, Mahajan, & Frías, 2010; Oliveira, Sousa-Gallagher, Mahajan, & Teixeira, 2012; Rico, et al., 2007).

According to published data (Brecht, Chau, Fonseca, & Oliveira, 2003; Fonseca, Oliveira, & Brecht, 2002), numerous factors may affect the effectiveness of minimal processing with MAP: (i) the initial state of freshness; (ii) the handling care; (iii) the procedure used for cutting, blanching, washing and disinfection; (iv) the species/varieties of vegetables (different vegetables display different behaviours towards the same MAP conditions), mostly due to specific respiration rates; (v) the type of cut (e.g., small pieces/big pieces/intact foods); and (vi) the type of atmosphere.

In what concerns to the type of atmosphere, several statements and conflicting guidelines can be found in many papers concerning the optimum gas combinations to store MP vegetables in MAP (Alasalvar, Al-Farsi, Quantick, Shahidi, & Wiktorowicz, 2005; Jacxsens, Devlieghere, Van der Steen, & Debevere, 2001; Kader & Ben-Yehoshua, 2000; Murcia, et al., 2009). For example, while some authors propose mild atmospheres (3-5 % O_2 and 3-10 % CO_2 with N_2 adding up to 100 %), others proclaim the benefits of using O_2 concentrations as high as around 70 %. Many of the works referred in previous sections consider the influence of MAP in minimally processed foods based on a limited set of quality parameters (Saltveit, 2003).

This work aimed to explain how MAP preservation may influence the quality of MP vegetables during storage time, as measured by a comprehensive set of parameters, namely pH, colour change, total antioxidant activity, texture, microbial growth and some important organoleptic properties, including the perception of some defects, and consumer acceptability.

2. Materials and Methods

This work was divided in two studies: a *first study* designed to compare the effect of passive and different active MAP conditions on MP vegetables commonly used as side dishes in Mediterranean and South Atlantic diets, preserved for 10 days; a *second study* was carried out in order to determine how vegetables behave over longer periods of time (17 days) under the influence of a selected atmosphere. In order to determine the

conditions to be used in industrial and/or commercial practices, canteens students were used as a case study in what concerns consumer acceptability.

2.1. *Samples and quality analysis*

Cabbage (*Brassica oleracea* L.), carrots (*Daucus carota* L.), green beans (*Phaseolus vulgaris* L.) and peppers (red, green and yellow fruits of *Capsicum annum*), obtained in local farmers after harvesting were the samples of the described research. Old leaves, stems and dark spots were removed before cutting. Vegetables were cut in 0.5 cm slices (cabbage was shredded). Washing and disinfection were carried out with potable water and a disinfectant solution, 0.3% VERCLOR (sodium hypochloride 5.4- 6.0% active chloride), over 5 minutes (according to supplier's instructions - Soro Internacional, SA, Spain). Then samples were stored in MAP as described below. Each package contained about 200 g of vegetables occupying 2/3 of the bag volume.

2.1.1.*Packaging materials*

First study. Samples were packed in multilayer polystyrene (EPS/HIPS/PE) trays (B22-50) and sealed with a selective medium barrier Technopack film (Pa/Pe, Miranda e Serra, Portugal).

S*econd study*. Vegetables were packed in Krehalon MLF40 (PA/PE) bags, with O_2 and CO_2 permeability of 90-130 cm^3/m^2 24 h and 750-850 cm^3/m^2 24 h, respectively.

2.1.2. *Modified atmosphere packaging equipment*

A Yang SR-I Oceania Jolly 20 thermossealer (Italy) was used for package sealing. Inside packaging atmosphere was created by a PBI Dansensor A/SMAP Mix 8000 EL (Denmark) gas mixer. Inside gas composition was verified with a gas analyser (O_2/CO_2 Checkmate II, PBI Dansensor, Ringsted, Denmark) all sampling days.

2.1.3. *Atmospheres, temperatures and storage times*

First study: Three atmospheres were tested. In terms of percentage of O_2 and CO_2 (with N_2 balanced to 100%) these atmospheres were 10/45, 15/45 and 15/40 (% O_2/ % CO_2). After packaging, samples were stored at 5 ºC for 10 days. Physicochemical and microbial growth evaluations were carried at the beginning of the experiments (day 0) and at 6th, 8th and 10th days after packaging. Sensory evaluation was performed at the 8th day.

Second study: For the development of this part of the work one of the atmosphere assayed in the 1st study was chosen (10% O_2/45% CO_2). Samples were kept at 5 ºC, and physicochemical, microbial and sensory parameters were evaluated at 0, 5, 10, 14 and 17 days after packaging.

2.1.4. *Sample preparation and physicochemical evaluation*

After opening the package, samples firmness and colour were determined at each sampling day. Colour measurements (n=10) were carried out with a MINOLTA CR300 (Konica Minolta, USA) and the total colour variation ($\Delta Eab=\sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$) determined using the Hunter Lab colour system. Firmness, a texture parameter defined as the maximum forced exerted in compression, was determined (n=20) with a TA-XT2i (Stable Micro Systems Ltd., U.K.) equipped with a 2 mm diameter cylindrical stainless steel probe and a compression speed of 1 $mm.s^{-1}$. Results were expressed as the median of all measurements.

To evaluate acidity, pH, moisture and ash contents, samples were grinded in a knife mill (GM 200, RETSCH, Haan, Germany). The pH evolution was monitored using a FC232 electrode coupled to a potentiometer HI99163 HANNA Instruments (USA) as described in the 981.12 AOAC method (AOAC, 2000). Acidity was determined by titration with 0.1N NaOH and the results expressed as g citric acid/100 g fresh weight, according to the official AOAC method 942.15 (AOAC, 2000). Moisture content was determined according to the AOAC 984.25 method (AOAC, 2000), in triplicate and results expressed as %. Briefly about 3 g of homogenized sample were dried at 103±2 ºC (UTG, Heroeus) to constant weight.

The ash content was determined by a gravimetric method (AOAC, 2000) by incineration of 3g of homogenized sample in a Heraeus muffle at 550 ºC, until complete carbonization. Determinations were performed in triplicate and the results expressed as percentage (%).

For the evaluation of the antioxidant activity (AA) the vegetables in study were freeze-dried and stored until analysis. Aqueous extracts were prepared with 1-3g of freeze-dried samples. The evolution of the AA contents over storage time was determined by DPPH radical-scavenging assay as described by Brand-Williams, Cuvelier, and Berset (1995). The aqueous extracts were added to a 50% MeOH solution and absorbance was determined after 30 minutes at 515 nm. The percentage of inhibition was calculated afterwards. Results were expressed as the antioxidant concentration needed to decrease the initial DPPH concentration by 50%, expressed as EC_{50} (labelled in literature also as IC_{50}). The calculation of EC_{50} value requires the determination of the reaction kinetics between DPPH and the antioxidant. The percentage of remaining DPPH against the sample concentration was plotted to obtain EC_{50} (Sánchez-Moreno, A. Larrauri, & Saura-Calixto, 1999).

2.2. *Microbial evaluation*

Microbial evaluation was performed just as indication of the deterioration extent based on microorganisms-colony-count technique at 30 ºC. Sampling was done in aseptic conditions according to ISO 6887-1:1999 (ISO, 1999). Small portions from different zones of the package were used to prepare the test sample for microbiological analysis. A mother suspension (10^{-1}) was prepared with 10 g of product sample. It was introduced in a sterile "Stomacher" bag, mixed with 90 ml sterilized Triptone salt solution, and homogenized for 1 minute in a Stomacher 400 (Seward, BA7021, UK). Decimal dilutions were prepared, 1ml of each pour plated on PCA medium (Plate Count Agar -Oxoid) and incubated at 30 ºC ± 1 ºC for 72 h ± 3 h, as recommended by ISO 4833:2003 (ISO, 2003).

2.3. *Sensory evaluation*

2.3.1. *Panel training*

Before the evaluation sessions, panel judges focused on the typical characteristics of fresh vegetables and also on the presence of defects caused by senescence. Fresh samples, and samples kept in air at 5ºC for different periods of time were used by judges in several discussion/training sessions, in order to understand the deterioration process and its effects on sensory quality. A list of attributes was also decided by consensus, containing the following attributes:

First study: general appearance, colour, odour, taste and texture (as hardness and mouth feel perception).

Second study: general appearance (including surface moisture; typical colour; browning, brightness; texture/shape), texture measured with forks (including elasticity, cohesiveness, hardness), aroma (typicity, intensity); texture during mastication (firmness at first bite, firmness at second bite), flavour and taste (including flavour typicity, taste intensity, sourness, bitterness, sweetness).

2.3.2. *Quantitative descriptive analysis (QDA)*

The sensory evaluation followed the QDA® methodology (Tragon Corporation, San Francisco, USA), as described by several authors (Meilgaard, Civille, & Carr, 2007; Stone & Sidel, 2004) and implemented in the Polytechnic Institute of Viana do Castelo (IPVC) following the methods of Alves and Oliveira (2005).

First study. Sensory evaluation was performed by a 7 member trained panel, on the 8th day of storage. The list of attributes with 9 point scales was used.

Second study. Products were evaluated by a 15 member trained panel, using the same attributes as before, but with 13 point scales. In all sessions, fresh-cut vegetables were presented as standards. Judges were instructed to start any session by tasting the standard

and consider point 7 of each attribute's scale as the standard's magnitude perceived. In this way, in all sessions and for all judges, evaluations were made against a fresh standard, reducing randomness in judgements.

2.3.3. *Acceptability studies*

Consumers' acceptability was evaluated on the 8th day of storage. Samples were tasted by 28 to 30 consumers to assess acceptability on a 9 point hedonic scale (Peryam & Pilgrim, 1957). Each consumer tasted only one sample. Consumer's evaluations were carried out during meals at lunch time at the IPVC canteen.

2.4. *Sample coding*

Samples were coded, in all tables and figures, as follows:

- Vegetable names represented by the first two letters (Cb = cabbage; Ct = carrots; Gb= Green Beans; Gp=Green peppers; Rp=Red peppers Yp=Yellow peppers)
- Storage or sampling time represented by numbers.
- Composition of the atmosphere is represented, only in the case of the *first study* by x, y, z and n, corresponding to a gas mixture of 10/45, 15/45, 15/40 and air (normal atmosphere), respectively.

2.5. *Statistical analysis*

Data mining was carried out with principal component analysis (PCA) to evaluate the main physicochemical characteristics and the way they changed with time. Kruskal-Wallis, Wilcoxon /Mann-Whitney, and canonical variate analyses (CVA) were used to evaluate, respectively, consumer's and judge's results, with groups representing products and judge's or consumer's opinions used as replications for each product. CVA enabled analysis and visualization of the significance of observed differences between products (Barbosa, Oliveira, & Alves, 2011). ANOVA, MANOVA, CVA and PCA were carried out using the Statistica for Windows software package, version 7 (Stat Soft Inc., Tulsa, USA).

3. Results

3.1. *First study - influence of MAP conditions*

As referred above, a first study was carried out in order to compare the effect of packaging under air (normal atmosphere), against selected, mild modified atmospheres, with 10% and 15% O_2 combined with 40% and 45% CO_2. The main objective was to verify if an initial active atmosphere could retard senescence and microbial growth. The film permeability used was low, in order to avoid gas exchanges between package and surrounding atmosphere, thus being able to observe the effect of the modified atmospheres on the quality parameters as well as the gas composition changes over time (Table 1).

Table 1 Evolution of physicochemical parameters and microbial growth of fresh-cut vegetables (cabbage and carrots) minimally processed, stored at 5ºC, under different MAP conditions (combinations of 10 and 15% O_2 with 40 and 45% CO_2 and N_2 to complete) and air, along different storage periods (0, 6, 8 and 10 days).

Sample	MAP %O_2/%CO_2	Codes	Time days	EC_{50} mg/ml	pH	CFU g^{-1}	ΔEab	% O_2	% CO_2	% N_2
Cabbage	10/45	Cb0	0	18.02	5.87	1.8E+01	-	10.8	45.6	43.6
		Cb6x	6	50.64	6.27	1.3E+03	3.34	0.5	56.3	43.2
		Cb8x	8	56.20	6.77	2.6E+03	1.88	0.0	57.6	42.4
		Cb10x	10	58.86	6.87	4.4E+03	2.30	0.0	59.2	40.8
	15/45	Cb0	0	18.02	5.87	1.8E+01	-	15.1	45.8	39.1
		Cb6y	6	35.46	6.33	1.2E+03	5.05	5.6	50.5	43.9
		Cb8y	8	41.58	6.96	2.3E+03	3.81	2.3	59.0	38.8
		Cb10y	10	46.61	7.02	3.9E+03	2.42	0.1	62.6	37.3
	15/40	Cb0	0	18.02	5.87	1.8E+01	-	15.7	40.8	43.5
		Cb6z	6	40.70	6.26	9.6E+02	2.76	4.8	54.2	41.0
		Cb8z	8	53.33	6.81	1.8E+03	6.51	1.9	58.2	39.9
		Cb10z	10	59.13	6.9	3.3E+03	2.35	0.0	60.8	39.2
	Air	Cb0	0	16.18	5.79	1.8E+01	-	20.9	0.0	79.1
		Cb6n	6	17.90	6.31	6.8E+03	2.58	10.5	7.8	81.6
		Cb8n	8	19.86	6.92	1.9E+04	3.64	7.2	13.9	78.9
		Cb10n	10	22.11	7.07	5.2E+04	1.18	4.0	19.0	77.0
Carrots	10/45	Ct0	0	15.98	5.71	6.8E+01	-	15.4	45.9	38.7
		Ct6x	6	45.65	5.91	2.0E+03	5.14	4.1	48.0	47.9
		Ct8x	8	44.73	6.5	4.1E+03	3.03	1.5	51.0	47.5
		Ct10x	10	46.82	6.73	8.3E+03	1.91	0.0	54.6	45.4
	15/45	Ct0	0	15.98	5.71	6.8E+01	-	15.1	45.8	39.1
		Ct6y	6	44.64	6.08	2.6E+03	4.14	7.0	51.7	41.3
		Ct8y	8	45.56	6.54	5.1E+03	2.94	3.9	55.4	40.8
		Ct10y	10	47.67	6.86	8.9E+03	3.68	1.7	59.3	39.0
	15/40	Ct0	0	15.98	5.71	6.8E+01	-	10.7	40.5	48.8
		Ct6z	6	37.86	5.98	2.6E+03	3.01	5.5	41.3	53.2
		Ct8z	8	38.89	6.46	4.2E+03	1.84	2.2	47.1	50.8
		Ct10z	10	36.56	6.72	8.8E+03	1.68	0.0	52.1	47.9
	Air	Ct0	0	15.98	5.67	6.8E+01	-	20.9	0.0	79.1
		Ct6n	6	20.24	5.98	5.1E+03	3.97	14.2	5.5	80.3
		Ct8n	8	22.05	6.62	1.1E+04	4.58	9.4	12.9	77.7
		Ct10n	10	24.33	6.97	5.3E+04	3.54	7.0	18.3	74.7

Table 1 shows the results of the evaluated physicochemical parameters and microbial growth, as well as the gases evolution inside packages, along the 10 days of the experiment. Some conclusions could be withdrawn directly from the expressed values in the table, but they were analysed by principal component analysis (PCA). Figure 1 shows the plane of components 1 *versus* 2 (PC1 *vs* PC2), which contains more than 80% of the information of the Table 1, and helps to depict the main features of the results.

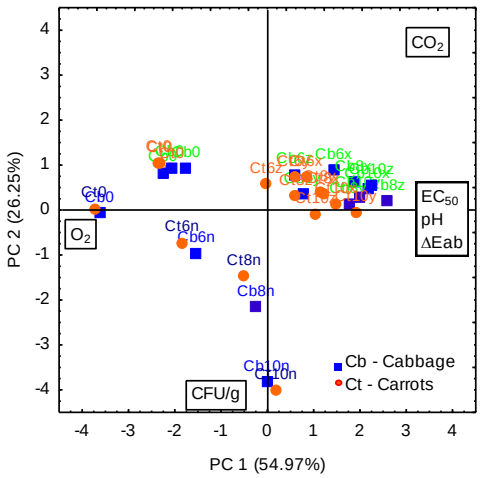

Figure1. Principal component analysis of physicochemical and microbial data from fresh-cut vegetables (cabbage and carrots) minimally processed, stored at 5ºC, under different MAP conditions (combinations of 10 and 15% O_2 with 40 and 45% CO_2 and N_2 to complete) and air, along different storage periods (0, 6, 8 and 10 days).

PC1, the horizontal axis of Fig. 1, represents the positive correlation between EC_{50}, CO_2, pH and colour change (increasing towards the right hand side of the graph), all negatively correlated with O_2 (increasing to the left side). Reminding that the lower the EC_{50} value, the higher the antioxidant activity, it is clear an initial loss of antioxidant capacity, which is positively correlated with respiration rates, and a slight increase in pH and colour change. As reported by Murcia, et al. (2009), the increase in CO_2 levels due to respiration rate, could decrease biologically active compounds, revealing a decrease in antioxidant activity as occurred in this study (EC_{50} increase). The magnitude of these correlations is shown in Table 2. For these reasons, samples at time 0 lie on the top left hand side of Fig. 1, while all the other samples tend to lie on the right hand side. These aspects are much less pronounced in vegetables packed in air, which spread downwards in Fig. 1. As PC2, the vertical axis, represents mainly the microbial growth variation (with counts increasing towards the bottom of the graph), it can be verified that the samples in normal atmosphere are the ones with the highest microbial proliferation. So, while a passive atmosphere tends to be less deleterious to nutritional quality, active atmospheres are much more efficient in what concerns microbiological control.

Table 2 – Table of correlations between physicochemical and microbial data from fresh-cut vegetables (cabbage and carrots) minimally processed, stored at 5ºC, under different MAP conditions (combinations of 10 and 15% O_2 with 40 and 45% CO_2 and N_2 to complete) and air, along different storage periods (0, 6, 8 and 10 days).

	EC_{50}	**pH**	**CFU/g**	**ΔEab**	**% O_2**	**% CO_2**
EC_{50}	1.000	-	-	-	-	-
pH	0.562	1.000	-	-	-	-
CFU/g	-0.157	0.518	1.000	-	-	-
ΔEab	0.525	0.469	0.112	1.000	-	-
% O_2	-0.854	-0.767	-0.154	-0.527	1.000	-
% CO_2	0.736	0.268	-0.348	0.168	-0.680	1.000

Another important conclusion is that after an initial variation in quality parameters, the stabilization of the modified atmospheres is quickly achieved, leading to an increase in shelf life. Figure 1 confirms all the facts discussed, showing an intermingling of points representing active MAP samples with times 6 to 10 days. According to Table 1 the best gas combination for carrots, concerning antioxidant activity behaviour, was 15/40. For cabbage, the samples stored under the atmosphere 15/45 had its antioxidant activity less affected during storage, in comparison with the other MAP conditions.

A high decrease on the EC_{50} values during the first 6 days of storage is evident in all tested samples under MAP conditions. This is a consequence of the vegetable's metabolic activity. However after this sampling day products reach stability as seen in Fig. 1 and Table 1 as a consequence of the atmosphere applied.

3.1.1. *Sensory evaluation*

Sensory results at the 8th storage day were analysed with canonical variates analysis (CVA). Each sample is faced as a group with judges' results for that sample as repetitions. A matrix was built with 21 rows (3 samples × 7 judges) and 5 columns (the 5 attributes described in section 2.7). Using well trained judges, a low dispersion means that all judges are in agreement, while a high dispersion means that judges do not recognize differences between samples and the observed differences are not significant. Fig. 2 shows the CVA results for carrots (2a) and cabbages (2b). In both cases, as there are only three groups, CVA develops only two canonical variates (CV1 and CV2), and therefore all main data structures are represented in Fig. 2a and 2b.

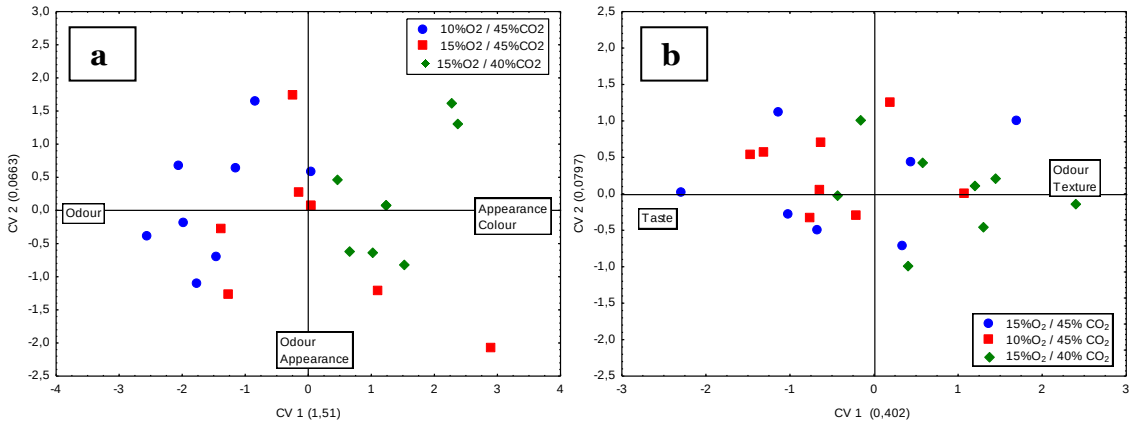

Figure 2. Canonical variates analyses of judges' appraisal of: a) fresh-cut carrots and b) fresh-cut cabbage, minimally processed, stored at 5ºC, on the 8th day of storage under 3 different atmospheres (combinations of 10 and 15% O_2 with 40 and 45% CO_2 and N_2 to complete).

For carrots (Fig. 2a), the MANOVA with a Wilk's Lambda of 0.3738 (p<0.0461) shows that judges discriminated the effects of different atmospheres on the organoleptic properties of the product. Judges were quite consistent, showing low dispersion along CV1 (horizontal axis), separating samples under 10/45 on the left hand side from samples under 15/40 on the right side hand, and samples under 15/45 spread along the variates. As shown in Fig. 2a, this gradient corresponds to a negative correlation between the changes in colour or appearance and typical odour. The samples stored under 10/45 were classified as the ones that have a more intense typical odour.

For cabbage, a MANOVA with a Wilk's Lambda of 0.66051 (p< 0.6441) shows that samples are faced as very similar. Graphically (Fig. 2b) the points are spreading left/right, with no group becoming distinct from the others, meaning that judges are not able to detect differences among the samples and scored them randomly.

Results of consumers' appreciation also showed that there are no significant differences among samples preserved under different atmospheres, as it was confirmed by Kruskal-Wallis tests. In the case of carrots, samples under 15/45, 10/45 and 15/40, each with 29 observations, were ranked 1072, 1505 and 1251, respectively, leading to a Kruskal-Wallis with H = 5.33 and p = 0.07, meaning that there is no significant difference in acceptability for any of the MAP conditions under study. Concerning cabbage the same conclusion can be extracted from consumers' acceptability test. Cabbage samples under 15/45, 10/45 and 15/40, ranked 1107.5, 1443.5 and 1277 (with 29, 28 and 30 observations), respectively,

resulting in no significant differences in acceptability according to a Kruskal-Wallis test (H= 4.13 p= 0.127).

From all results obtained with carrots and cabbage, it seems that the best gas combination concerning antioxidant activity changes (EC_{50}) was 15/40, but in the case of sensory evaluation the best one seems to be 10/45. So, due to the fact that the sensory evaluation is of utmost importance for acceptability of the product, the chosen combination for these two products was 10/45.

3.2. *Second study - time extent*

The second study included in this work aimed at analysing the behaviour of fresh-cuts during a longer storage time (17 days) and to extend the study to other vegetables, i.e., green beans and red, green and yellow peppers. Samples were under MAP of 10% O_2 and 45% CO_2, using Krehalon MLF40 (PA/PE) bags, a material more permeable to CO_2 then the commonly used low density polyethylene (LDPE) or medium density polyethylene (MDPE) (Manolopoulou, Xanthopoulos, Douros, & Lambrinos, 2010). The choice for this material aimed at exposing fresh-cuts to an initial impact caused by a relatively high CO_2 percentage, but allowing a posterior stabilization with less severe conditions due to CO_2 outward migration. This avoided undesirable anoxia conditions (Mir & Beaudry, 2004; Sandhya, 2010) as happened in the first study at the 10th day of storage in all samples, except for cabbage in MAP with 10/45 where this condition was attained after the 5th day.

3.2.1. *Physicochemical parameters*

Results obtained in this second study for physicochemical data are shown in Table 3. Correlations between variables were calculated and are shown in Table 4.

Table 3- Evolution of physicochemical parameters and microbial growth of fresh-cut vegetables (Gb-green beans; Rp-red pepper; Yp- yellow pepper; Gp – Green pepper; Ct-carrots and Cb-cabbage) minimally processed, stored at 5ºC, under MAP condition (10% O_2/45% CO_2 and N_2 to complete) along a storage periods of 17 days (0, 5, 10, 14 and 17 days).

Prod	Codes	Time (day)	EC_{50} (mg/ml)	pH	CFU g^{-1}	Acidity g/100 g	Ash %	W %	L	a	b	Firmness (N)	% O_2	% CO_2	% N_2
	Gb0	0	7.57	5.89	5.0E+02	0.11	0.60	94.93	42.91	-10.37	18.68	38.43	10.0	45.0	45.0
	Gb1	5	6.53	6.17	8.7E+02	0.08	0.45	95.05	37.60	-11.95	20.86	64.44	5.5	30.7	63.9
Gb	Gb2	10	7.86	6.15	1.7E+03	0.07	0.46	93.79	35.12	-12.27	21.18	40.73	7.8	22.6	69.6
	Gb3	14	11.19	5.93	1.6E+03	0.08	4.94	95.06	43.87	-12.85	22.40	33.26	10.2	19.0	70.8
	Gb4	17	12.57	6.14	3.0E+03	0.06	0.52	93.87	39.22	-11.38	19.87	27.34	13.8	9.7	76.5
	Rp0	0	1.14	5.26	1.4E+03	0.09	0.65	94.67	38.79	31.37	27.81	10.99	10.0	45.0	45.0
	Rp1	5	1.29	5.06	1.9E+04	0.17	0.56	92.88	38.99	33.73	26.35	26.98	17.5	6.6	75.9
Rp	Rp2	10	1.43	5.13	1.7E+05	0.08	0.33	93.46	41.32	30.02	26.40	11.51	15.7	7.9	76.4
	Rp3	14	1.86	4.92	6.3E+06	0.09	5.56	94.44	39.70	30.89	26.15	9.80	16.6	5.2	78.2
	Rp4	17	1.77	5.09	2.2E+07	0.08	0.59	93.67	37.61	28.79	25.36	10.96	11.9	12.6	75.5
	Yp0	0	1.70	5.28	5.9E+02	0.19	0.98	94.81	61.39	4.98	49.98	10.64	10.0	45.0	45.0
	Yp1	5	1.26	5.37	5.5E+02	0.14	0.70	93.84	54.51	-0.09	40.64	18.31	12.5	22.8	64.8
Yp	Yp2	10	1.43	5.17	9.5E+02	0.14	1.31	92.12	55.79	-0.09	46.47	10.07	18.5	3.4	78.1
	Yp3	14	1.49	5.32	9.5E+02	0.15	5.08	94.92	52.47	-0.21	43.46	9.35	15.6	9.5	74.9
	Yp4	17	1.22	5.16	2.0E+04	0.14	0.66	93.73	57.24	-0.46	41.82	8.91	18.8	1.5	79.7
	Gp0	0	1.09	6.08	3.2E+03	0.07	0.51	96.43	33.70	-8.58	16.93	10.84	10.0	45.0	45.0
	Gp1	5	1.06	6.17	1.6E+03	0.08	0.33	94.99	33.60	-7.78	14.40	17.84	11.1	21.4	67.5
Gp	Gp2	10	1.17	5.85	1.7E+03	0.07	0.18	95.85	39.38	-8.94	18.65	9.46	14.0	14.0	72.0
	Gp3	14	1.20	6.05	1.5E+04	0.06	4.11	95.89	33.83	-7.81	15.26	9.83	16.4	5.7	77.9
	Gp4	17	1.69	5.94	5.0E+02	0.07	0.39	95.31	34.58	-7.49	14.95	10.13	16.3	5.4	78.4

Table 3- (continued from previous page)

Prod	Codes	Time (day)	EC_{50} (mg/ml)	pH	CFU g^{-1}	Acidity g/100 g	Ash %	W %	L	a	b	Firmness (N)	% O_2	% CO_2	% N_2
Ct	Cto	0	9.21	5.53	6.9E+03	0.11	1.06	91.24	52.48	30.18	38.94	34.20	10.0	45.0	45.0
	Ct1	5	5.67	6.25	5.0E+04	0.14	0.81	89.77	48.33	20.59	32.91	62.14	14.9	14.9	70.1
	Ct2	10	10.21	5.86	1.3E+05	0.09	0.69	90.18	47.80	18.29	30.34	39.40	4.7	27.2	68.2
	Ct3	14	9.42	5.90	2.0E+06	0.08	8.38	91.62	54.34	27.65	40.35	33.66	10.6	14.3	75.1
	Ct4	17	11.89	5.97	2.3E+07	0.10	0.94	90.46	51.27	20.26	31.39	32.03	10.3	10.9	78.8
Cb	Cbo	0	-	6.15	8.7E+02	0.17	0.52	95.78	70.71	-4.53	17.05	12.28	10.0	45.0	45.0
	Cb1	5	-	6.35	1.7E+03	0.10	0.48	94.90	63.76	-4.80	16.19	19.64	19.6	1.6	78.8
	Cb2	10	-	6.34	1.6E+03	0.14	0.39	95.21	59.62	-2.97	14.62	12.24	19.4	1.1	79.5
	Cb3	14	-	6.13	3.0E+03	0.11	3.77	96.23	64.82	-4.15	20.41	11.14	19.2	1.2	79.6
	Cb4	17	-	6.58	1.4E+03	0.14	0.52	95.65	58.97	-3.59	17.24	8.59	17.1	3.1	79.8

Table 4 - Correlations between physicochemical and microbiological data from fresh-cut vegetables (cabbage, carrots, green beans, green, red and yellow pepper) minimally processed, stored at 5ºC, under MAP with 10 % O_2 / 45 % CO_2 with N_2 to complete), along a storage period of 17 days (0, 5, 10, 14 and 17 days).

	Ac	pH	Ash	W	L	a	b	Ec_{50}	Txt	% O_2	% CO_2	CFU/g
Ac	1.000	-	-	-	-	-	-	-	-	-	-	-
pH	-0.254	1.000	-	-	-	-	-	-	-	-	-	-
Ash	0.470	-0.368	1.000	-	-	-	-	-	-	-	-	-
W	-0.110	0.203	-0.610	1.000	-	-	-	-	-	-	-	-
L	0.668	0.116	0.399	-0.064	1.000	-	-	-	-	-	-	-
a	0.131	-0.585	0.356	-0.611	-0.026	1.000	-	-	-	-	-	-
b	0.508	-0.616	0.842	-0.538	0.347	0.408	1.000	-	-	-	-	-
Ec_{50}	-0.257	0.432	0.184	-0.447	0.096	-0.037	-0.040	1.000	-	-	-	-
Txt	-0.123	0.321	0.133	-0.528	-0.170	0.042	0.025	0.638	1.000	-	-	-
% O_2	0.268	-0.100	-0.093	0.215	0.287	-0.024	-0.051	-0.444	-0.534	1.000	-	-
% CO_2	0.049	-0.011	0.175	0.033	-0.072	0.014	0.091	0.122	0.250	-0.765	1.000	-
CFU/g	-0.131	-0.160	0.154	-0.275	-0.060	0.377	0.032	0.156	-0.047	-0.080	-0.174	1.000

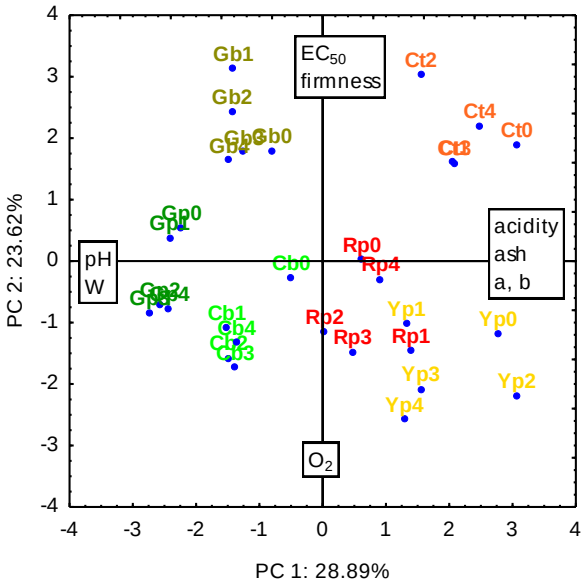

Figure 3. Principal component analysis of physicochemical and microbial data from fresh-cut vegetables (Gb-green beans; Rp-red pepper; Yp- yellow pepper; Gp – Green pepper; Cb-cabbage; and Ct-carrots) minimally processed, stored at 5ºC, under MAP condition (10% O_2/45% CO_2 and N_2 to complete) along a storage periods of 17 days (0, 5, 10, 14 and 17 days).

Based on these correlations, a principal component analysis was carried out to enable a "visualization" of the main data structures, which are displayed in Fig. 3 as the plot of PC1 vs PC2. The following facts are evident from Table 3 and Fig. 3:

(i) Microbial proliferation is observed for all vegetables along time, but never exceeding 10^8 recommended levels (Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market, established by the Health Protection Agency). This aspect is not accounted for in the PCA display mainly because microbiological counts are independent of all other variables of the study, also perceived in correlations results (see Table 4);

(ii) All other parameters analyzed are very stable along the time of study for all vegetables;

(iii) EC_{50} is very stable along time, being very high for green beans and carrots. These vegetables are therefore relatively poor in antioxidant activity in contrast to the other vegetables in evaluation. This characteristic is positively correlated with firmness (r = 0.638) and negatively correlated with O_2 levels (r=-0.444 in Table 4). This is the second data structure well represented by PC2, the vertical axis in Fig. 3;

(iv) The main data structure is the separation of green vegetables from the others, being negatively loaded in PC1, i.e., displaced towards the left hand side of Fig. 3. Colour parameters (a and b) is negatively correlated with pH and water content and positively correlated with acidity and ash contents, with correlation coefficients of -0.616, -0.538,

0.508 and 0.842, respectively. This is the main data structure that represents more than 50% of the data information.

(v) All points representing different sampling times of the same vegetable cluster together in the PC1 vs PC2 display, showing that all parameters maintained stable its values during the studied period. It can therefore be concluded that the procedure used was very protective, preventing physicochemical changes, and keeping microbial proliferation at acceptable low levels.

In this study some correlations are revealed, such as: - colour parameters (L and b) and acidity. This correlation was also verified and explained by Artés, Gómez, and Artés-Hernández (2007). Enriched atmosphere with CO_2 prevents chlorophylls degradation and at same time slightly decreases pH, due to some CO_2 solubilization; - Ash and water contents and texture as reported by Smith, Waldron, Maness, and Perkins-Veazie (2003) and discussed by Manolopoulou, et al. (2010). They stated that mass loss and firmness are closely related; - AA and texture are somehow related as reviewed by Rojas-Graü, Oms-Oliu, Soliva-Fortuny, and Martín-Belloso (2009).

3.2.2. *Sensory evaluation*

As it was done before, results from sensory judgements were subjected to a canonical variates analysis (CVA). This analysis considers that each vegetable analysed in a given time period is a group, with data from different judges being faced as repetitions. For example, cabbage in time 4 (coded as Cb4) was tasted by 14 judges. Thus, in the CVA display shown in Fig. 4a Cb, one see 14 triangles, each triangle representing one judge (or one group repetition). If groups are dispersed in the display, that means that judges are not in agreement. If points corresponding to different groups overlap, it means that judges do not see differences among the products. It is also important to verify that if differences exist, eigen values corresponding to canonical variates (indicated between brackets following CV1 or CV2) will be clearly higher than 1, meaning that differences between groups (differences between vegetables/times) are greater than differences within groups (attributed to product heterogeneity or lack of agreement among judges).

There are three main reasons that can lead to lack of agreement among judges: (i) judges are not well trained, (ii) products are very similar, or products/samples are heterogeneous. As judges were very experienced and had special training for this work, the first hypothesis must be discarded, while the other two must be kept in mind.

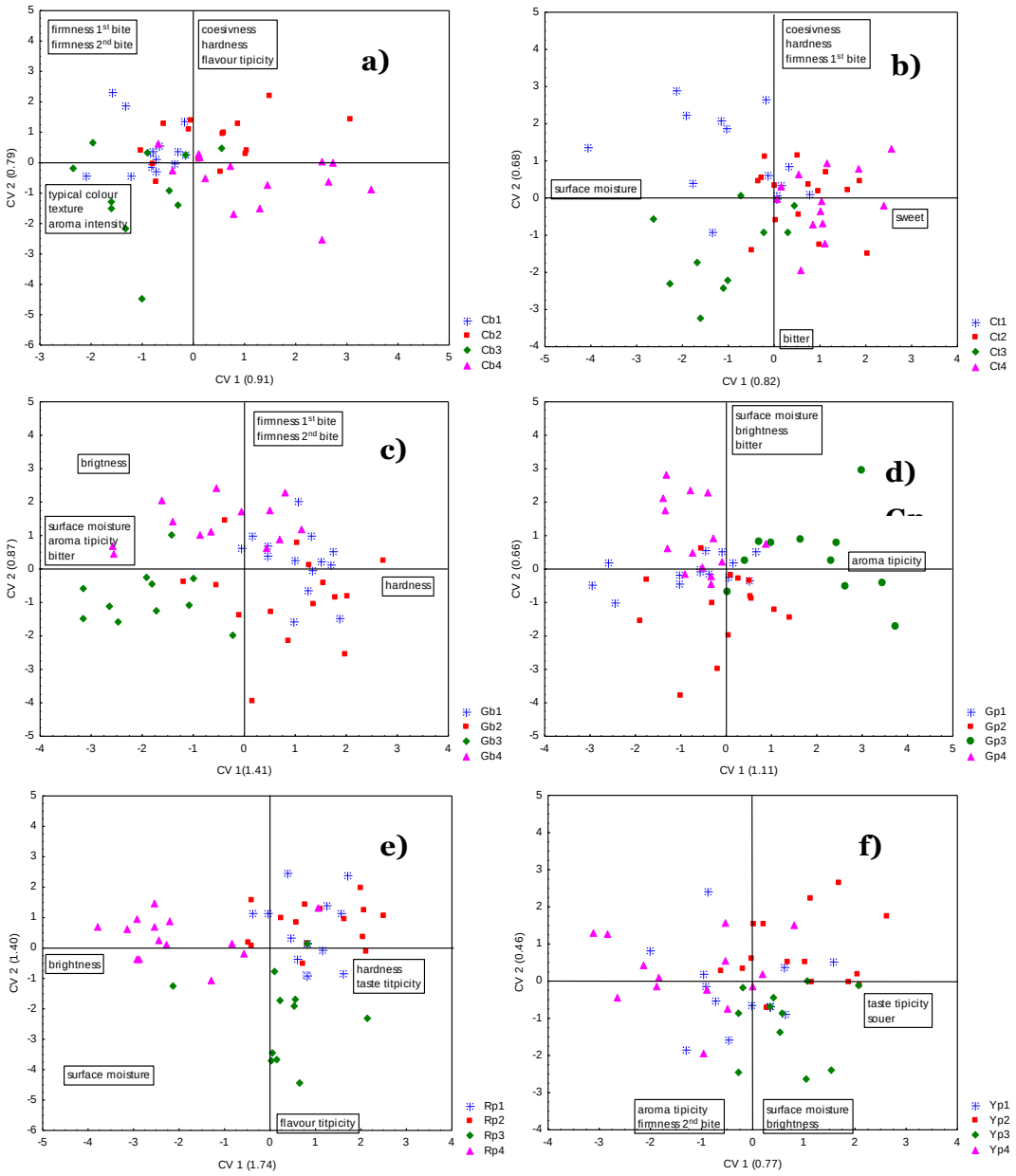

Figura 4. CVA of sensory data sets of the six studied vegetables: Cb-cabbage; Ct-carrots; Gb-green beans; Gp-green peppers; Rp-red peppers; Yp-yellow peppers, at different storage times (1-5days, 2-10 days; 3-14 days; 4-17 days)

Figure 4 show CVA displays for the sensory analysis of the six vegetables studied at times 5th to 17th. As it can be seen, dispersion of points representing any vegetable/time is very high, due to the lack of agreement among judges and/or sample heterogeneity. All second canonical variates (CV2) have eigen values inferior or close to 1 and therefore differences observed between the top and the bottom of all figures are not significant.

Amanatidou, Slump, Gorris, and Smid (2000) reported a study with carrots stored in MAP, under different gas combinations (20 % N_2 / 50 % O_2 / 30 % CO_2 and 89 % N_2 /1 % O_2 / 10 % CO_2). Although our gas ratios were different, our judges noted similar behaviour of the vegetables along the study. According to Amanatidou, et al. (2000) low levels of O_2 combined with high levels of CO_2 are beneficial to keep high standards of quality. The same trend was reported by Conesa, Artés-Hernández, Geysen, Nicolaï, and Artés (2007) confirming that low O_2 and high CO_2 levels prevent microbial spoilage, production of browning promoting compounds and loss in general appearance, quality, colour, crunchiness and flavour.

Only for green beans (Gb), green peppers (Gp) and red peppers (Rp) the first canonical variates (CV1) have eigen values slightly greater than 1. Projecting all sample points on the horizontal axes, the following conclusions can be withdrawn: green beans at time 3 seem to have higher surface moisture, aroma tipicity and bitterness than at time 1; green peppers at time 3 display a more typical aroma than at other times; red peppers at time 4, and according to judges opinion, were brighter and softer than in the other times of the study.

As a conclusion, the results of the study show that judges are faced with similar products, not being able to perceive definite changes along time, a fact that is probably enhanced by sample heterogeneity. It is worth noting that some of the differences observed do not point to a steady change in characteristics, i.e., there is no sequence from the initial to the last time of analysis.

4. Conclusions

Considering all results obtained with the two studies carried out with fresh-cut vegetables, it can be concluded that an atmosphere with low O_2 (around 10%) and high CO_2 (around 40-50%) levels, together with a relatively permeable film, will cause in the product an initial impact that decreases respiration activity and metabolic reactions, as well as microorganism proliferation.

The film permeability is a very important issue since it enables a progressive decrease of the aggressiveness of initial packaging conditions, mainly avoiding possible CO_2 deleterious actions, and promoting an increase in the product shelf life, well balanced in what concerns to physicochemical, microbiological and organoleptic properties.

The increase in shelf life achieved is around 40%, i.e., from 8 (some cases 10) to 14 days, which is very important, since it enables an extended period in the commercial chain. More important is the possibility enabled to the reorganization of harvesting and

packaging operations, in small farms, specialized in small productions of a variety of vegetables.

The evaluation of the different parameter changes must be deepened, and a comprehensive study of different microorganisms in different vegetables and along time is also advisable, since in this work only total counts of mesophylls were measured.

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V Modified atmosphere packaging of precooked vegetables: effect on physicochemical and sensory quality

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A semelhança do capítulo anterior, no artigo aqui apresentado pretendeu-se avaliar a possibilidade de atribuir um prazo de validade mais alargado a legumes pré-cozidos prontos-a-comer, apenas necessitando de uma etapa de aquecimento se o consumidor final assim o pretender.

Mais uma vez, e tendo em atenção que os legumes são comummente usados como acompanhamento de refeições, sobretudo nas dietas atlântica e mediterrânica, a possibilidade de extensão do tempo de vida útil destes produtos será uma mais-valia não só para o consumidor final, mas também para os operadores da restauração e sobretudo da coletiva (cantinas ou outros serviços de *catering*). O alargamento do prazo de validade permite que se disponibilize uma maior variedade de produtos prontos-a-consumir, que podem ser armazenados (a temperaturas de refrigeração), minimizando custos de mão-de-obra na sua confeção diária, e também as perdas de produtos confecionados desnecessariamente.

Nesta fase de estudo, o trabalho consistiu na monitorização, ao longo do armazenamento, de parâmetros da qualidade sensorial, físico-química e microbiológica de legumes (couve coração, feijão verde, cenoura e pimentos verde, vermelho e amarelo) cozidos, embalados em AM. Procurou-se reproduzir práticas industriais, no que diz respeito às técnicas de preparação das amostras, embalagem e armazenamento. Experimentalmente procedeu-se do seguinte modo:

- seleção de atmosferas adequadas à conservação dos legumes cozidos;
- monitorização de parâmetros organoléticos, físico-químicos (atividade antioxidante, pH, acidez, humidade, cinzas, cor e textura) e crescimento microbiano ao longo do tempo de armazenamento, mais alargado que o habitualmente atribuído pela indústria (28 dias);
- avaliação da aceitabilidade;

Os resultados obtidos permitem validar a possibilidade de alargamento de prazo de validade dos legumes, sem colocar em risco a qualidade e segurança dos produtos em estudo.

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under review

Modified atmosphere packaging of precooked vegetables: effect on physicochemical and sensory quality

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Abstract: This study aims to verify the effect of MAP conditions on the quality preservation of precooked vegetables. Physicochemical parameters (pH, acidity, moisture and ash contents, antioxidant activity, colour, and texture), microbial growth, organoleptic properties and consumer acceptability were assessed. Firstly three atmospheres (%O_2/%CO_2: 0/40; 2.5/40 and 2.5/60) with cabbage and green beans over 20 days (sampling at 0, 10, 15 and 20 days) were tested. The atmosphere with the best performance (0%O_2/40%CO_2) was then assayed with other vegetables (carrots, green, red and yellow peppers) along an extended storage period (28 days, 6 sampling times). Concerning physicochemical parameters and microbial growth only small changes, without any consistent tendency were observed. This was also confirmed by the trained panel that was not able to discriminate samples with different storage times. Results suggest the suitability of the used preservation conditions in shelf life extension of the evaluated vegetables.

Keywords: MAP, ready-to-eat vegetables, precooked vegetables, antioxidant activity, sensory evaluation, acceptability.

Running title: MAP of precooked vegetables: effect on physicochemical and sensory quality

1. Introduction

The daily requirement for nutrients such as minerals, vitamins and dietary fibres, is often accomplished by consumption of vegetables. Fresh and cooked vegetables are important sources of biologically active micronutrients and other health benefit compounds. Fruits and vegetables consumption is recommended due to the strong relationship between its intake and the protective properties against diseases like cancer, cataract and macular degeneration (Flood, Velie, Chaterjee, Subar, Thompson, Lacey, et al., 2002; Roy, Takenaka, Isobe, & Tsushida, 2007; Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009; Murcia, Jiménez-Monreal, García-Diz, Carmona, Maggi, & Martínez-Tomé, 2009). Besides being great health promoter, the vegetable bioactive compounds are responsible for many of their organoleptic characteristics and are also important for their maintenance during shelf life (Jiménez-Monreal, et al., 2009).

The vegetable's nutritional profile is highly dependent on processing procedures. Considerable changes are described when vegetables undergo processing techniques, affecting its nutritional quality and degrading antioxidant constituents (Jiménez-Monreal, et al., 2009). Even mild thermal treatments, like blanching, have impact on functional components (Roy, et al., 2007). Due to the importance of these processing techniques for food preservation and nutritional integrity, more work is advisable in this topic.

In Mediterranean and Southern European Atlantic diets vegetables are greatly used fresh or cooked and frequently eaten as side dishes (Guallar-Castillon, Oliveira, Lopes, Lopez-Garcia, & Rodriguez-Artalejo, 2013). Portuguese eating habits contain aspects of both Atlantic and Mediterranean diet, probably due to its geographical situation, with fresh vegetables being mainly consumed in summer, while cooked vegetables are preferred in colder seasons. The small size of Portuguese producing areas and the variety of products in each area enhance quality, but make logistic operations complex and tend to raise prices. Thus work is being carried out to increase product shelf life, allowing a better rationalization of harvesting and post-harvesting procedures as well as packaging and distribution, while satisfying the consumer's demand for freshness and high quality standards.

In canteens, restaurants, hospitals and similar institutions, vegetables may be considered as ready-to-eat and precooked (PC) products. PC vegetables may be coupled to modified atmosphere packaging (MAP), a technique aiming to slow down senescence and microbial growth through the utilization of specific gas combinations inside the packages. Gas compositions with residual levels of O_2 and high amounts of CO_2 and N_2, submitted to adequate refrigerating temperatures will prevent the growth of microorganisms, delay compositional decay and at the same time maintain the organoleptic properties (general

appearance, aroma, taste and texture). These procedures will fulfil a set of processes that constitute the hurdle technology, a term commonly used in the literature referring to foods preserved by a combination of processes (Galić, Ščetar, & Kurek, 2011). It promotes, consequently, the increase in shelf-life of ready-to-eat products (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007; Murcia, et al., 2009; Galić, et al., 2011).

In the case of PC vegetables, keeping high quality over extended shelf life is a challenge. The gas mixtures selection must take into consideration the intrinsic parameters of the food product to preserve (pH, water activity, fat content and type of fat), that will influence the susceptibility to microbial spoilage and chemical and enzymatic alterations (Devlieghere, Gil, & Debevere, 2002). According to Amanatidou, Slump, Gorris, and Smid (2000) and Conesa, Artés-Hernández, Geysen, Nicolaï, and Artés (2007) low levels of O_2 combined with high levels of CO_2 were reported as beneficial to keep high quality standards, in preventing microbial spoilage, production of compounds that promotes browning and loss in general appearance quality, colour, crunchiness and flavor.

Cooked vegetables are non-respiring products, susceptible to microbial spoilage due to the development of Gram-negative bacteria and yeasts. Therefore, they should be packed in atmospheres with high CO_2 concentration. O_2 must be excluded from the gas mixture, allowing only residual amounts to prevent risk of anaerobic microorganism's growth (Al-Ati & Hotchkiss, 2002; Devlieghere, et al., 2002; Galić, et al., 2011).

Few data have been published for PC vegetables, fact also confirmed by Porter (2012). As far as we know, only Murcia and collaborators (2003 and 2009) have reported studies on ready-to-eat food storage with special packaging conditions, namely MAP and vacuum. In their work proximate composition and microbiological quality were monitored. Other works already referred in previous sections discuss the influence of MAP on minimally processed and precooked foods based on a limited set of quality parameters.

This study aims at explaining how MAP preservation may affect a wide set of parameters, namely pH, colour change, microbial growth, total antioxidant activity, texture and some important organoleptic properties, including the perception of some defects, and consumer acceptability. The effect of MAP conditions in PC vegetables commonly used as side dishes in diets was studied in the canteens of the Polytechnic Institute of Viana do Castelo (IPVC), in order to determine the best conditions to be used in a real application.

2. Materials and Methods

2.1. *Samples*

Cabbage (*Brassica oleracea* L.), carrots (*Daucus carota* L.), green beans (*Phaseolus vulgaris* L.) and peppers (red, green and yellow *Capsicum annum*), obtained in local farmers were cut and sliced. Old leaves, stems and dark spots were removed before

cutting. Washing was carried out with potable water. After this preparation they were boiled for 8 min, in salted water (1 % p/v).

2.1.1.*Packaging materials*

PC vegetables were packed in multilayer polystyrene (EPS/HIPS/PE) trays and sealed with a 55□m high barrier Technopack film (OPEX 55 AB – PA/EVOH/PE) supplied by Miranda e Serra, Portugal, with O_2 and CO_2 permeability of 2 cm^3m^{-2} 24 h and 5.4 cm^3m^{-2} 24 h, respectively.

2.1.2. *Modified atmosphere packaging equipment*

A Yang SR-I Oceania Jolly 20 thermossealer (Italy) was used for package sealing. Inside packaging atmosphere was substituted using a PBI Dansensor A/SMAP Mix 8000 EL (Denmark) gas mixer. On sampling days, inside gas composition was verified with a gas analyser (O_2/CO_2 Checkmate II, PBI Dansensor, Ringsted, Denmark).

2.2. *Atmospheres, temperatures and times*

First study: Three atmospheres and two vegetables (cabbage and green beans) were tested. In terms of % O_2 and % CO_2 (with N_2 balanced to 100 %) these atmospheres were 0/40, 2.5/40 and 2.5/60 (% O_2 / % CO_2). After packaging, samples were stored at 5ºC for 20 days. Physicochemical, and microbial growth evaluations were carried out at the beginning of the experiments (day 0) and on the 10^{th}, 15^{th} and 20^{th} days after packaging. Sensory evaluation and consumer acceptability were carried out on the 10^{th} day of storage.

Second study: The best atmosphere determined in the first study (MAP of 0 % O_2/ 40 % CO_2) was used. All samples were kept at 5 ºC, and physicochemical, microbial and sensory parameters were evaluated at the beginning of the experiment (day 0) and at 7^{th}, 14^{th}, 20^{th}, 24^{th} and 28^{th} days after packaging. In both studies samples were prepared in duplicate.

In tables and figures related to the second study samples were coded as follows: Vegetable names represented by the first two letters (Cb = Cabbage; Ct = Carrots; Gb = Green Beans; Gp = Green peppers; Rp = Red peppers Yp = Yellow peppers); Storage or sampling time represented by numbers (0 to 5).

2.3. *Physicochemical evaluation and sample preparation*

At each sampling day, after opening the package, samples firmness and colour were determined. Colour measurements were carried out with a MINOLTA CR300 (Konica Minolta, USA) to determine colour parameters (L*, a*, b*) and total colour variation as $\Delta Eab=\sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$, using the Hunter Lab colour system. The determinations were performed 10 times for each sample. Firmness, a texture parameter defined as the

maximum force exerted in compression, was determined using a TA-XT2i (Stable Micro Systems Ltd., U.K.) equipped with a 2 mm diameter cylindrical stainless steel probe. The compression test speed was 1 $mm.s^{-1}$. For each sample, about 20 measurements were performed.

To evaluate acidity, pH, moisture and ash contents samples were mill in a knife mill (GM 200, RETSCH, Haan, Germany). Evolution of pH was monitored using a FC232 electrode coupled to a potentiometer HI99163 HANNA Instruments (USA) as described in the 981.12 AOAC method (AOAC, 2000). Acidity was determined by titration with 0.1N NaOH. The results were expressed as g citric acid/100 g fresh weight according to the official 942.15 AOAC method (AOAC, 2000). Moisture content was determined gravimetrically according to the 984.25 AOAC method (AOAC, 2000), performed in triplicate and results expressed as %. About 3 g of homogenized sample were dried at 103±2 ºC (UTG, Heroeus) to constant weight. The ash content was determined by a gravimetric according to the 935.42 AOAC method (AOAC, 2000). 3g of homogenized sample were incinerated in a Heraeus muffle at 550 ºC until complete carbonization, cooled and weighed. Determinations were performed in triplicate and the results expressed as percentage (%).

For the evaluation of the antioxidant activity (AA), the vegetables in study were freeze-dried and stored until analysis. Aqueous extracts were prepared with 1-3 g of freeze-dried samples. The evolution of the AA values, over storage time, was determined by the DPPH radical-scavenging. The aqueous extracts were added to a 50 % MeOH solution and absorbance was determined after 30 minutes at 515 nm. The percentage of inhibition was calculated afterwards. Results were expressed as the antioxidant concentration needed to decrease the initial DPPH concentration by 50 %, expressed as EC_{50} (described in literature also as IC_{50}). The calculation of EC_{50} value required the determination of the reaction kinetics between DPPH and the antioxidant according to (Sánchez-Moreno, A. Larrauri, & Saura-Calixto, 1999).

2.4. *Microbial evaluation*

Microbial evaluation was performed just as indication of the deterioration extent based on microorganisms-colony-count technique at 30 ºC. Sampling was done in aseptic conditions. Small portions from different zones of the package were used to prepare the test sample for microbiological analysis. A mother suspension (10^{-1}) was prepared with 10 g of each sample. It was introduced in a sterile “Stomacher” bag, mixed with 90 ml sterilized Triptone salt solution, and homogenized for 1 minute in a Stomacher 400 (Seward, BA7021, UK). Decimal dilutions were prepared, 1ml of each pour plated on PCA

medium (Plate Count Agar - Oxoid) and incubated at 30 ºC±1 ºC for 72 h ± 3 h, as recommended by ISO 4833:2003 (ISO, 2003).

2.5. *Sensory evaluation*

2.5.1. *Panel training*

Before the evaluation sessions, panel judges focused on the typical characteristics of PC vegetables and also on the presence of defects caused by senescence. Freshly cooked vegetables (prepared for each training session), and cooked vegetables kept in air at 5ºC for different periods of time were used by judges in several discussion/training sessions, in order to understand the course of deterioration and its effects on sensory quality, while comparing to freshly cooked vegetables. This training step was performed in group sessions. A list of attributes was also decided by consensus, containing the following attributes:

First study: appearance (as general appearance), colour, odour, taste and texture (hardness and mouth feel perception).

Second study: general appearance (surface moisture; typical colour; browning, brightness; texture/shape), texture with forks (elasticity; cohesiveness; hardness), aroma (aroma typicity; aroma intensity), texture during mastication (firmness at first bite; firmness at second bite), flavour and taste (flavour typicity; taste intensity; sour; bitter; sweet).

2.5.2. *Quantitative descriptive analysis (QDA)*

The sensory evaluation followed the QDA® methodology (Tragon Corporation, San Francisco, USA), as described by several authors (Stone & Sidel, 2004; Meilgaard, Civille, & Carr, 2007) and implemented according to Alves and Oliveira (2005).

First study. Sensory evaluation was performed by a 7 member trained panel on the 10th day of storage. A list of attributes with a 9 point scale was used.

Second study. Products were evaluated by a 15 member trained panel, using the same attributes as in th first study, but with a 13 point scale. In all sessions, freshly cooked vegetables were available as standards. Judges were instructed to start any session by tasting the standards and consider point 7 of each attribute's scale as the standard's magnitude perceived for that attribute. In this way, in all sessions and for all judges, evaluations were made against a freshly boiled standard, reducing bias in judgements.

2.5.3. *Consumer acceptability studies*

Samples from study 1 were tasted by 83 consumers to assess their acceptability, on a 9 point hedonic scale (Peryam & Pilgrim, 1957). Consumer's sensory evaluation was carried out on the 10th day of storage, during meals at lunch time at the IPVC canteen. Small

portions of vegetable (approximately 20 g) were served and distributed randomly among consumers.

2.6. *Statistical analysis*

Data mining was carried out with principal component analysis (PCA) to evaluate the main characteristics and their changes along storage time. Kruskal-Wallis, Wilcoxon /Mann-Whitney, and canonical variates analyses (CVA) were used to evaluate consumers and judges results, respectively, with groups representing products and judges or consumers opinions used as replications for each product. CVA enabled analysis and visualization of the significance of observed differences between products (Barbosa, Oliveira, & Alves, 2011). ANOVA, MANOVA, CVA and PCA were carried out using the Statistica for Windows software package, version 7 (Stat Soft Inc., Tulsa, USA).

3. Results and Discussion

MAP is referred by many authors as an efficient option for ready-to-eat foods preservation (Kader & Ben-Yehoshua, 2000; Jacxsens, Devlieghere, Van der Steen, & Debevere, 2001; Devlieghere, et al., 2002; Spencer, 2005; Rico, et al., 2007; Manolopoulou, Xanthopoulos, Douros, & Lambrinos, 2010). Different gas compositions have been described as suitable for products previously submitted to thermal processing (non-respiring products) (Devlieghere, et al., 2002).

This work was divided in two studies. The results obtained in the first study, consisting in the application of three different gas mixtures in two PC vegetables (cabbage and green beans) were discussed in order to explore the best one to apply in a wide variety of precooked vegetables, simulating industry practices. In the second study, the best MAP condition selected was used to study how vegetables behave over a longer period of time.

The study of the evolution of the quality parameters is summarized in Tables 1 and 2. The discussion of the relationships between MAP gas composition and quality parameters such as total antioxidant activity, pH, colour change, some important organoleptic properties (also the perception of some defects), and microbial growth is supported by multivariate analysis.

3.1. *First study - influence of MAP conditions*

As it was referred before, a first study was carried out in order to compare the effect of selected atmospheres, with 0 % and 2.5 % O_2 combined with 40 % to 60 % CO_2. It is important to clarify that 0% of O_2, corresponds in general to a residual amount of 0.5% ± 0,2% of O_2, which is desirable so it can be avoided an anaerobic environment, preventing anaerobic bacteria growth.

3.1.1. *Physicochemical and microbial quality parameters*

The EC_{50}, pH, ΔEab, and microbial growth results are summarized in Table 1. Antioxidant activity was measured by DPPH assay and expressed as EC_{50}. It is clear the increasing values of EC_{50} along all the experiments as discussed below. Some changes were also verified in pH and total colour change.

To simplify the analysis of data sets obtained from these quality parameters multivariate statistical techniques of well recognised application in food analysis were used (Oliveira, Alves, & Ferreira, 2001; Alves & Oliveira, 2005; Barbosa, et al., 2011). Some conclusions could be withdrawn directly from the expressed values in the Table 1, but they were analysed by principal component analysis (PCA). Fig. 1 shows the plane of components 1 (horizontal axis) *versus* 2 (vertical axis) referred to as PC1 vs PC2, which contains more than 94% of the information of Table 1, and helps to depict the main features of the results.

Results showed that green beans at 0/40 atmosphere presented lower values of EC_{50} for all sampling times indicating this as the ideal gas composition in what concerns antioxidant activity. This is explained by PC1 in the plot presented in Fig. 1. Also, the evolution of microbiological indicator (CFU/g) and colour variation (ΔEab) showed the same behaviour.

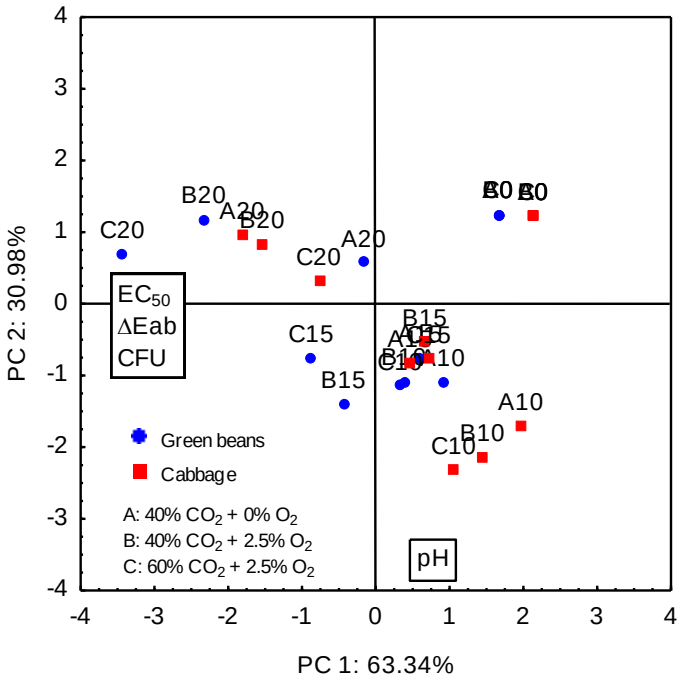

Fig 1. Principal component analysis of physicochemical (pH, antioxidant activity (EC_{50}), total colour change (ΔEab)) and microbial data from precooked vegetables (cabbage and green beans), stored at 5 ºC, under different MAP conditions (0/40, 2.5/40 and 2.5/60 as % O_2/ % CO_2) along different storage periods (0, 10, 15 and 20 days).

Table 1 Evolution of physicochemical parameters (antioxidant activity (EC_{50}), pH, total colour change (ΔEab)) and microbial growth of precooked vegetables (cabbage and green beans), stored at 5ºC, under different MAP conditions (0/40, 2.5/40 and 2.5/60 as % O_2/ % CO_2), along different storage periods (0, 10, 15 and 20 days)

Sample	MAP %O_2 / %CO_2	Time	EC_{50} (mg/mL)	pH	ΔEab	CFU/g
Cabbage	0/40	0	13.16	5.90	0.00	< 10
		10	22.30	6.55	7.02	< 10
		15	28.99	6.11	16.98	< 10
		20	35.38	5.78	19.52	> 3.0E+04
	2.5/40	0	13.16	5.90	0.00	< 10
		10	27.44	6.57	11.59	< 10
		15	33.94	6.09	11.85	< 10
		20	41.60	5.87	14.42	> 3.0E+04
	2.5/60	0	13.16	5.90	0.00	< 10
		10	35.06	6.56	12.90	< 10
		15	35.94	6.16	11.11	2.0 E+02
		20	39.98	5.80	17.81	8.8 E+03
Green beans	0/40	0	24.53	5.84	0.00	< 10
		10	42.70	6.28	7.37	< 10
		15	49.01	6.17	6.72	1.4 E+02
		20	50.33	5.76	9.44	1.3 E+03
	2.5/40	0	24.53	5.84	0.00	< 10
		10	48.20	6.20	10.42	< 10
		15	56.13	6.15	16.54	< 10
		20	57.71	5.68	15.11	2.9 E+04
	2.5/60	0	24.53	5.84	0.00	< 10
		10	46.14	6.20	12.02	< 10
		15	49.72	6.04	18.18	8.5 E+03
		20	79.89	5.67	18.18	3.0 E+04

In the case of cabbage packed in 0/40, EC_{50}, microbial growth and total colour variation levels presented a slower evolution of these parameters. A similar conclusion can be extracted in relation to green beans, confirming the best atmosphere as 0/40 %O_2/%CO_2. Variable correlations are presented in Supplementary Table 1, and as expected, a strong correlation was observed between antioxidant activity and colour variation. This is probably due to the degradation of colour pigments such as anthocyanins, carotenoids and chlorophylls (Volden, Borge, Bengtsson, Hansen, Thygesen, & Wicklund, 2008; Porter, 2012).

3.1.2. *Sensory evaluation*

Sensory results on the 10th storage day were analysed with canonical variates analysis (CVA), as described by Barbosa, et al. (2011). Judges assessed green beans and cabbage. Concerning cabbage no discrimination was achieved as revealed by a Wilk's Lambda of 0.735 ($p < 0.90$). This is illustrated in Fig. 2A, where each point corresponds to one judge opinion and the observed dispersion means that the panellists are confused due to the similarity of the samples.

CVA from green beans sensory data (Fig. 2B) revealed that the panel was able to discriminate samples under different MAP conditions (Wilk's Lambda of 0.0998, $p < 0.013$). CV1, horizontal axis, describes the attributes related to tipicity (aroma and taste) that the panel considered important whose values increase from left to the right hand side of the graph. CV2 represents manly attribute "firmness", increasing from top to bottom of the display.

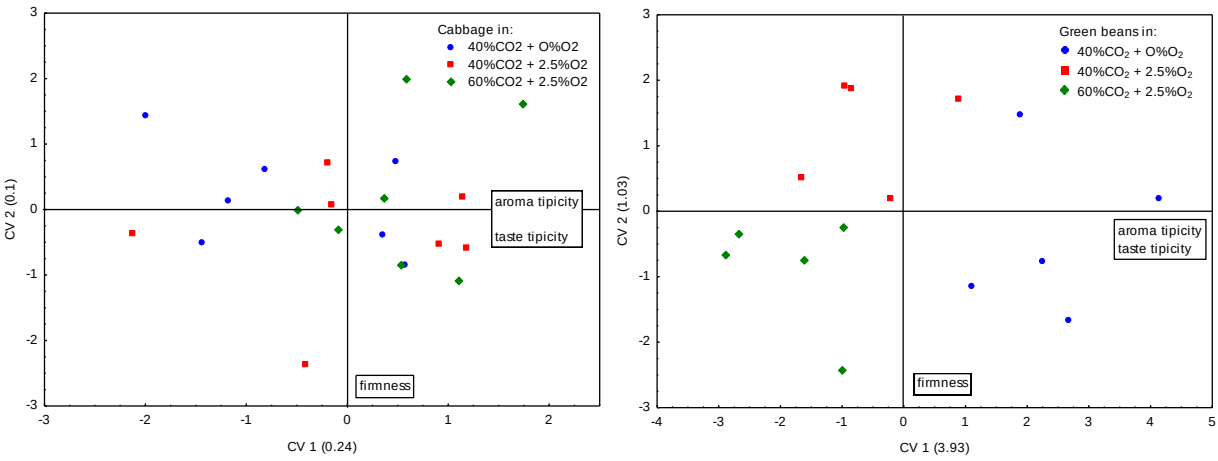

Fig 2. Canonical variates analyses of judges' appraisal on: PC cabbage and PC green beans, on the 10th day of storage under 3 different atmospheres (0/40, 2.5/40 and 2.5/60 % O_2/ % CO_2).

With respect to taste and aroma tipicity of green bean samples, those packed in 0/40 were better appreciated, as expected since antioxidant activity (Ec_{50}) until the 15th day follows the same pattern. Some authors reported correlations between antioxidant activity and organoleptic properties preservation (L. Tijskens, Simčič, & Schouten, 2007; Shahidi, Chandrasekara, & Zhong, 2011).

The consumers' opinion data was analysed by a nonparametric Kruskal-Wallis test, comparing multiple independent samples. The Boxplot of the results presented in Supplementary Figure 1 shows that cabbage in 0/40 is better classified by the majority of the consumers. However the difference is not statistically significant (Kruskal-Wallis with H = 0.79 and p = 0.68) (Spurrier, 2003). Consumer acceptability of green bean samples in

MAP was also similar for all tested samples, with no significant differences detected by a Kruskal-Wallis test (H = 3.05 and p = 0.218).

If a criterion to accept/reject was to be implemented based on consumers acceptability as proposed by Tijskens (2000), then any average score between 1 and 5 would lead to a rejection and between 6 and 9 would lead to acceptance. Applied to these results such a criterion would result in acceptance of studied samples in 0/40.

Despite the fact that no significant differences were observed, results showed an expected tendency towards a decrease in antioxidant activity over storage time, together with small changes in organoleptic properties, as also Rico et al. (2007) reported. The best MAP combination for precooked vegetables seems to be 0 % O_2/ 40 % CO_2. This atmosphere meets both antioxidant capacity and organoleptic preservation over storage.

Concerning microbial growth all tested samples were considered quite acceptable according to the guidelines for assessing the microbiological safety of ready-to-eat foods on the market. The Health Protection Agency (HPA) established a maximum acceptable value for total mesophilic counts for ready-to-eat foods of 1×10^8 (CFU g^{-1}).

3.1.3. *Second study - time extent*

The following stage of this work aimed at analyzing the behavior of the products towards a longer storage time (28 days), in terms of some physicochemical parameters and sensory quality, using the gas combination selected from the previous study (0 % O_2/ 40 % CO_2). Moreover, more vegetables were included, i.e., carrots and red, green and yellow peppers. Samples were stored under MAP with the gas combination 0/40/60 (residual amounts of O_2, as referred before, with 40 % of CO_2 and N_2 to balance the gas composition inside package), using the same packaging material as in the earlier study. Results are presented in Table 2.

Once more, statistical analysis on physicochemical data revealed that over storage time samples remain quite stable. Loss of antioxidant activity is generally verified but not at a large extent except for carrots, that seem to have higher losses over time. Other authors reported the same behavior in similar studies with carrots (Alasalvar, Al-Farsi, Quantick, Shahidi, & Wiktorowicz, 2005; Berger, Küchler, Maaßen, Busch-Stockfisch, & Steinhart, 2008).

Microbiological growth was quite acceptable for all the samples except for carrots with 20 days of storage (Ct4). Once carrot samples with 28 days (Ct5) presented values less than 10 CFU/g, a probable contamination during the handling of Ct4 samples may have occurred. All other samples were acceptable according to the HPA guidelines.

3.1.3.1. Physicochemical quality parameters

With regard to PC vegetables, the studied quality parameters (Table 2) presented small changes over time, but in a consistent way, after the first sampling period.

PCA plot (Fig. 3) representing PC1 *vs* PC2 summarizes the most important information (~58 %) and explains the importance of some variables. Acidity is one of these variables to distinguish the type of vegetable, as well as pH, both highly negatively correlated. Along PC1 (left to right-hand side of the plot), it is possible to observe pepper samples (green, red and yellow) separated from the other studied vegetables, mainly due to its acidity. This parameter increased towards the right-hand side of the plot in opposition to cabbage, carrots and green bean samples that showed higher pH, EC_{50} and ash content.

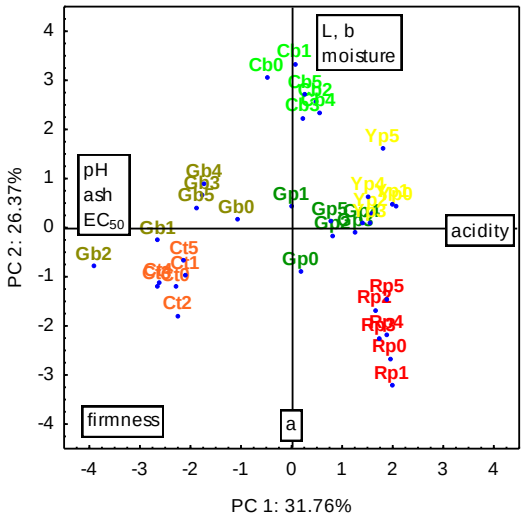

Fig 3. Principal component analysis of physicochemical (pH, ash content, acidity, antioxidant activity (EC_{50}), instrumental firmness, colour parameters L, a^a and b*) and microbial data from precooked vegetables (Cb- cabbage; Gb-green beans; Ct-carrots; Rp- red pepper; Gp- green pepper and Yp - yellow pepper), stored at 5ºC, under MAP condition (0 % O_2/ 40 % CO_2 and N_2 to complete) along a storage periods of 28 days (where numbers from 0 to 5 correspond to 0, 7, 14, 20, 24 and 28 storage days, respectively).

Table 2 Evolution of physicochemical parameters (antioxidant activity (EC_{50}), pH, acidity (Ac), ash content, moisture content (MC), colour parameters L, a* and b* and instrumental firmness) and microbial growth (CFU) of precooked vegetables (Cb- cabbage; Gb- green beans; Ct- carrots; Rp- red pepper; Gp- green pepper and Yp- yellow pepper), stored at 5ºC, under MAP condition (0% O_2 with 40% CO_2 and N_2 to complete) along a storage periods of 28 days (where numbers from 0 to 5 correspond to 0, 7, 14, 20, 24 and 28 day of storage, respectively)

Product	Code	EC_{50} (mg/ml)	pH	Ac (g /100 g prod)	Ash (%)	MC (%)	L	a*	b*	Firmness (N)	CFU/g
Cabbage	Cb0	2.80	6.76	0.92	1.14	94.09	56.94	-4.66	8.01	0.88	<10
	Cb1	3.43	6.63	0.51	0.84	95.16	60.21	-3.80	6.60	0.25	<10
	Cb2	3.07	6.28	1.01	0.92	94.50	52.73	-3.19	7.86	0.39	<10
	Cb3	3.50	6.45	0.74	0.88	94.35	47.62	-2.10	7.50	0.89	<10
	Cb4	4.66	6.27	0.98	0.79	95.14	56.73	-3.46	4.82	0.56	<10
	Cb5	4.21	6.31	0.88	0.88	94.48	57.90	-2.72	6.86	0.55	<10
Green bean	Gb0	17.34	6.43	0.56	0.63	93.00	41.93	-8.53	3.54	8.80	<10
	Gb1	26.96	6.33	0.37	1.04	94.35	42.05	-4.26	2.68	12.33	<10
	Gb2	52.81	6.13	0.46	1.10	93.87	42.44	-2.72	3.68	14.15	<10
	Gb3	16.56	6.34	0.37	1.11	94.20	43.32	-1.96	3.98	6.47	<10
	Gb4	20.75	6.40	0.46	1.07	95.41	46.50	-1.90	3.05	6.55	4.90E+02
	Gb5	17.34	6.34	0.60	1.16	93.85	44.35	-1.89	3.54	7.10	2.65E+02
Carrot	Ct0	17.88	6.14	0.92	1.26	90.58	48.48	18.78	2.12	6.28	<10
	Ct1	20.94	6.17	1.01	1.14	90.31	51.56	23.64	3.91	6.36	<10
	Ct2	20.85	6.15	1.06	1.06	90.17	48.34	20.16	1.91	9.18	<10
	Ct3	29.13	6.10	1.08	1.10	89.96	51.81	21.17	5.85	10.83	<10
	Ct4	16.83	6.15	0.84	1.12	92.52	48.74	18.74	2.65	6.99	1.08E+05
	Ct5	23.37	6.20	0.82	1.16	90.33	48.75	18.77	3.89	3.96	<10

Table 2 (continued from previous table)

Product	Code	EC_{50} (mg/ml)	pH	Acidity (g/100 g prod)	Ash (%)	MC (%)	L	a*	b*	Firmness (N)	CFU/g
Red pepper	Rp0	5.17	5.09	2.22	0.53	93.28	36.56	29.72	1.83	5.14	<10
	Rp1	2.61	4.04	2.11	0.78	92.83	36.56	29.72	1.83	5.47	<10
	Rp2	2.88	5.05	2.24	0.79	93.11	40.42	24.09	4.67	4.80	<10
	Rp3	2.08	5.02	2.28	0.80	93.17	37.18	27.55	3.15	4.19	<10
	Rp4	2.06	4.98	2.29	0.78	94.03	37.33	25.12	1.98	3.89	<10
	Rp5	2.39	5.00	2.41	0.80	92.83	41.70	28.49	6.76	4.31	<10
Green pepper	Gp0	1.44	5.52	0.77	0.67	93.30	36.65	-7.72	1.53	8.90	1.50E+02
	Gp1	1.23	6.33	0.70	0.73	93.64	40.14	-2.49	3.96	5.69	3.25E+02
	Gp2	1.11	5.61	0.74	0.61	94.03	39.38	-3.49	1.71	4.34	<10
	Gp3	1.28	5.45	1.11	0.62	94.17	38.19	-2.82	3.45	4.08	<10
	Gp4	1.40	5.50	1.14	0.58	95.31	41.09	-3.69	2.10	4.27	<10
	Gp5	2.21	5.71	0.91	0.61	93.78	41.86	-3.73	3.25	5.08	<10
Yellow pepper	Yp0	1.24	4.95	2.17	0.68	95.43	49.67	-3.22	6.15	7.06	<10
	Yp1	1.29	5.01	2.26	0.67	92.87	54.02	-2.86	6.53	4.01	<10
	Yp2	1.55	5.01	1.79	0.71	93.08	48.31	-2.28	6.93	5.38	<10
	Yp3	1.70	4.98	1.66	0.70	93.25	50.05	-3.33	3.57	3.27	<10
	Yp4	1.52	4.94	1.51	0.67	93.74	55.29	-2.13	4.80	4.64	<10
	Yp5	1.61	4.97	1.48	0.64	93.42	55.84	-1.97	9.72	4.10	<10

Observing PC2 (top/down variation on the plot of Fig. 3), samples were separated in terms of moisture content and colour. Also firmness is explained by the PC1 vs PC2, increasing from the top right quarter of the graph to the left bottom one. From the top to down on the plot, vegetables are firmer, being carrots the hardest, as expected.

Over time (from 0 to 5) samples seem to become softer, but this difference is not statistically significant ($p > 0.05$) (data not shown), exception made for yellow peppers from the latest sampling time (Yp5). This situation contrasts with moisture content which is somehow expected. Concerning antioxidant capacity, results revealed little variation along the time. Very little information is published concerning evolution of EC_{50} values for precooked vegetables. However some studies on radical scavenging of raw and thermal treated vegetables have reported similar results to the ones presented here, with some variations that are attributed not only to natural products and season variability but also to the methodology itself (Mishra, Ojha, & Chaudhury, 2012).

The PCA table of correlations (Supplementary Table 2) reveals the relationships between variables and help understanding the products' response towards the preservation method. Some of them are obvious and well known like acidity and pH. In this study some other correlations are revealed, such as: colour parameters, L* and b*, correlated with acidity and ash content; moisture content correlated with texture as it was also reported by Smith and collaborators (2003) and discussed by Manolopoulou, et al. (2010) stating that mass loss and firmness are closely related.

As it can be seen in the correlations obtained, a moisture content and/or pH vary; the antioxidant activity and texture are also affected. This observation is in accordance to several authors that studied biologically active compounds and its antiradical properties (Alasalvar, et al., 2005; Berger, et al., 2008; Li, Deng, Zhu, Hu, Liu, Young, et al., 2012).

3.1.4. *Sensory evaluation*

Generally, judges consider vegetable samples quite similar, visible from the cloudy results presented in Fig 4. Apparently only small differences were perceived throughout the storage period almost until the 24th day.

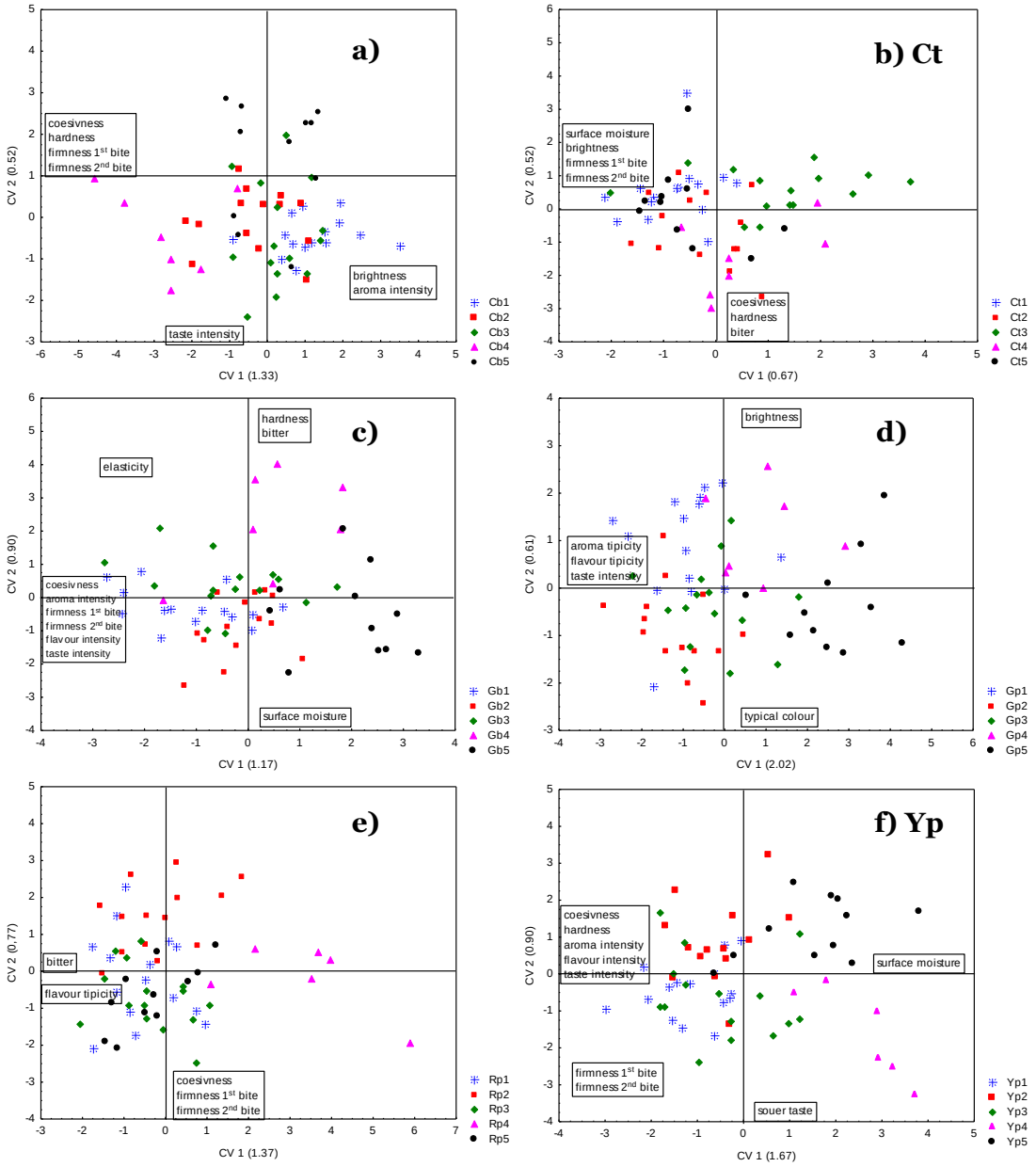

Fig 4. CVA of sensory data sets of the six studied PC vegetables: a) Cb- cabbage; b) Ct- carrots; c) Gb- green beans; d) Gp- green peppers; e) Rp- red peppers; f) Yp- yellow peppers, at different storage times (1- 7 days, 2- 14 days; 3- 20 days; 4- 24 days and 5- 28 days).

In Fig 4 it can be seen that CV2, in all product studied have an eigen value (shown between brackets) lower than 1, meaning that the top down scatters observed do not correspond to significant differences. On the other hand all CV1 have an eigen value slightly higher than one, expressing very small, yet significant differences, in all cases but for carrots. Thus in this analysis only CV1 (left to right variation on the observed scatterplots) is important, except in the case of carrots (Ct). Although in every sensory evaluation session judges had

a freshly cooked sample used as a standard to reduce randomness, judges had difficulties in discriminating the samples and therefore it can be assumed that no significant changes were detected over time.

Along the CV1 axis from Fig 4 c), d), e) and f), the panel detected some changes in samples corresponding to 24 and 28 days of storage (Rp4, Yp4 and Gb5, Gp5, respectively). These small differences are mainly related to texture and tipicity attributes.

4. Conclusions

MAP with residual O_2 and high CO_2 percentages enable minor quality changes in all studied parameters during storage of precooked vegetables. Over more than twenty days of storage, antioxidant activity loss is higher in green beans samples than in cabbage. Since microbiological results showed values below the legal limits in the late storage days, again, it may be indicative of negligible microbial growth and indicative that shelf life of this products can be extended to 20-24 days without danger to the consumers, in addition to the fact that no additives were added.

Small sensory and physicochemical changes were observed, which influenced consumer acceptability although not statistically significant. The relationship between sensory and consumer data is of utmost importance to define the optimum handling procedures, once chemical degradation is quite slow in this study.

Regarding the evolution of the sensory attributes, panelists sometimes detected aging starting from the 24^{th} to 28^{th} day of PC products storage time. However, this freshness lost varies from product to product and at a different extent. These observations are not clearly reflected in the studied physicochemical parameters.

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Supplementary Material

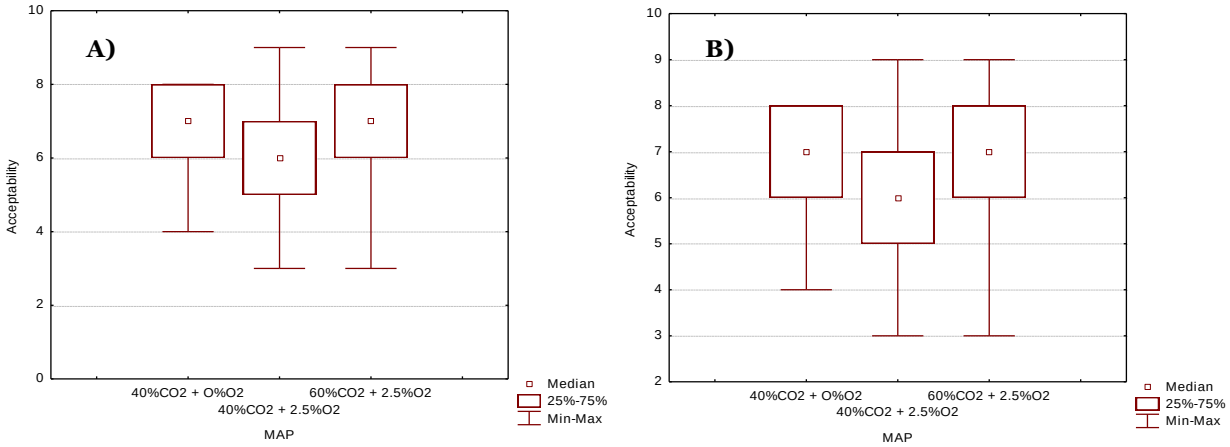

Figure 1 Consumers acceptability concerning precooked vegetables: A) cabbage and B) green beans, on the 10th day of storage under 3 different atmospheres (0/40, 2.5/40 and 2.5/60 % O_2/ % CO_2).

Table 1 Table of correlations between physicochemical and microbial data from pre-cooked vegetables (cabbage and green beans), stored at 5ºC, under different MAP conditions (0/40, 2.5/40 and 2.5/60 as % O_2/ % CO_2), along different storage periods (0, 10, 15 and 20 days)

	EC_{50}	**pH**	**ΔEab**	**CFU**
EC_{50}	1.000	-	-	-
pH	-0.099	1.000	-	-
ΔEab	0.886	0.055	1.000	-
CFU	0.692	-0.618	0.577	1.000

Table 2 Correlations between physicochemical and microbiological parameters from all pre-cooked vegetables under study, stored at 5ºC, in MAP with 0 % O_2/ 40 % CO_2), along different storage periods (0, 7, 14, 20, 24 and 28 days)

	Ac	**pH**	**Ash**	**MC**	**L**	**a***	**b***	**EC_{50}**	**CFU**	**F**
Ac.	1.000	-	-	-	-	-	-	-	-	-
pH	-0.852	1.000	-	-	-	-	-	-	-	-
Ash	-0.458	0.603	1.000	-	-	-	-	-	-	-
MC	-0.086	0.012	-0.410	1.000	-	-	-	-	-	-
L	-0.185	0.352	0.276	-0.012	1.000	-	-	-	-	-
a*	0.538	-0.352	0.219	-0.606	-0.299	1.000	-	-	-	-
b*	0.097	0.100	-0.003	0.175	0.682	-0.286	1.000	-	-	-
EC_{50}	-0.496	0.467	0.689	-0.373	-0.002	0.100	-0.229	1.000	-	-
CFU	-0.102	0.109	0.222	-0.096	0.055	0.165	-0.136	0.114	1.000	-
F	-0.215	0.033	0.262	-0.333	-0.396	0.087	-0.451	0.729	0.091	1.000

Ac – acidity; F-Firmness; MC – moisture content

VI Active MAP improves shelf life of fresh cut bell peppers as assessed by a broad set of quality parameters

Carla Barbosa, M. Rui Alves, M. Beatriz P.P. Oliveira

Neste capítulo, o artigo apresentado centra-se na avaliação da evolução de pimentos verdes, vermelhos e amarelos ao longo de 17 dias de armazenamento embalados em AM com uma combinação inicial de 10% de O2 e 45% de CO2. O objetivo principal é o aumento do tempo de vida útil destes produtos recorrendo a uma alteração brusca da atmosfera (elevado teor de CO2). A utilização desta concentração inicial de CO2 tem a intensão de promover um efeito desacelerador do metabolismo dos pimentos e ao mesmo tempo bacteriostático. Contudo, para não se correr o risco de elevadas percentagens de CO2 prejudicarem os produtos, como referido por alguns autores referidos na parte I desta tese, e ao mesmo para não se correr o risco de anoxia no interior da embalagem, como verificado também em estudos anteriores, foi selecionada uma embalagem com um filme de permeabilidade razoável, que permitiu uma estabilização da composição dos gases dentro da embalagem. Para além disto, pretende-se com este estudo, uma alargada recolha de dados acerca do comportamento dos produtos que permite uma visão mais abrangente e segura da sua evolução.

O efeito deste tratamento foi analisado ao longo de 17 dias de armazenamento, monitorizando a evolução de parâmetros da qualidade e segurança, nomeadamente:- as propriedades organoléticas, por um painel de provadores treinados; a textura e os parâmetros de cor L*, a* e b*, avaliados instrumentalmente; pH, acidez, cinzas e humidade; atividade antioxidante e os teores totais de compostos bioativos (fenólicos, flavonoides, carotenoides e antocianinas); e crescimento de microrganismos totais a 30ºC.

Assim a quantidade de dados recolhidos acerca da qualidade dos pimentos em estudo é vasta e de difícil congregação. Recorrendo à análise multivariada (PCA com *biplots*), foi possível reduzir a informação obtida e compreender a evolução dos produtos bem como a diferenças entre eles, recorrendo a representações gráficas, e as correlações patentes entre os parâmetros analisados.

Com o trabalho verificou-se o tratamento utilizado na conservação dos três tipos de pimentos se mostra eficaz sem perdas acentuadas de qualidade até pelo menos ao 15º dia de armazenamento.

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Submitted

Active MAP improves shelf life of fresh cut bell peppers as assessed by a broad set of quality parameters

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ABSTRACT

Green, red and yellow peppers were stored in actively modified atmosphere packaging (MAP, 10% O_2 and 45% CO_2) at 5 ºC, aiming to increase current commercial shelf-life time. CO_2% used is higher than the commonly used, decreasing progressively over time due to the reasonable permeability of the selected polyethylene film. An initial shock with high CO_2 level followed by stabilization after 5 days of storage occurred. The effect of the treatment was evaluated over 17 days of storage through the analysis of an important set of quality parameters such as pH, acidity, moisture and ash contents, bioactive compounds (total phenols, flavonoids, anthocyanins and carotenoids), total antioxidant activity, color (L*, a* and b*), texture, organoleptic properties and microbial growth. A PCA with predictive biplots confirmed the existence of significant correlations between these parameters and showed that the products retain their initial characteristics without severe loss of quality until at least the 15th day of storage. Sensory analysis shows that panelists also could not find great differences between sampling periods. An increase in 30% of pepper shelf life was enabled by the described treatment.

Keywords: Bell peppers, MAP, sensory analysis, bioactive compounds, principal component analysis

1. INTRODUTION

Minimally processed and ready-to-use vegetables are products with increasing demand, not only for convenience but also for the importance in healthy diets. As highly perishable, their appearance, texture, flavor and nutritional value are factors that influence consumer attitudes and affect purchase decisions. It is important to give detailed information to the processing industries enabling them to adjust their procedures in order to ensure high quality levels from process to consumption.

Consumption of bell peppers (*Capsicum annuun*) has increased, either as major ingredients in salads or as side dishes. They give color, flavor and pungency to the dishes (González-Aguilar et al., 2004; Horvitz and Cantalejo, 2013). Peppers are rich in biological active compounds (phenols, flavonoids, anthocyanins, carotenoids and vitamins A and C) (Manolopoulou et al., 2010) correlated to health benefits, protection properties against diseases like cancer, cataract and macular degeneration (Murcia et al., 2009; Roy et al., 2007) and organoleptic properties (Jiménez-Monreal et al., 2009). However, the phytochemical content is affected by the maturation stage and genotype, the processing operations and storage conditions that the fresh-cut products are subjected to (Zhang and Hamauzu, 2003).

According to Kader and Ben-Yehoshua (2000) more investigation is needed on gas combinations and their effects on senescence and biological activity of the products. Moreover, maintaining the optimum range of temperature and relative humidity during postharvest handling, the use of high CO_2 atmospheres might be a determinant factor to maintain fresh produce quality.

A great research effort has been made regarding the preservation of vegetables with modified atmospheres. Most of these studies involved whole samples (Singh et al., 2014) packed in passive MAP (Barbagallo et al., 2012) or long storage periods in controlled atmospheres (Conesa et al., 2007). Studies involving fresh cuts preserved in active MAP during large periods and supported by a broad set of parameters are scarce. For this reason, this work aimed at evaluating the changes in several physicochemical parameters and bioactive compounds, as well as the evolution of texture, microbial growth and organoleptic properties of green, red and yellow peppers, minimally processed and packed in MAP (10% O_2 and 45% CO_2), seeking the extension of the "best before" presently adopted by industry.

The CO_2 % used is higher than the usually described for controlled atmosphere or MAP (Fernández-León et al., 2013). The high introduced CO_2 level and a *breath-away* film allow an initial extreme atmosphere followed by the gradual release of the CO_2, thus minimizing its possible deleterious effects.

2. MATERIALS AND METHODS

2.1. *Samples*

The samples studied were freshly harvested green (Gp), red (Rp) and yellow (Yp) bell peppers (*Capsicum annuun* L.) obtained in local farmers. Old stems and dark spots were removed before cutting. Washing and disinfection was carried out with potable water and a disinfectant solution (0.3% VERCLOR, Soro Internacional, SA, Spain), over 5 minutes. Peppers were cut into transversal slices after disinfection.

2.1.1. *Minimally processed vegetables*

About 200 g of each sample were packed in Krehalon MLF40 (PA/PE) bags (O_2 and CO_2 transmission rates of 90-130 $cm^3\ m^{-2}$ 24 h and 750-850 $cm^3\ m^{-2}$ 24 h, respectively) and sealed using a VacuumMit P20 (Germany) gas flushing and thermossealer, coupled to a PBI Dansensor A/SMAP Mix 8000EL (Denmark) gas mixer. Samples were prepared in triplicate for each sampling day.

Packaging atmosphere (established in a previous study) was obtained by an active way with initial gas composition of 10% O_2 /45% CO_2 (with N_2 balanced to 100%). The minimally processed samples were stored over 17 days at 5 ºC. Sampling for overall quality evaluation was made at the beginning of the experiment and at the 5^{th}, 10^{th}, 14^{th} and 17^{th} days in storage.

On each sampling day, packaging gas composition was monitored with a gas analyser (O_2/CO_2 CHECKMATE II, PBI Dansensor, Ringsted, Denmark).

For physicochemical analyses samples were grinded in a knife mill. Sample extracts for determination of bioactive compounds were obtained from previously freeze-dried vegetables, and extractions were performed as described in section 2.3.

2.2. *Physicochemical evaluation*

2.2.1. *Moisture content*

Moisture content was determined according to the 984.25 AOAC method (AOAC, 2000). About 3 g of homogenized sample were dried at 103±2 ºC (UTG, Heroeus) to constant weight. Determinations were performed in triplicate and results expressed as percentage of total weight.

2.2.2. *Ash content*

The ash content was determined by 935.42 AOAC method (AOAC, 2000). 3 g of homogenized sample were incinerated in a muffle at 550 ºC until complete carbonization, cooled and weighed. Determinations were performed in triplicate and the results expressed as percentage of total weight.

2.2.3. *Titratable Acidity*

Acidity was determined by titration with 0.1 N NaOH. The results were expressed as g citric acid/100 g fresh weight (942.15 AOAC method) (AOAC, 2000).

2.2.4. *pH analysis*

Evolution of pH values was monitored using a FC232 electrode coupled to a potentiometer HI99163, HANNA Instruments (USA) (981.12 AOAC method) (AOAC, 2000).

2.2.5. *L*, a* and b* color*

Colour measurements were carried out with a MINOLTA CR300 (Konica Minolta, USA), using the Hunter L*, a*, b* colour system. Measurements were performed longitudinally joining four pepper slices, obtaining a sufficiently large area, in such a way that the 8 mm diameter measuring head was completely surrounded by the product surface. CIE L*, a*, b* readings were calibrated against a standard white plat and measurements repeated 10 times for each sample.

2.2.6. *Texture analysis*

The texture parameter analyzed was firmness using a TA-XT2i (Stable Micro System Ltd, UK) equipped with a 2 mm diameter cylindrical stainless steel probe. The compression speed was 1 mm s^{-1} on the cut side of the slice. For each sample, about 20 measurements were done.

2.3. *Extraction of bioactive compounds*

For extraction of bioactive compounds, extracts were prepared according to Porter (2012), with some modifications. About 1.5 g of freeze-dried sample were extracted with 15 ml of 80% methanol (Zhang and Hamauzu, 2004) and filtered. The residue was extracted 2 fold again with 80% methanol and filtrates were centrifuged at 4000 rpm for 30 min. Supernatants were collected and stored at -20 ºC for no longer than 4 weeks.

2.3.1. *Total phenolic content (TPC)*

The Folin-Ciocalteau method (Singleton and Rossi, 1965) was the methodology applied for the determination of TPC. A calibration curve of gallic acid with concentrations varying from 5-100 ppm was used as standard. The results were expressed as milligrams of gallic acid equivalent (GAceq) per liter of extract. 500 µl of sample extract were added to 2.5 ml of Folin reagent (1:10) and 2 ml de $NaCO_3$ (7.5%). Absorbance was measured at 765 nm after 15 min incubation at 45 ºC followed by 30 min at room temperature.

2.3.2. *Total anthocyanins (ANT)*

ANT were determined following Li et al. (2012). 2.5 ml of sample extract were dissolved in 45 ml of methanol, then placed in a ultra-sound bath for 10 min, and heated at 40ºC during 30 min. Absorbencies (Abs) were read at 528 nm. The anthocyanin contents were calculated using the equation:

$$\text{mg Cyd-3-Glu eq/ Lext} = \frac{Abs \times MW \times DF \times 10^3}{\varepsilon \times l}$$

Where Abs = Absorbance; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (Cyd-3-Glu); DF = dilution factor (1:10); l = path length in cm; ε is the 26900 molar extinction coefficient, in L mol^{-1} cm^{-1}, for cyd-3-glu; and 10^3 = factor for conversion from g to mg. Results were expressed in mg Cyd-3-Glu eq/L extract solution.

2.3.3. *Total flavonoids content* (TFL)

TFL content of extracts was determined using a methodology previously described by Costa et al. (2014) with modifications. Catechine standard solutions in the range of 0-400 ppm were prepared for the calibration curve. A white essay was used to calibrate the zero in the equipment and absorbance was measured at 510 nm.

2.3.4. *Total carotenoids* (CT)

CT quantification was performed as described by Yuan et al. (2009). About 1.5 g of freeze-dried sample was mixed with 40 ml of pure acetone, stirred for about 15 min, protected from light, and filtered. 30 ml of petroleum ether was used to wash the residue. This step was repeated twice. The filtrate was transferred to an ampoule and washed for several times with distilled water. The upper phase was collected and volume measured. CT content was determined by spectrophotometry, at 450 nm, using petroleum ether as white standard. The results were expressed in μg of carotenoids /g fresh sample.

2.3.5. *Antioxidant activity - DPPH• assay*

Evolution of antioxidant activity over storage time was determined by DPPH· radical scavenging according to Brand-Williams et al. (1995) with modifications (Porter, 2012; Zhang and Hamauzu, 2003, 2004). A 0.1 mM DPPH· solution (in 80% methanol) was used and added to the extracts (4 ml DPPH solution to 0.2 ml of extract). A control was prepared with 0.2 ml of distilled water instead of the extract. Absorbance was measured at 521 nm using a 96-multiwell plate reader spectrophotometer. 25 μl of sample or control were applied into each well followed by 200 μl of DPPH solution. Radical scavenging activity (RSA %) was calculated as the percentage of discoloration of DPPH solution, using the following equation:

$$\text{RSA } \% = \frac{Abs\ (control) - Abs(sample)}{Abs\ (control)} \times 100$$

The antiradical activity is defined as the amount of sample needed to reduce the initial DPPH· concentration to 50% (EC_{50}). This concentration was obtained from the plot of RSA % as a function of sample concentration.

2.4. *Total microorganisms counting*

Microbial evaluation was performed just as indication of the deterioration extent based on microorganisms-colony-count technique at 30 ºC. Sampling was done in aseptic conditions according to ISO 6887-1:1999 (ISO, 1999) and microbial growth determined following ISO 4833:2003 (ISO, 2003). Small portions from different zones of the package

were used to prepare the test sample for microbiology analysis. Results were expressed in colony forming units (CFU) /g of sample.

2.5. *Sensory evaluation*

The sensory evaluation followed the QDA® methodology (Tragon Corporation, San Francisco, USA), as described by several authors (Meilgaard et al., 2007; Stone and Sidel, 2004) and implemented according to Alves and Oliveira (2005). The methodology involves a simultaneous selection of judges and attributes. Before the evaluation sessions, panel judges focused on the typical characteristics of fresh vegetables and also on the presence of defects caused by senescence. Fresh samples and samples kept in air at 5ºC for different periods of time were used by judges in several discussion/training sessions, in order to understand the course of deterioration and its effects on sensory quality. A list of attributes was decided by consensus, containing the following attributes: general appearance (including surface moisture (A1), typical colour (A2), browning (A3), brightness (A4), texture/shape (A5)); texture measured with forks (including elasticity (A6), cohesiveness (A7), hardness(A8)), aroma typicity (A9), intensity (A10)); texture during mastication (firmness at first bite (A11), firmness at second bite (12)), flavour and taste (including flavour typicity (A13), taste intensity A(14), sourness (A15), bitterness (A16), sweetness (A17)). 13 point scales were used for all attributes.

Products were evaluated by 15 trained judges. In all sessions, fresh cut vegetables were presented as standards. Judges were instructed to start any session by tasting the standard and consider point 7 of each attribute's scale as the standard's magnitude perceived for that attribute. In this way, in all sessions and for all judges, evaluations were made against a fresh standard, reducing randomness in judgments.

2.6. *Statistical analysis*

Evaluation of the main characteristics and the way they changed with time was carried out with Principal Component Analysis (PCA) with predictive biplots, using the Alves (2012) *AutoBiplot.PCA* function written in R (The R project for Statistical Computing, http://www.r-project.org/). Biplot axes, representing initial variables equipped with appropriate measurement scales, were automatically drawn in the biplot displays using a mean standard predictive error (MSPE) of 0.5. R was also used to evaluate the significance of observed differences among several groups through parametric univariate ANOVA tests and Tukey HSD post-hoc tests, as well as with their corresponding non-parametric counterparts, Kruskal-Wallis and Wilcoxon/Mann-Whitney tests (Barbosa et al., 2011). All graphs were produced with R programs specially built by the authors.

3. RESULTS AND DISCUSSION

Peppers are popular vegetables for their combination of color and taste. Peppers are sensitive to chilling temperatures, not being suitable for long, cold storage periods. Adverse storage conditions (T=5±2 ºC) promote water loss and surface pitting, shrinkage, softening, physiological disorders and/or fungal infections are some other phenomena reported to occur during cold storage (Manolopoulou et al., 2010).

To evaluate changes in peppers' quality during refrigeration with MAP several parameters concerning the physicochemical, textural, microbiological and organoleptic characteristics of the product were measured in order to obtain a picture, as complete as possible, of the product's evolution.

3.1. *Gas Composition*

Combining the right temperature and an adequate packaging film is now largely recognized as an efficient preservation method to overcome senescence. In this study the evolution of CO_2 and O_2 levels inside packages during storage of Gp, Rp and Yp in MAP at 5 ºC was monitored (Fig. 1). The CO_2 percentage along the evaluation period decreased gradually and kept quite low after the second sampling moment, near the recommended 2-5% (Saltveit, 2001, 2003).

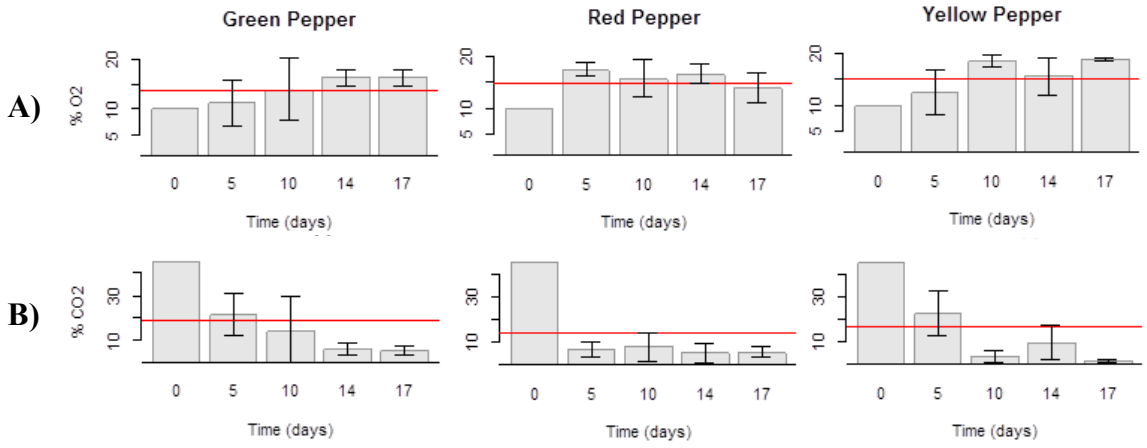

Fig.1. Atmosphere gas composition evolution inside the package: % of O_2 (A) and % CO_2 (B) along storage periods over 17 days (0, 5, 10, 14 and 17 days).

At the end of the study CO_2 levels attain a minimum of 5.3% in Gp, 1.5% in Yp and about 5% in Rp samples (Fig. 1B). This reduction in CO_2 levels is due to diffusion outwards across the film, and to dissolution in the product.

In the beginning of the study the O_2 concentration was 10%, but after 5 days of storage all packages had around 15% O_2 (Fig. 1A). Its concentration in all products and sampling times did not drop to low levels avoiding harmful physiological reactions.

Transpiration did not occur at a concerning level as no condensation inside the package was observed, only in the latest sampling periods some exudates were observed.

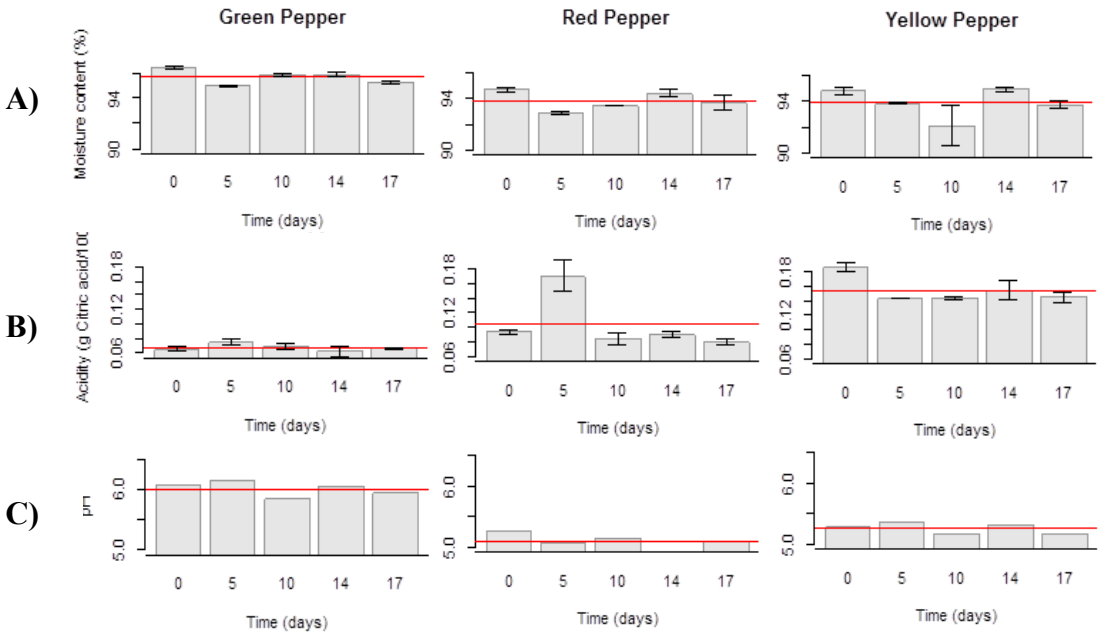

Fig.2. Moisture content (A), acidity (B) and pH (C) values of minimally processed peppers (Gp-green pepper; Rp-red pepper; Yp- yellow pepper), stored at 5ºC, under an initial MAP condition of 10 % O_2 and 45 % CO_2 (with N_2 to complete) along storage periods over 17 days (0, 5, 10, 14 and 17 days).

3.2. *Moisture and ash*

Water loss generally results in a reduction of fresh weight causing degradation of appearance and loss freshness and firmness (Manolopoulou and Varzakas, 2011). The obtained results (Fig. 2A) showed no significant changes in moisture content over the storage time (p>0.05) with losses of 1-2%, except on the 5th day of storage (p<0.05). Concerning ash content, it was quite stable over storage time and no significant changes were determined (p>0.05).

3.3. *Titratable Acidity and pH*

The organic acids are among the compounds considered in this parameter, which greatly influence taste, aroma, color and overall stability of the vegetables. Fig. 2B/C shows the evolution of the values of acidity and pH along the storage time.

Acidity in Gp samples did not vary significantly over storage time (p>0.05). Concerning Rp samples, acidity varied over storage time. According to a post-hoc Tukey HSD, it was significantly increased on the first 5 days of storage (p<0.05), followed by a decrease and stabilization. In the case of the Yp acidity decreased significantly (p<0.05) in the first five days of storage, but stabilized during the remaining storage time. Comparing acidity

values among the three types of peppers, Yp presented the highest values followed by the Rp and Gp. Castro et al. (2008) also reported that Rp presented higher acidity values than Gp, probably due to the different organic acids in their composition.

The pH values determined in this study are in accordance with other authors (Castro et al., 2005; Estrada et al., 2000). As it can be observed in Fig. 2C, Rp presented the lowest pH values, quite stable over time. The pH of Yp was slightly above and also stable over the study time. Gp presented a small variation after the 10th day of storage.

3.4. *Texture*

Significant changes in fresh cut bell peppers firmness were observed only in the first 5 days of sample storage (Fig. 3A). After that time the rupture force decreases to initial values and tends to be constant along the remaining days. This might be due to the washing procedures performed on the fresh cut pepper slices, which improve firmness probably by removing from the cut surfaces some solutes and "stress-related signaling compounds"(Barbagallo et al., 2012; Toivonen and Stan, 2004). Moreover, these results can be due to the initial choc with high CO_2 concentrations, leading to the firmness changes in the initial days of storage. Kruskal-Walis chi-squared tests and Mann-Wittney (Wilcoxon) tests revealed that the 5th day samples were the ones presenting a significant texture difference (p<0.05).

3.5. *Colour evaluation*

The L*, a* and b* values were used to monitor the changes in pepper color during storage. Small changes were observed, following different patterns depending on the type of pepper. Darkening, reflected in a decrease in L* values, occurs at a higher extent in Yp when compared to Gp and Rp. A moderate decrease in L* values over time is observed, which probably reflects an increase in enzymatic activity, in response to mechanical shock (Barbagallo et al., 2012; Castro et al., 2008). Nevertheless, color results from non-parametric analysis revealed no significant differences over storage time (Fig. 3 B/C/D), with the Kruskal-Walis chi-squared test p>0.05 in the case of Rp and Gp. Significant differences, in the case of Yp, are mainly verified between fresh and 17th days samples (Wilcox test, p<0.05). L* is highly correlated with acidity, which in turn correlates with pH and this one influences the enzyme activity as referred above (Table 1). In all cases, for a* and b* values no significant differences were observed during the experiment time (p>0.05).

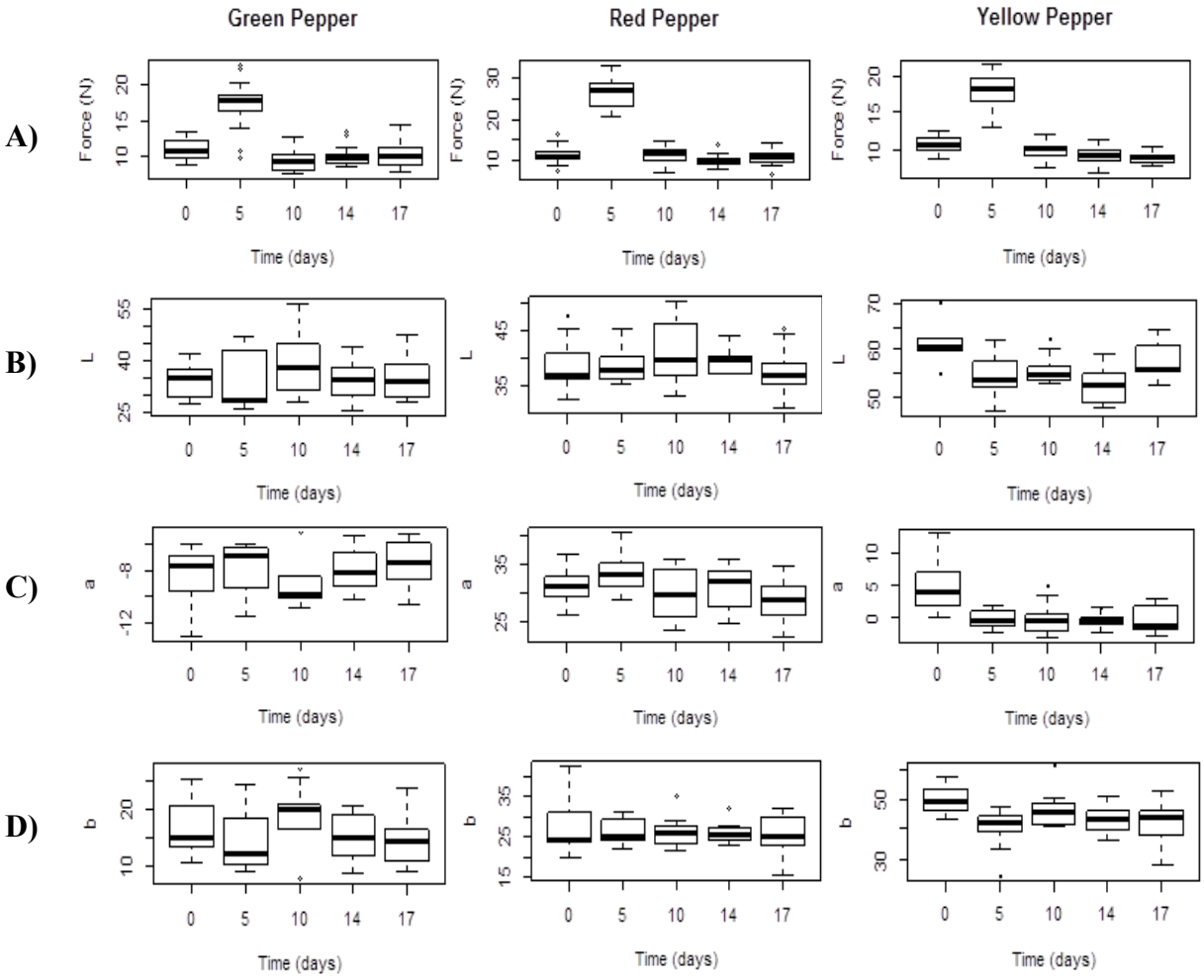

Fig.3. Instrumental firmness (A); L*, a* and b* color values (B, C and D, respectively) of minimally processed green, red pepper and yellow pepper), stored at 5ºC, under an initial MAP condition of 10 % O_2 and 45 % CO_2 (with N_2 to complete) along storage periods over 17 days (0, 5, 10, 14 and 17 days).

3.6. *Antioxidant activity and bioactive compounds evaluation*

Bioactive compounds play a protective role against harmful free radicals and peppers are known to be important sources of these antioxidant compounds (Zhang and Hamauzu, 2003). In this study, bioactive compounds (total phenols, flavonoids, anthocyanin and carotenoids) and antioxidant activity (EC_{50}) were evaluated and any trend in the evolution of these parameters could not be pointed out.

EC_{50} for the three pepper samples and over storage time were not found to be significantly different (p>0.05) (Fig. 4E) exception made to the latest sampling days for Gp and Rp visible on the plot (Fig. 4E). This is an important result as losses in total antioxidant activity are negligible.

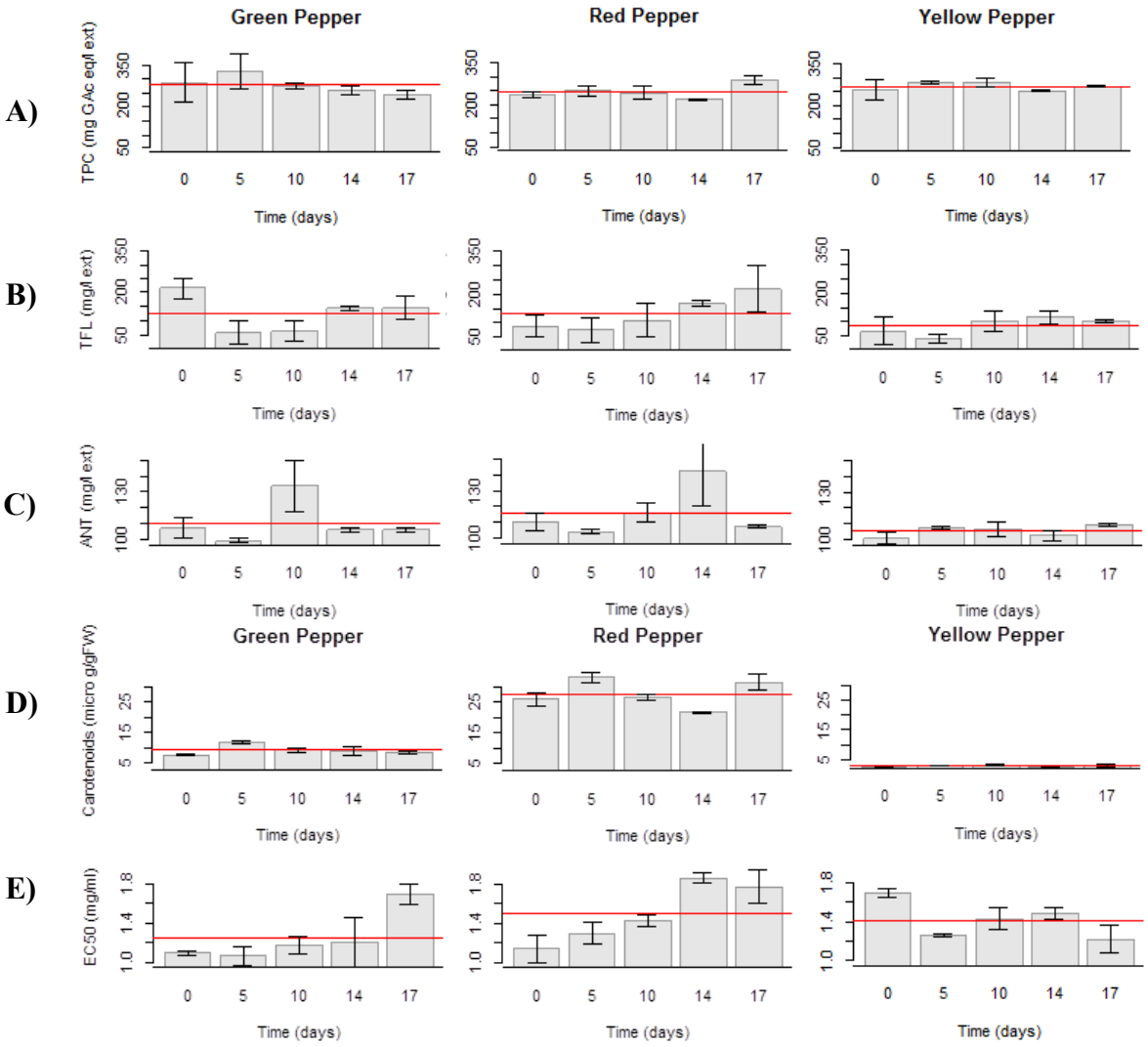

Fig.4. A) Total phenol content (TPC); B) Total flavonoids content (TFL); C) Total anthocyanins content (ANT); D) Total carotenoids content and E) Antioxidant activity (EC_{50}) in minimally processed green, red pepper and yellow pepper), stored at 5ºC, under an initial MAP condition of 10 % O_2 and 45 % CO_2 (with N_2 to complete) along storage periods over 17 days.

3.6.1. *Total phenols content*

Fig. 4A shows that Gp samples have higher TPC than Rp and Yp, although with no significant difference (p>0.05). These results are similar to the ones reported by Zhang and Hamauzu (2003).

3.6.2. *Total Flavonoids content*

The evolution of TFL content is presented in Fig. 4B, with samples maintaining their levels without changes along the storage period (p>0.05). Yp are the poorest samples in what concerns these bioactive compounds.

3.6.3. *Anthocyanin content*

Although some small changes were observed during the storage of peppers (Fig. 4C), these changes seemed to be random, with no apparent trend. In the case of de Gp and Rp small differences are graphically observed (Tukey HSD $p<0.05$ for samples with 10 and 14 days of storage, respectively), and no significant alteration for Yp was registered ($p>0.05$).

3.6.4. *Carotenoid content*

The CT content in Yp and Gp is lower than in Rp not following the tendency described by Zhang and Hamauzu (2003), but in accordance to Navarro et al. (2006), who stated that Rp presented higher values of biologically-active carotenoids. CT evolution along the study time (Fig. 4D) is also different in each case. Rp samples presented small changes over the storage time probably due to the advanced maturation stage. Yp and Gp samples changes were not observed ($p>0.05$), except in samples with five days of storage, that had slightly high values, as happened with other parameters.

3.7. *Microbial growth control*

The results obtained from microbial growth control are presented in Fig.5. Total microorganisms count at 30 ºC remain below the admitted limits within the microbiological criteria of the Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market, established by the Health Protection agency. It is worth noting that total microorganism's count at 30 ºC was only used to help deciding if samples were safe for sensory studies. Authors are aware that this microbiological parameter alone is not enough to assure microbiological safety.

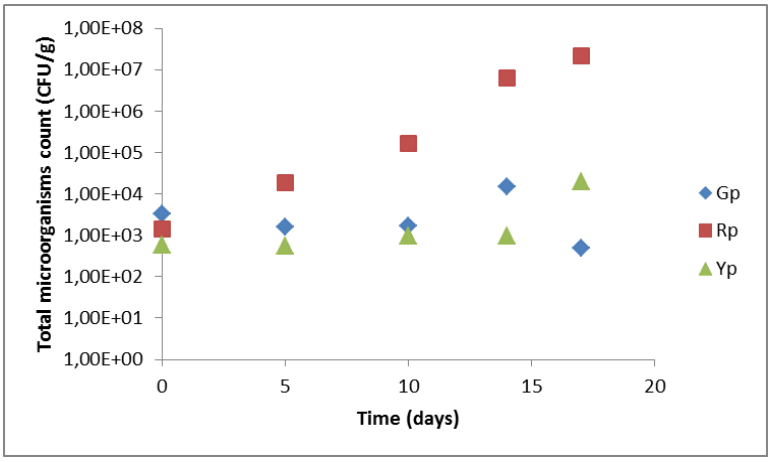

Fig.5. Total microorganisms count at 30 ºC of minimally processed peppers (Gp- green pepper; Rp-red pepper; Yp- yellow pepper), stored at 5ºC, under an initial MAP condition of 10 % O_2 and 45 % CO_2 (with N_2 to complete) along storage periods over 17 days.

3.8. *Interplay of parameters*

Due to the high number of parameters and individual variations observed, it was decided to perform a multivariate analysis to look for main data structures and possible trends (Fig. 6). PCA with predictive biplots was chosen since it enables to carry out interpretations based on initial parameters and respective correlations. To interpret the biplot displays, a straight, orthogonal line is drawn from a sample point to a variable axis, and the value of the variable in that sample is read directly in the display. In this way, principal components do not need to be interpreted and judgments are based on the original data.

Fig. 6 shows that over time the evaluated parameters presented only slight variations. The results corresponding to samples with five days of storage (Gp1, Rp1 and Yp1) deserve some mention, since they generally presented different values from the fresh ones (0 days of storage).

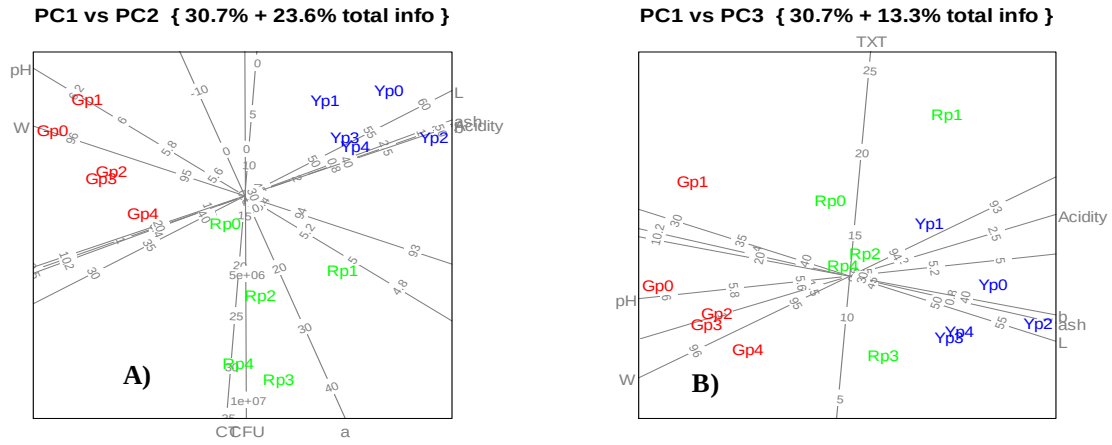

Fig.6. Predictive biplots of physicochemical and microbiological parameters used to evaluate quality changes over storage time applied to the planes: **A)** PC1*vs*PC2 plot presenting most important variables and its relations (pH, moisture content (W), acidity (%), ash content, microorganisms (CFU), total carotenoids (CT) and colour parameters (a and L)); **B)** PC1*vs*PC3 plot of presenting important variables and its relations (pH, moisture content (W), acidity (%), ash content, and colour parameters b and L and texture). Letters identify product type: Gp=green peppers, Rp=red peppers, Yp= yellow peppers associated to numbers (0 to 4) corresponding to sampling/storage time (fresh; 5; 10; 14; 17 days, respectively). Axis scales correspond to initial variables values.

In the display of Fig. 6B, it is seen that on variable TXT (firmness) sample Rp1 projects to 23 N while the other Rp samples project to between 7 and 17 N, sample Gp1 to 17 N and

the other Gp samples to between 7 and 11 N, and finally Yp1 to 16 N, while the others project to between 9 and 12 N. As it is seen this kind of interpretation is very easy and helpful. These results are in agreement with the discussions presented on physicochemical parameters and Fig. 2, 3 and 4.

Concerning samples other than samples with 5 days of storage, no trend was identified. These samples are projected in the graph in the neighborhood of the fresh samples, with a negligible distance. Most of the analyzed parameters show no significant differences between the storage times under study (p>0.05).

The analysis shows the existence of three important data structures visible in Fig. 6A: acidity, ash, b* and L* which are highly correlated (respective axes are almost collinear); pH and moisture content, highly correlated between themselves; a lower correlation between these two structures can also be inferred; a third structure representing the correlation between CFU, a* and CT. In Fig. 6B it becomes clear that TXT is not correlated with other parameters, behaving as a data structure by itself.

Generally, moisture loss is followed by an increase in pH and a decrease in acidity. Strong correlations were observed between the ash content and acidity, presumably due to combinations between minerals such as calcium, phosphorus, and potassium with the organic acids which could influence the buffering capacity (Kader, 2008).

Color parameter b* is negatively correlated with moisture content and pH, although, as it is seen in Fig. 6A, this correlation is relatively small as represented by an increased angle between the respective axes. As expected, PCA differentiates Gp from Rp in terms of the parameter a*, placing them in opposite ends of the a* axis drawn on the PC1 *vs* PC2 plot (Fig 6A). Similarly, Yp samples project onto the b* axis edge corresponding to the highest values of b*. It is also seen that the units projecting to higher values of a* also present lower pH values. These lower pH values may be indicative of some inactivation of cell wall enzymes which may lead to some loss of firmness (Castro et al., 2008; Rao et al., 2011).

Concerning texture, in the plot of PC1*vs*PC3 (Fig 6B) it becomes clear that the first sampling time samples presented higher values of firmness.

PCA reveals no significant changes in TPC content over time except in the case of the 5th day Rp and Gp samples, for the same reasons explained before.

Table 1 Correlations table of all variables studied.

	Acidity	pH	ash	W	L	a	b	EC_{50}	CT	TPC	ANT	TFL	TXT	$\%O_2$	$\%.CO_2$	CFU
Acidity	1,000															
pH	-0,579	1,000														
ash	0,725	-0,491	1,000													
W	-0,547	0,761	-0,529	1,000												
L	0,830	-0,511	0,763	-0,454	1,000											
a	0,144	-0,763	0,024	-0,518	-0,099	1,000										
b	0,865	-0,637	0,857	-0,542	0,969	0,073	1,000									
EC_{50}	0,158	-0,513	0,272	-0,246	0,193	0,372	0,260	1,000								
CT	-0,214	-0,402	-0,343	-0,305	-0,521	0,869	-0,374	0,168	1,000							
TPC	-0,116	0,494	-0,006	0,056	-0,032	-0,534	-0,107	-0,447	-0,300	1,000						
ANT	-0,329	-0,238	-0,339	0,099	-0,174	0,269	-0,197	0,247	0,218	-0,439	1,000					
TFL	-0,486	0,041	-0,112	0,240	-0,435	0,128	-0,351	0,379	0,241	-0,109	0,127	1,000				
TXT	0,328	-0,075	-0,102	-0,351	-0,134	0,274	-0,096	-0,269	0,385	0,183	-0,292	-0,413	1,000			
$\%.O_2$	0,181	-0,310	0,109	-0,462	0,169	0,030	0,143	0,175	-0,046	-0,324	0,201	-0,017	-0,015	1,000		
$\%.CO_2$	0,030	0,217	0,096	0,412	0,014	-0,033	0,046	-0,255	-0,075	0,135	-0,228	-0,058	-0,007	-0,900	1,000	
CFU	-0,213	-0,330	-0,028	-0,186	-0,195	0,432	-0,098	0,538	0,503	0,106	0,139	0,595	-0,118	-0,151	-0,129	1,000

In the case of flavonoids, PCA only gives them relevance on the plane PC2*vs*PC3. The corresponding axis (TFL) does not appear on the plane PC1*vs*PC2 because it does not follow the trends of other variables in the plot PC1*vs*PC2 and does not contribute to separate varieties or sampling times. The table with correlations (Table 1) shows a relation between the TPC and EC_{50}.

These results show that the preservation technique allows conservation and it is able to maintain the levels of flavonoids, an important conclusion from the nutritional point of view.

3.9. *Sensory evaluation*

Concerning sensory quality, the panel evaluation confirmed the discussed about the evolution of the physicochemical quality parameters. PCA with VARIMAX rotation of sensory data (Fig. 7) shows some strong correlations between attributes, such as flavor typicity (A13) and taste intensity (A14). This analysis also removes from the plot the axis of the attributes with a mean standard predictive error higher than 0.5. These attributes are A3, A5, A6, A9, A10, A12, A15, A16 and A17 related to all the categories of characteristics evaluated (appearance, texture, mouth feel, aroma and taste). This illustrates the difficulty experienced by the panel during the evaluation process, due to the difficulty in pointing out differences between samples or finding out problems in the samples, when comparing to standard (fresh product) always present in every session.

The majority of the samples are projected together close to the center of the sensory evaluation scale in the neighbour of point 7 corresponding to the classification attributed to the fresh samples.

The PCA plot PC1vs PC2 (Fig. 2) shows that Rp samples with 10 (Rp3) and 17 (Rp4) days of storage stand out from the remaining. The former has high values for attributes A15 and A2, and low values for A6 and A7. The latter is more extreme that A3, and also shows very high values in A4 and low values in A8. due to increasing sour taste (A15), in Rp3 samples and excessive brightness (A4) and surface moisture (A1) revealing an abnormal release of exudative liquids in Rp4. However, sour taste is considered typical in peppers and as it can be seen in the plots the A15 axis has the same orientation and is close to A13 and A14. Gp samples with 17 days of storage (Gp4) are also judged as slightly different from the others mainly due to low values in A8, although not as extreme as Rp4 that seems to be separated also from the main group because the panelists found these samples more soft and also with usual brightness and surface humidity.

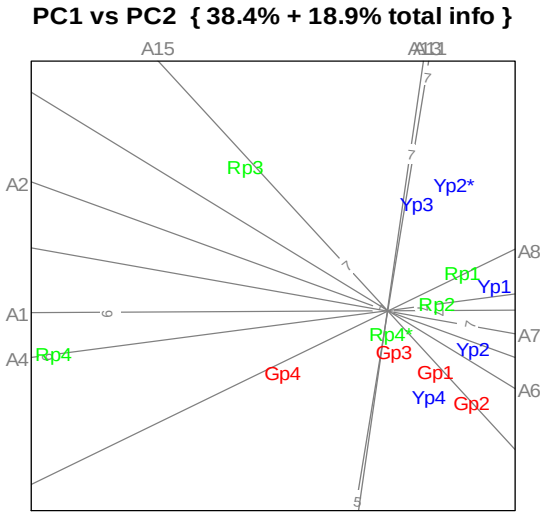

Fig.7. Predictive biplots of sensory data over storage time applied to the plane PC1*vs*PC2. Inside the plot letters identify product type: Gp=green peppers, Rp=red peppers, Yp= yellow peppers associated to numbers (0 to 4) corresponding to sampling/storage time (fresh; 5; 10; 14; 17 days, respectively). Letters around the graph represent attribute biplots: surface moisture (A1), typical colour (A2), browning (A3), brightness (A4), texture/shape (A5), elasticity (A6), cohesiveness (A7), hardness(A8), aroma typicity (A9), intensity (A10), firmness at first bite (A11), firmness at second bite (12), flavour typicity (A13), taste intensity (A14), sourness (A15), bitterness (A16), sweetness (A17). Axis scales correspond to initial attributes values representing the scale used.

Apart from these differences, in judges' opinion, samples were quite similar to the standard and no major problems were found.

4. **CONCLUSIONS**

The effect of the initial high CO_2 percentage powers the barrier effect of MAP, by slowing down metabolic reactions in peppers. Small physical and chemical changes were observed only during the first sampling period (5 days). Changes tended to stabilize, with no significant differences between sampling periods till the end of the study. Sensory analysis confirmed the results with experienced panelists not being able to discriminate products between sampling periods. It can be concluded that the treatment used enabled an increase in almost 30% of pepper shelf life time.

Although the initial CO_2 concentration used was high (45%), causing an initial shock, the final levels of CO_2 were small due to the porosity of the film used, allowing higher gas exchange rates and the release of CO_2 excess, thus minimizing possible deleterious effects.

In light of the hurdle technology, severe changes in the atmosphere gas composition, low storage temperatures and the use of a package composed by polymer with higher permeability than those commonly used in the industry allowed the deceleration of quality loss.

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VII Read-to-eat (fresh-cut and precooked) cabbage and green beans: effect of MAP on mineral and microstructure, bioactive compounds and sensory quality

Carla Barbosa, M. Rui Alves, O. Morais e M. Beatriz P.P. Oliveira

O trabalho apresentado neste capítulo teve como objetivo estudar a evolução da qualidade de legumes verdes (couve e feijão verde), minimamente processados (MP) e pré-cozidos (PC) conservados em AM. Para tal, foram avaliadas as propriedades organoléticas, relacionadas com a evolução da textura e microestrutura, e ainda com os compostos bioativos e o teor de minerais. Estes últimos mostraram-se de importância relevante na manutenção da qualidade nutricional destes produtos.

Os requisitos de embalagem são diferentes nos dois tipos de processamento, tanto ao nível da composição de gases da atmosfera envolvente como do tipo de filme usado na embalagem. Neste sentido, foram usadas embalagens de baixa permeabilidade e uma atmosfera de 10% O2 e 45% de CO2 para os legumes MP. No caso dos PC o filme usado era de alta barreira e usou-se uma combinação de gases de 0% O2 e 40% de CO2.

O procedimento experimental para a determinação dos teores de minerais foi a microscopia eletrónica de varrimento (SEM). Trata-se de uma análise rápida, que pode fornecer informação válida e comparável com as tendências observadas nos demais parâmetros analisados.

A variedade de parâmetros envolvidos nesta fase do trabalho e a quantidade de dados obtidos justificou, mais uma vez, o recurso a técnicas quimiométricas. Neste caso, para além da análise componentes principais com *biplots*, foi feita uma análise de correlações canónicas para investigar as correlações entre as variáveis (de diferente natureza) em estudo.

O trabalho permitiu concluir que as perdas de minerais são reduzidas ao longo do tempo de armazenamento e que se correlacionam com a avaliação sensorial. A perda de humidade ou a inclusão de etapas de cozedura conduz a perdas de minerais mais acentuadas.

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Submitted

Read-to-eat (fresh-cut and precooked) cabbage and green beans: effect of MAP on mineral and microstructure, bioactive compounds and sensory quality

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Abstract: The effect of storage under modified atmosphere packaging (high % CO_2 and low %O_2) on bioactive compounds (total phenols, flavonoids, carotenoids and anthocyanins), mineral content and microstructure, and sensory quality of ready-to-eat cabbage and green beans was assessed. Vegetables were stored fresh-cut and precooked in MAP conditions of 10/45 and 0/40 (% O_2/ %CO_2) during 17 and 28 days, respectively, at 5 °C. A low barrier film was used for fresh-cut samples. Precooked vegetables were packaged in trays sealed with a high barrier polyethylene film. Sensory evaluation was carried out by a trained panel performing a QDA with attributes related to general appearance, color, flavor (aroma and taste) and texture (hardness and mouth feel perception). Elemental analysis and microstructure were evaluated by SEM. Canonical correlation analysis (CCA) was used to investigate how parameters correlate over the storage period. Mineral losses were very small over storage time and correlated with sensory data. CCA outputs showed that PC samples were grouped due to Na and Cl contents. Samples with higher moisture surface and brightness presented loss in Mg. MP cabbage showed a better retention of P, S and Ca. Although moisture content was related to mineral loss it had no impact on flavor.

Keywords: MAP, Ready-to-eat vegetables, mineral content and bioactive compounds, sensory quality, microstructure, canonical correlation

1. Introduction

Consumers expect industrialized foods to be not only safe, but also good in flavour, convenient and with health-enhancing properties (Bruhn, 2010; Tomás-Barberán & Espín, 2001). Moreover, daily consumption of fresh fruits and vegetables is recommended to prevent major health disorders such as cardiovascular diseases and certain cancers (Ekholm et al., 2007). Vegetables are rich sources of vitamins, biologically-active compounds and also excellent dietary sources of minerals. Little attention has been paid to this last issue (López, Fenoll, Hellín, & Flores, 2013) specially because the handling procedures (washing and cutting) of raw vegetables and the thermal treatment (boiling) of precooked vegetables can induce lixiviation and the consequent loss of nutritional value (Radziejewska-Kubzdela, Czapski, Czaczyk, & Bieganska-Marecik, 2014). Thus, food safety and preservation of chemical composition should be of major concern when preservation methods are applied.

Modified atmosphere packaging (MAP) of ready-to-eat (RTE) vegetables has been extensively used as a preservation procedure. RTE vegetables may be presented to consumers as minimally processed (MP) vegetables, usually only washed or possibly cut and packaged, or included in soups or other dishes. RTE foods are also presented as precooked (PC) to be consumed as it is or after warming.

The natural variability of vegetables and their response to processing and storage conditions leads to uncertainties and difficulties in the identification of optimal storage conditions. Many studies have been published without a clear definition of the processing and storage conditions necessary to achieve higher quality standards, safety assurance, convenience and pleasure. Thus knowledge is needed on the correlation of data from processing procedures, organoleptic properties, nutritional value, microstructure and physical characteristics, simultaneously with the type of package and the initial nutritional value of these products (Hussein et al., 2000). According to additional refinements in recommendations for modified atmosphere (MA), storage of vegetables will continue to grow guided by empirical observations derived from many independent experiments being carried out in this subject (Saltveit, 2003). Understanding how vegetables behave when exposed to different storage conditions and the impacts on their nutritional value, structure and organoleptic characteristics is of utmost importance for the food industry in order to fulfil consumer's demands.

The control of minerals and bioactive compounds in vegetables is also important for industrial logistic and commercial procedures, in order to extend vegetables' shelf lives.

This work aimed at studying the sensory quality of minimally processed (MP) and precooked (PC) green beans and cabbages, stored in MAP (CO_2 rich atmosphere) during 17

and 28 days, respectively, and the influence of packaging on some physicochemical parameters, bioactive compounds, minerals and microstructure.

2. Materials and Methods

2.1. *Sample preparation*

Cabbage (*Brassica oleracea* L.) and green beans (*Phaseolus vulgaris* L.) obtained in local farmers were cut and sliced. Old leaves, stems and dark spots were removed before cutting. MP samples were washed and disinfected using potable water and a disinfectant solution (0.3% VERCLOR, Soro Internacional, SA, Spain), over 5 minutes. PC vegetables were obtained by boiling for 8 minutes, in salted water (1% p/v) after washing with potable water.

2.2. *Atmospheres and sampling*

MAP conditions were defined taking into consideration previous studies developed by the team (submitted data). MP cabbage (Cb) and green beans (Gb) were stored in MAP with 10 % O_2 and 45 % CO_2 (with N_2 balanced to 100%). Samples were kept at 5 ºC, and physicochemical, SEM and sensory parameters were evaluated at 0, 5, 10, 14 and 17 days after packaging.

PC cabbage (Cb*) and green beans (Gb*) were stored in MAP with 0 % O_2 and 40% CO_2 (with N_2 balanced to 100%). Samples were kept at 5ºC, and physicochemical, SEM and sensory parameters were evaluated at 0, 7, 14, 20, 24, and 28 days after packaging.

2.3. *Modified atmosphere packaging equipment and materials*

MP vegetables were packed in Krehalon MLF40 (PA/PE) bags, with O_2 and CO_2 permeability of 90-130 $cm^3\ m^{-2}$ 24 h e 750-850 $cm^3\ m^{-2}$ 24 h, respectively.

PC vegetables were packed in multilayer polystyrene (EPS/HIPS/PE) trays (B22-50) and sealed with a selective high barrier Technopack film (Pa/Pe, Miranda e Serra, Portugal). A Yang SR-I Oceania Jolly 20 thermossealer (Italy) was used for package sealing.

Inside package atmosphere was substituted in an active way using a PBI Dansensor A/SMAP Mix 8000 EL (Denmark) gas mixer. On sampling days, inside gas composition was verified with a gas analyser (O_2/CO_2 Checkmate II, PBI Dansensor, Ringsted, Denmark).

2.4. *Physicochemical evaluation*

After opening the package at each sampling day, sample moisture contents (MC) and firmness were determined. For MC samples were grinded in a knife mill (GM 200, RETSCH, Haan, Germany), determined in triplicate according to the 984.25 AOAC

method (AOAC, 2000), and results expressed as %. Briefly about 3 g of sample homogenate were dried at 103±2 ºC (UTG, Heroeus) to constant weight. Firmness, a texture parameter defined as the maximum force exerted in compression, was determined (n=20) with a TA-XT2i (Stable Micro Systems Ltd., U.K.) equipped with a 2 mm diameter cylindrical stainless steel probe and a compression speed of 1 mm s^{-1}.

For the evaluation of bioactive compounds, vegetables were freeze-dried and stored until analysis. For extraction of bioactive compounds, extracts were prepared according to (Porter, 2012), with some modifications. About 1.5 g of freeze-dried sample were extracted with 15 ml of 80% methanol and filtered. The residue was extracted 2 fold again with 80% methanol and then filtrates were centrifuged at 4000 rpm for 30 min. Supernatants were collected and stored at -20 ºC for no longer than 4 weeks. Methanolic extracts were chosen based on the studies of (Zhang & Hamauzu, 2003) who concluded that values of anti-radical scavenging activity of this type of extracts were higher compared to other solvents.

Total phenolic content (TPC) determination: The Folin-Ciocalteau method (Singleton & Rossi, 1965) with gallic acid as a standard was the methodology applied for this determination. A calibration curve of gallic acid with concentrations ranging from 5 to 100 ppm was used. The results were expressed as milligrams of gallic acid equivalent per L of extract solution. 500 µl of sample extract were added to 2.5 ml of Folin reagent (1:10) and 2 ml de $NaCO_3$ (7.5%). After incubation for 15 min at 45 ºC and 30 min at room temperature, absorbance was measured at 765 nm.

Total anthocyanin determination: Anthocyanins (ANT) were determined according to Li et al. (2012). 2.5 ml of sample extract were dissolved in 45 ml of methanol, then placed in an ultra-sound bath for 10 min, and heated at 40 ºC during 30 min. Absorbance (A) was read at 528 nm. The anthocyanin contents were calculated as cyanidin-3-glucoside equivalent, mg/L, using the equation:

$$\text{mg /L ext}=\frac{A\times MW\times DF\times 10^3}{\varepsilon\times l}$$

where A = Absorbance; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (Cyd-3-Glu); DF = dilution factor (1:10); l = path length in cm; ε (26900 molar extinction coefficient, in $L.mol^{-1}\ cm^{-1}$, for cyd-3-glu); and 10^3 = factor for conversion from g to mg. Results were expressed in mg Cyd-3-Glu equivalent per L extract solution.

Flavonoid determination: Total flavonoid (FL) content of the extracts was determined using a methodology previously described by Costa et al. (2014) with modifications. Catechine standard solutions in the range of 0-400 ppm were prepared for the calibration

curve. The absorbance was measured at 510 nm and to calibrate the zero of the equipment a blank control was used. Results were expressed as mg of catechine equivalent per L extract solution.

Total carotenoid determination: Total carotenoids (CT) quantification was performed generally as described by Yuan, Sun, Yuan, and Wang (2009). About 1.5 g of freeze-dried sample was mixed with 40 ml of pure acetone, stirred for about 15 min, protected from light, and filtered. 30 ml of petroleum ether was used to wash the residue. This step was repeated two times. The filtrate was transferred to an ampoule and washed for several times with distilled water. The upper phase was collected and volume measured. Total carotenoid content was determined by spectrophotometry UV/Vis, at 450 nm, using petroleum ether as blank standard. The results were expressed as μg/g of fresh sample (FW).

2.5. *Microstructure and Mineral content determination*

Minerals (Ca, P, Mg, Na, K, Fe, Cl and S) and microstructure were analyzed by scanning electron microscopy using a Hitachi SU1510 (Japan) coupled to a Si-based semiconductor X-ray detector Bruker NanoXFlash 5010 (UK) for microanalysis of minerals. Before SEM analysis, MP and PC samples were freeze-dried and coated in a rotary pumped carbon coater (Quorum Q150R E, UK.). About five images for each sample were taken at 4 different magnifications and 4 different distance shoots (×50 / 1.0 mm; ×100 / 500 μm; ×200 / 200 μm; ×750 / 50.0 μm). Elemental analysis was performed using the X-ray detector after getting the SEM images. The electron beam stimulates the atoms in the sample with uniform energy and they instantaneously send out X-rays of specific energies for each element. This radiation gives information about the elemental composition of the sample. X-ray spectrum is converted to a quantitative analysis by ESPRIT 1.9 software that compares the sample mineral spectrum to a set of standards stored in a database. Data is expressed as compositional weight percent (Wt %) of Ca, P, Mg, Na, K, Fe, Cl and S normalized to the total signal from the detected elements.

2.6. *Sensory evaluation*

2.6.1. *Panel training*

Before the evaluation sessions, panel judges focused on the typical characteristics of freshly prepared MP and PC vegetables and also on the presence of defects caused by senescence. Freshly prepared samples and samples kept in air at 5 ºC for different periods of time were used by judges in several discussion/training sessions, in order to understand the deterioration process and its effects on sensory quality. A list of attributes was also decided by consensus, containing the following attributes: general appearance (including

surface moisture (A1), typical colour (A2), browning (A3), brightness (A4), texture/shape (A5), texture measured with forks, including elasticity (A6), cohesiveness (A7) and hardness (A8), aroma typicity (A9) and intensity (A10), texture during mastication including firmness at first bite (A11) and firmness at second bite (A12), flavour and taste including flavour typicity (A13), taste intensity (A14), sourness (A15), bitterness (A16) and sweetness (A17).

2.6.2. *Quantitative descriptive analysis (QDA)*

The sensory evaluation followed the QDA® methodology (Tragon Corporation, San Francisco, USA), as described by several authors (Lawless & Heymann, 1999; Meilgaard, Civille, & Carr, 2007; Stone & Sidel, 2004) and implemented in the Polytechnic Institute of Viana do Castelo (IPVC) according to M. Rui Alves and Oliveira (2005).
Products were evaluated by a 15 member trained panel, using the same attributes as before, but with 13 point scales. In all sessions, freshly prepared vegetables were presented as standards. Judges were instructed to start any session by tasting the standard and consider point 7 of each attribute's scale as the standard's perceived magnitude. In all sessions and for all judges, evaluations were made against a fresh standard, reducing randomness in judgments.

2.7. *Statistical analysis*

Data mining was carried out with canonical correlation analysis (CCA) to describe the correlations between data sets used to relate and simplify information. CCA enabled analysis and visualization of the significance of observed differences between products (Barbosa, Oliveira, & Alves, 2011). Evaluation of the main characteristics and the way they changed with time was carried out with Principal Component Analysis (PCA) with predictive biplots, using the M. R. Alves (2012) *AutoBiplot.PCA* function written in R (The R project for Statistical Computing, http://www.r-project.org/). This function allows the calculation of the average standard predictive error (*mspe*) for each axis and automatically draws the axis in the PCA graph, with a convenient measurement scale, if the *mspe* is not exceeded. Canonical variates analyses were used to evaluate judges' results, and were carried out using the Statistica for Windows software package, version 7 (Statsoft Inc., Tulsa, USA).

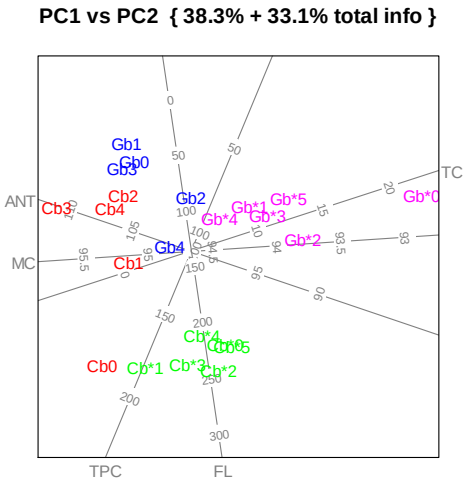
3. Results

A PCA with predictive biplots (Figure 1- physicochemical data (I), mineral content evaluation (II) and sensory data (III)) was performed as described by M. R. Alves (2012) and Barbosa et al. (2011) to evaluate the main characteristics and their changes with time.

Any biplot axis represents one initial variable equipped with a scale for appropriate measurement, which allows inferring the initial values of samples in that variable directly from the readings carried out in the graph. The *mspe* controls the selection of an axis to be displayed or not, based on the average accuracy of readings. The biplots are accompanied by a table of outliers, which are defined as samples for which a reading may result in an erroneous assumption.

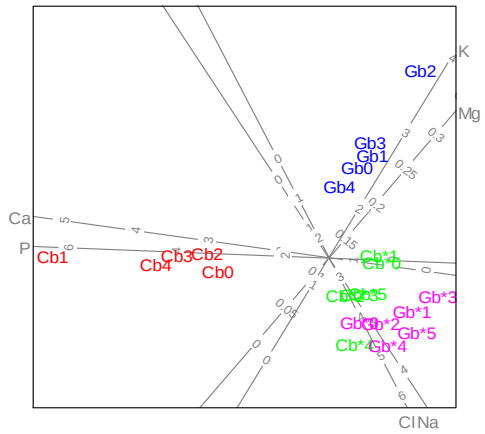
PCA of bioactive compounds, firmness and MC data (Fig. 1 (I)) shows only slight variations along the experiment. In what concerns the firmness axis, it does not appear on the plot (*mspe* > 0.75) meaning that this parameter is not relevant for sample discrimination. Nevertheless differences between PC (Cb* and Gb*) and MP (Cb and Gb) vegetables are visible on the biplot. Carrying out the orthogonal projecting of PC samples onto TPC and FL axes (Figure 1 I), it is seen that these samples have higher values then the MP samples. For example, TPC for Cb* project to 150-200 mg/L ext. and Cb to 100-125 mg/L ext., except for Cb1 considered an outlier as shown in the corresponding table of outliers. FL for Cb* varies between 200-250 mg/L ext. and for Cb between 80-120 mg/L ext. This is in accordance to some published studies (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Porter, 2012) being markedly higher in the case of cabbage samples as also referred by Volden et al. (2008). Concerning natural pigments (ANT and TC) the observed losses are mostly due to cooking procedures (ANT for Cb is 102-107 mg/L and for Cb* is 95-100 mg/L ext., except the outlier Cb*3). However along storage differences did not present any tendency in both cases. Concerning firmness, although statistically less important in this analysis (axis not drawn in the plot), differences between PC and MP follows the same pattern.

I)



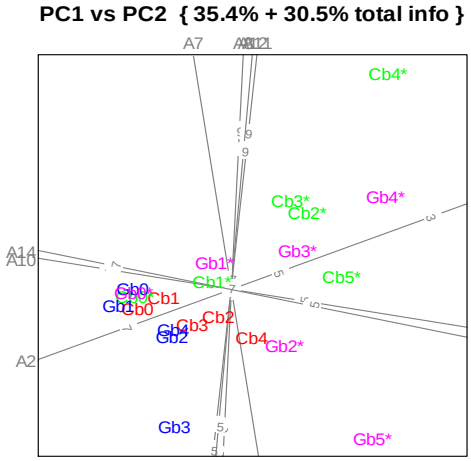
	Biplot Axis	Outlier	Unit.pe
1	MC	Gb2	1.11
2	MC	Gb4	1.26
3	MC	Gb*4	1.24
4	TC	Cb0	0.98
5	TC	Gb*0	1.46
6	TPC	Cb1	0.88
7	ANT	Cb*3	0.77
8	ANT	Gb1	1.06
9	ANT	Gb4	1.10
10	ANT	Gb*0	0.96
11	ANT	Gb*4	0.83
12	FL	Cb*0	0.80

II)



	Biplot Axis	Outlier	Unit.spe
1	Ca	Cb1	0.79
2	Ca	Cb2	0.95
3	P	Cb1	0.83
4	Mg	Gb1	0.79
5	Mg	Gb*0	0.82
6	Mg	Gb*3	0.85
7	Cl	Cb1	0.96
8	Cl	Cb*0	0.98

III)



	Biplot Axis	Outlier	Unit.spe
1	A2	Gb1*	0.86
2	A2	Gb4	0.91
3	A10	Cb4	1.08
4	A11	Cb5*	0.78
5	A11	Gb4*	0.87
6	A12	Cb4	0.89

Figure 1 – PCA with autobiplots applied to: physicochemical (I), mineral content (II) and sensory data (III) over storage time and respective table of outliers on PC1 vs PC2. Samples Cb- MP cabbage; Gb- MP green beans; at different storage times (0- 0 days, 1- 5days, 2- 10 days; 3- 14 days; 4- 17 days); and PC vegetables (Cb*- cabbage; Gb*- green beans; at different storage times (1- 7days, 2- 14 days; 3- 20 days; 4-24 days; 5- 28 days)) are projected to the biplots axis with a mean

standard predictive error < 0.5. Higher values are considered outliers being presented on the right side tables, next to each plot.

Mineral composition by SEM analysis is a practical method to rapidly identify mineral phases, using integrated computer software, producing information on minerals composition and distribution, along the images generated by the scanning electron microscope. Figure 2 is an example of the spectra obtained during the observation of a PC cabbage sample with five days of storage.

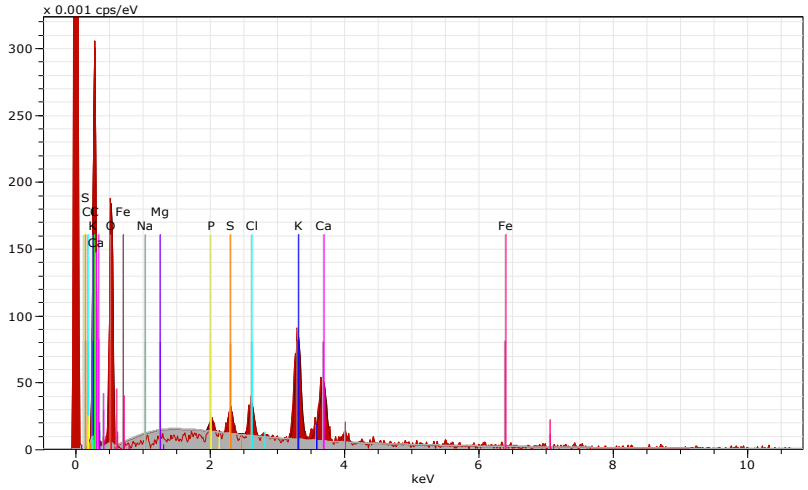


Figure 2 – Example of a mineral composition spectrum in PC cabbage samples with five days of storage.

Being a kind of semi-quantitative information, the apparent concentration of low atomic number elements tends to be slightly underestimated while the apparent concentration of elements with relatively high atomic number tends to be slightly overestimated as reported by Laskin and Cowin (2001). Organic particles are difficult to analyze due to their susceptibility to beam damage, low contrast in the SEM images, low atomic mass, and possible contamination of the carbon signal from the film background (Laskin & Cowin, 2001). Nevertheless, performing an adequate number of replications with an appropriated statistical analysis, it can rapidly give useful information about mineral distribution along the microstructure and about mineral losses during processing or storage. Concerning cabbage and comparing MP with PC samples, losses in Ca and P content were observed (Fig. 1. II). In Cb samples P ranged from 3 to 5 % wt, except in outlier samples with 5 days of storage (Cb1). In precooked Cb products, P values were around 1 %wt. These low values are probably due to leaching into the cooking medium. In PC cabbage a small increase in

K, Mg and Fe was also observed when comparing to MP samples, as reported in studies comparing fresh with boiled, microwaved or steamed food products (Hosseini et al., 2014; Rickman, Barrett, & Bruhn, 2007; Rickman, Bruhn, & Barrett, 2007; Santos et al., 2014). According to Rickman, Bruhn, et al. (2007) minerals are resilient nutrients in general, stable to processing and storage. Therefore changes in their content are generally negligible unless they are added during processing or lost in peeling or cutting operations or by leaching during boiling. They are heat stable under typical processing conditions although P, Na and Ca might be affected by hard water used in processing.

Physical properties (firmness) and bioactive compound levels, studied over the storage period, did not present a clear tendency (decrease or increase levels). According to the results obtained it can be shown that plant tissue reacts against the MAP conditions but generally no significant differences are observed over storage time. This was also felt by the panelists in sensory analysis. Canonical variates analysis of sensory data are presented in Figure 3 A) and B). Each point corresponds to one judge opinion, and it is clear their difficulty in discriminating samples or even see differences when comparing to standards (freshly prepared samples). In this analysis only CV1 (left to right variation on the scatterplot) is important, meaning that no significant alterations were detected over time. On plot B for PC green beans a separation ($p<0.05$) of the marks corresponding to the last sampling time (28 days) is observed, meaning that at this point some problems in sensory quality appeared.

SEM images of MP and PC vegetables' microstructure also support these observations (Figure 4). Concerning MP cabbage and green beans, both show quite stable microstructures, remaining turgid, ordered and with well-defined cellular structures over storage time (Fig 4, A to E MP images). Comparing to cooked samples, softening involving cell separation is patent on the SEM images of PC vegetables (Figure 4). This fact is in agreement with the decrease in firmness (Fig 1. I). Concerning evolution during storage, SEM images of PC cabbage do not differ significantly, while in the case of green beans microstructure softening is observed after 7 days, but maintaining the same structure afterwards, until the 24th day of storage. On the 28th day an increase in softening is again observed. These facts are in agreement to instrumental firmness (Fig 1.I).

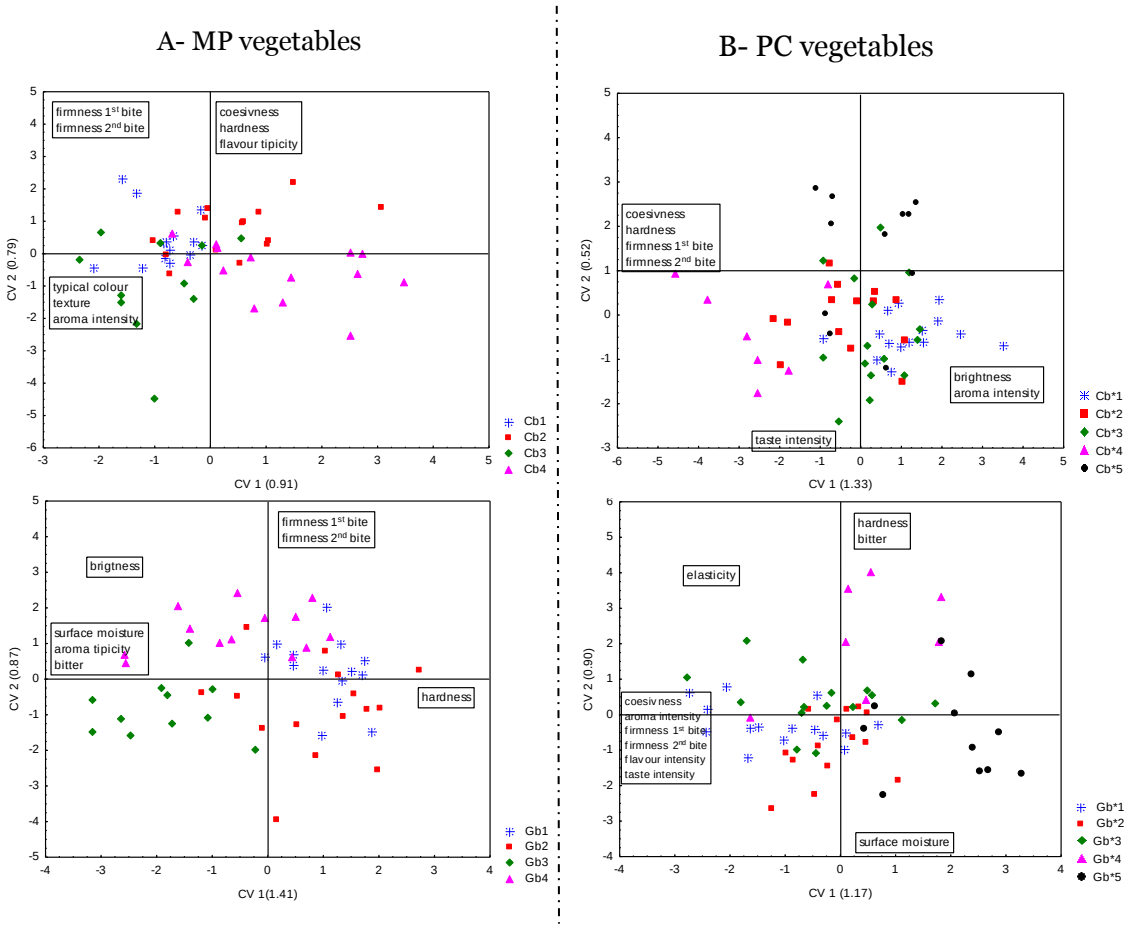


Figure 3 – CVA of sensory evaluation of :A) MP vegetables (Cb-cabbage; Gb-green beans; at different storage times (1-5days, 2-10 days; 3-14 days; 4-17 days)); and B) PC vegetable (Cb*-cabbage; Gb*- green beans; at different storage times (1- 7days, 2- 14 days; 3- 20 days; 4-24 days; 5- 28 days)).

Data mining was carried out by a CCA where data was conveniently arranged in multivariate matrices. Canonical dimensions describe data structures in one data set that are correlated with data structures in other data set, and were used to simplify and relate information (Figure 5).

In this study, 3 data matrices were obtained: one relative to the sensory evaluation of all sample products over time; another relative to the analysis of some bioactive compounds, moisture content and texture; and another one relative to mineral contents. Data sets were analyzed combining: I) Mineral content related to bioactive compounds, texture and moisture content; II) Bioactive compounds, texture and moisture content related to sensory data; III) Mineral content related to sensory data.

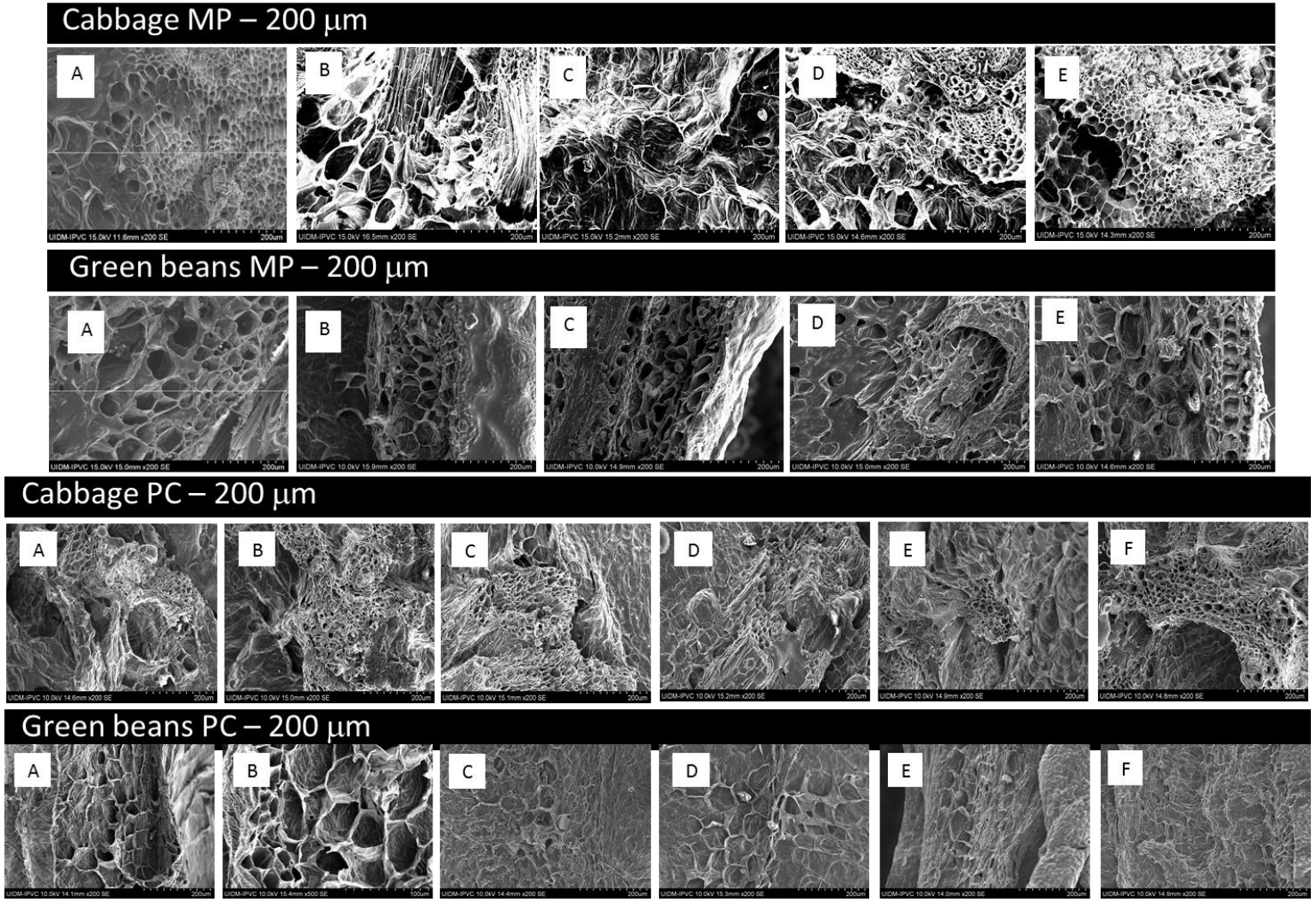


Figure 4 – SEM images of minimally processed (MP) cabbage and green beans stored for different periods over 17 days: A- 0 days; B – 5 days; C- 10 days; D- 14 days; E-17 days and precooked samples (PC) stored over 28 days: A- 0 days; B – 7 days; C- 14 days; D- 20 days; E-24 days; F- 28 days

It can be extracted that mineral losses are very small over storage time, as expected, but when observed, they correlate with sensory data. CCA outputs show that generally PC samples are grouped due to high Na and Cl contents, added as NaCl during cooking procedures (Figure 5, plots I and III). This trend is also pointed out by the panelists and visible on the correspondent pair of roots, where browning of these samples is also above the average. Samples presenting higher moisture surface and brightness (highly correlated) also presented loss in Mg (Figure 5, plot II). On the opposite side of the graph are plotted MP cabbage samples that better retain P, S and Ca over storage time. Although moisture content is related to mineral loss, this has no impact on flavour.

Canonical correlation analysis between sensory quality and bioactive compounds levels, texture and moisture content reveals that samples losing typicity (colour and flavor) undergo browning reactions, also correlated to the observed loss in firmness. Concerning bioactive compounds, only changes in flavonoids content seems to be important and is correlated to browning increase.

Regarding evolution of the sensory attributes, panelists sometimes detected aging starting from the 14^{th} to the 17^{th} day of storage, this freshness lost is not patent on SEM images (Figure 3) and again not clearly reflected in the studied physicochemical parameters as shown in Figure 1.

Samples Cb4 and Gb4 with 14 days of storage stand out slightly. As referred before, PCA with physicochemical data also does not detect significant differences throughout the entire study period. This somehow justifies the confusion felt by the judges, who can hardly distinguish the samples, perceiving only cases of great lost in sensory quality and well-marked defects.

Graphically, it is possible to observe a tendency in the judges' response. However, the small observed differences between products are overshadowed by the differences between the panelist observations, revealed by CVA of judges' data ($p>0.05$ and Wilks' Lambda > 0.2, respectively). This result reveals panel difficulty in detecting differences between the samples and the standards (fresh products presented in every sessions). This kind of results is common when differences between products are small, yielding confusion and dispersing their punctuation consequently increasing the differences between them.

I)

II)

III)

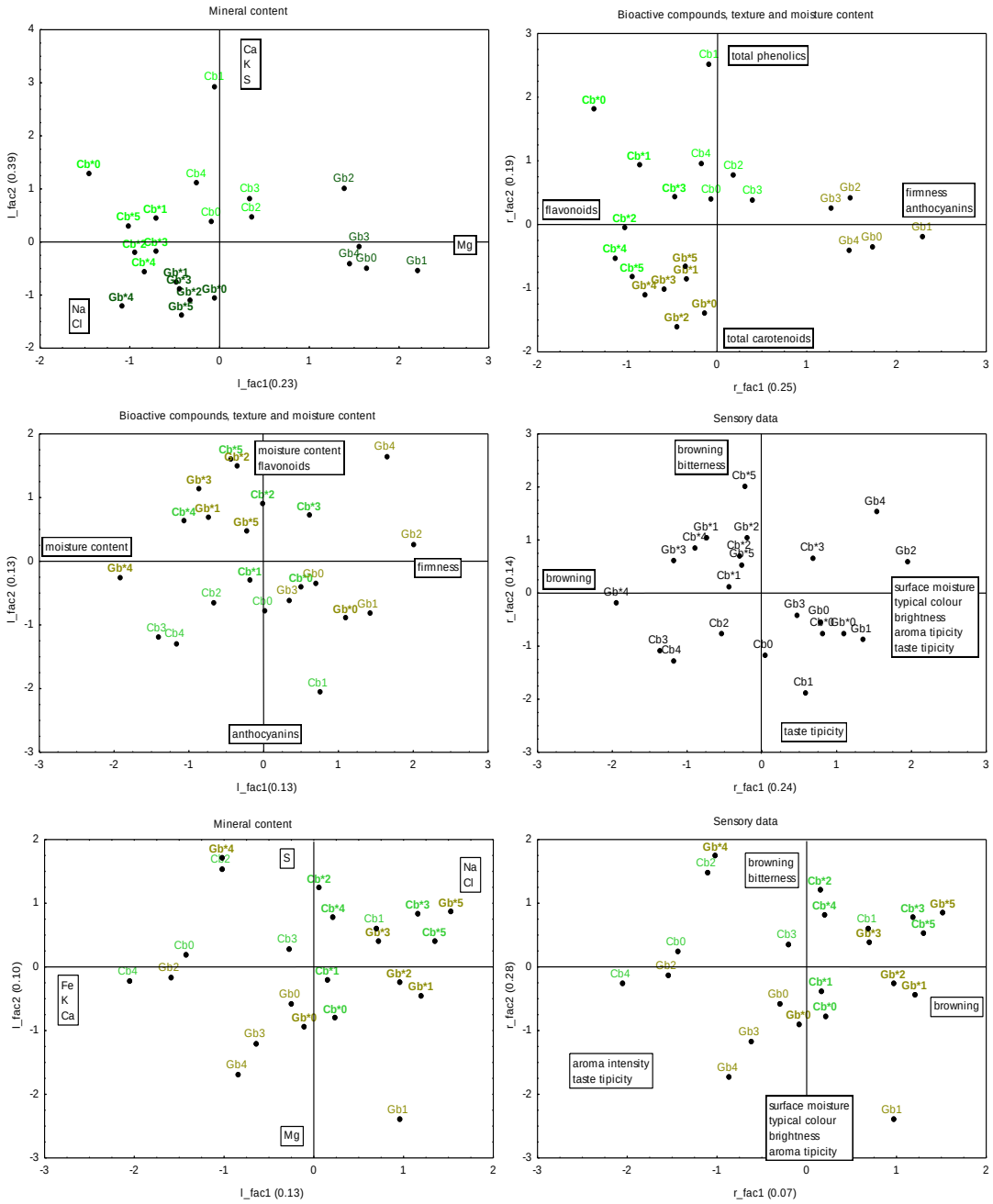


Figure 5 - Canonical correlations analysis of: I) Mineral content related to bioactive compounds, texture and moisture content; II) Bioactive compounds, texture and moisture content related to sensory data; III) Mineral content related to sensory data, over 17 and 28 days of storage of MP and PC vegetables, respectively. Units projections on the plot correspond to samples: Cb - MP cabbage and Gb – MP green beans, where numbers are sampling time corresponding to storage periods (0- 0 days, 1-5days, 2-10 days; 3-14 days and 4-17 days) ; Cb* - PC cabbage and Gb*- PC green beans and numbers correspond to sampling time: 0- 0 days, 1-7 days, 2-14 days; 3-20 days; 4-24 days and 5- 28 days of storage periods.

Concerning green beans (Gb), Gb4 and Gb3 samples, with17 and 14 days of storage, respectively, are again slightly separated from the others. Here, again, judges considered more intense typical aroma of these samples, although at this time, losses in firmness are also pointed out (Wilks' Lambda: 0.15558 (p< 0.0135). Once again, CV2 has little importance.

Sensory evaluation of cabbage (Cb) gave results (Wilks' Lambda=0.27072 with a p< 0.3338) meaning once more judges had difficulties in distinguishing storage samples. Even graphically it is possible to see how confused they were.

Amanatidou, Slump, Gorris, and Smid (2000) reported a similar study with carrots stored in MAP. They compared different gas combinations (20 % N_2 + 50 % O_2 + 30 % CO_2 and 89 % N_2 + 1 % O_2 + 10 % CO_2), although our gas ratios were different, our panelists noted similar behavior of the vegetables along the study.

4. Conclusions

The used MAP conditions, 10/45 and 0/40 (% O_2/ %CO_2) are adequate to preserve minimally processed and precooked cabbage and green bean samples, respectively.

Nutritional parameters and sensory quality remained quite stable over storage, which is a good indication for producers and industry. This preservation technique can extend logistic operations for a few days without compromising quality. Bioactive compounds and mineral losses are negligible over storage time which correlates with the judges opinions. Texture/microstructure are maintained along the experimental period in both processing methodologies (MP and PC).

Acknowledgments

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PARTE III – CONCLUSÕES E CONSIDERAÇÕES FINAIS

Nesta parte serão salientadas a principais conclusões do trabalho desenvolvido e sugeridos trabalhos futuros no sentido de vir a completar a informação aqui obtida e também tendo em atenção as tendências desta área de investigação.

VIII Conclusões Gerais

O trabalho desenvolvido nesta tese foi planeado e desenvolvido no sentido de incrementar o tempo de vida útil de produtos prontos a consumir, nomeadamente legumes em atmosfera modificada, minimamente processados ou pré-cozidos, estes últimos, necessitando de uma etapa de aquecimento. A ideia de uma metodologia de conservação, aplicável numa indústria (unidade fabril ou mesmo cozinhas industriais de restauração coletiva) com o mínimo de variáveis de processo, adequada aos diferentes produtos, esteve sempre presente. Todas as etapas de processamento realizadas seguiram as boas práticas de fabrico, incluindo a qualidade das matérias-primas e a seleção de materiais de embalagem.

O prazo de validade habitualmente dado pela indústria é de aproximadamente 7 dias para os produtos frescos e cerca de 10-12 dias para refeições prontas a consumir. O objetivo principal consistiu no aumento do tempo de vida útil dos vários legumes (couve, feijão verde, cenoura, pimentos verde, vermelho e amarelo) tanto minimamente processados (MP) como pré-cozidos (PC). Neste sentido, foram estudados materiais e misturas de gases (CO_2, O_2 e N_2) a usar na embalagem dos produtos MP e PC. Esta fase permitiu selecionar a atmosfera ideal, com base nos resultados obtidos, e avaliar o comportamento ao longo de diferentes tempos de armazenamento. Os produtos foram monitorizados, tendo em consideração parâmetros de qualidade sensorial, nutricional e microbiológica, avaliados de forma integrada por ferramentas estatísticas de análise multivariada.

No que diz respeito às opções de compra dos consumidores de produtos prontos a consumir, MP ou PC, a avaliação sensorial é relevante na sua aceitabilidade. Aparência, textura e sabor são fatores decisivos que, a par com a segurança, são indispensáveis na tomada de decisões, por parte dos industriais na otimização dos seus processos.

O treino de um painel para a avaliação quantitativa descritiva (QDA) das propriedades organoléticas é uma fase essencial neste tipo de trabalhos. No entanto, e tal como esperado, não se revelou um tarefa fácil nem tão pouco célere. Apesar das dificuldades, foi possível constituir um painel de provadores capaz de avaliar os legumes MP e PC em estudo, cumprindo os requisitos de repetibilidade e reprodutibilidade que a metodologia exige. Cumpriu-se, assim, um dos objetivos deste trabalho (a implementação de processos eficazes para proceder à seleção e treino do painel de provadores para avaliação segundo a

metodologia QDA). Os resultados foram aferidos através de *biplots* aplicados à análise multivariada (análise de componentes principais). Este painel é capaz de avaliar as propriedades organoléticas dos vegetais MP e PC armazenados em atmosfera modificada.

No caso específico da conservação dos legumes MP, esta tem requisitos de embalagem no que concerne à permeabilidade dos filmes e mistura de gases a usar. Os diferentes produtos em estudo mantêm os seus processos metabólicos, pelo que o sistema de embalagem a propor deve manter os parâmetros da qualidade referidos como relevantes para o consumidor, dentro de níveis aceitáveis. Numa primeira abordagem estes parâmetros foram monitorizados ao longo de 10 dias de armazenamento de couve e cenoura MP embalados em AM. A melhor combinação de gases foi 10 %O2/45 %CO2, com perdas mínimas de atividade antioxidante, sem alteração relevante nas propriedades organoléticas e desenvolvimento microbiano aceitável.

Atendendo a que as alterações ao longo do tempo de armazenamento não apresentaram perdas relevantes da qualidade, a avaliação sensorial foi a que teve mais peso na eleição da atmosfera a prosseguir o estudo. A experiência foi repetida, para um tempo de armazenamento de 28 dias, com um número mais alargado de legumes, e a embalagem consistiu num saco de filme plástico laminado com uma permeabilidade elevada ao CO2 e O2 com AM de 10/45. Verificou-se a preservação do teor de compostos bioativos e da atividade antioxidante. A textura, apesar de se ressentir num período muito inicial, manteve-se estável ao longo do armazenamento, bem como os parâmetros de cor. Relativamente ao crescimento microbiano, este ocorreu, mas manteve-se sempre dentro de limites aceitáveis. No que diz respeito à avaliação das propriedades organoléticas, parâmetro preponderante, é importante referir que os provadores classificaram os produtos sem registos de defeitos maiores e com dificuldades de perceber diferenças ao longo do tempo. Os provadores tinham sempre na sua presença uma amostra fresca dos produtos em avaliação, permitindo a comparação entre a amostra e a respetiva referência. Este trabalho permitiu concluir pela adequação do sistema de embalagem aos legumes em estudo, e a extensão em mais de 40% do tempo de vida útil, em relação aos habituais prazos de validade atribuídos pela indústria.

Os requisitos em relação ao sistema de embalagem dos legumes pré-cozidos (PC) são bem distintos dos produtos MP. Neste caso, o estudo centrou-se numa atmosfera (pobre em O2) que permitisse a extensão do tempo de vida, sem recurso a aditivos. Na fase de seleção da AM foi possível concluir que os dois produtos em estudo (feijão verde e couve cozida) apresentaram níveis de qualidade físico-química, sensorial e microbiológica aceitáveis, quando armazenados em embalagens de alta barreira a gases e com uma atmosfera de 0% O2 e 40% CO2, durante um período de 20 dias. Numa segunda etapa do

estudo, estas condições foram usadas na conservação destes e outros legumes (cenoura, pimento verde, vermelho e amarelo) por um período de 28 dias, o que permitiu confirmar a adequabilidade do sistema de embalagem preconizado. Quimicamente, os produtos evoluíram lentamente e sem uma tendência bem definida. A evolução da atividade antioxidante, textura e cor foi influenciada pelas pequenas oscilações de pH e do teor de água verificadas ao longo do tempo de armazenamento. No entanto, estas alterações não foram detetadas pelo painel de provadores treinados antes do 24º dia de armazenamento.

Relativamente à evolução de alguns parâmetros nutricionais relevantes para este tipo de produtos, nomeadamente os compostos bioativos e minerais, foram apresentados dois estudos: o primeiro relativo ao efeito da embalagem, em AM com elevado teor de CO_2, em pimentos verdes, vermelhos e amarelos; e o segundo onde se procurou avaliar a correlação entre estes parâmetros, a qualidade sensorial e a sua evolução ao longo de 17 e 28 dias de armazenamento de legumes MP e PC, respetivamente.

No primeiro trabalho foram avaliados, entre outros parâmetros físico-químicos, a evolução do teor em compostos fenólicos totais, flavonoides totais, antocianinas e carotenoides totais e a atividade antioxidante, ao longo de 28 dias de armazenamento de pimentos verdes, vermelhos e amarelos em AM com 10/45. O efeito inicial da elevada percentagem de CO_2 potencia o efeito barreira dos princípios subjacentes a esta técnica de conservação, desacelerando o metabolismo dos pimentos. Observaram-se pequenas alterações físico-químicas apenas nos primeiros cinco dias, seguindo-se uma estabilização entre os tempos de amostragem até ao final dos 28 dias. Os compostos fenólicos totais, flavonoides totais, antocianinas e carotenoides totais, correlacionados com a evolução da atividade antioxidante, são influenciados pelas pequenas oscilações de pH e do teor de água. A textura e cor, ao longo do armazenamento, são muito estáveis. Os resultados obtidos com o painel, já experiente nestes produtos, revelam a dificuldade que este teve em perceber diferenças entre amostras correspondentes aos diferentes tempos de amostragem. Concluiu-se que a técnica de conservação preconizada se adequa aos produtos em estudo.

No segundo caso, as condições de AM usadas foram 10/45 e 0/40, otimizadas anteriormente na conservação de couve e feijão-verde MP e PC, respetivamente. Mais uma vez verificou-se a estabilidade dos parâmetros físico-químicos ao longo do tempo de armazenamento, o que foi corroborado pela avaliação sensorial. Estes factos revelam mais uma vez a adequação da mistura de gases introduzida na embalagem. Relativamente ao processamento, verificou-se que os legumes PC apresentam teores totais em compostos bioativos mais elevados. No que diz respeito ao teor de minerais, as perdas ao longo do armazenamento são reduzidas, no entanto ocorrem perdas, genericamente por lixiviação.

As correlações destes parâmetros foram avaliadas pela análise de correlações canónicas, que revelou fortes correlações entre o teor de bioativos e as propriedades organoléticas, sobretudo as relacionadas com a tipicidade e entre a textura e o teor em alguns minerais. Apesar da perda de água, neste estudo não se verificou impacto ao nível do sabor. A análise da microestrutura veio também confirmar as observações anteriormente comentadas.

Estas conclusões são sustentadas pelo elevado número de parâmetros avaliados e analisados com recurso a técnicas estatísticas que permitiram reduzir a informação, sendo apenas usada a mais relevante para cada caso estudo. É de salientar o recurso à função AutoBiplot.PCA( ), implementada no R, que facilita a leitura da variabilidade e permitiu a interpretação da informação com rigor.

Pelo exposto é possível concluir que, no caso dos produtos prontos a consumir, as técnicas de conservação estudadas, permitem um alargamento do prazo de validade, genericamente em cerca de 30% no caso de legumes MP e de 50% no caso dos PC, sem comprometer a qualidade organolética e nutricional, e apresentando níveis aceitáveis de crescimento microbiológico. Esta informação considera-se de especial utilidade para a indústria que vê, assim, facilitadas algumas das operações de logística. O consumidor poderá passar a ter à sua disposição produtos com tempos de vida mais alargados, sem perda da qualidade, e atendendo sempre às suas expectativas sensoriais.

IX Perspetivas Futuras

Relativamente às questões da embalagem, sugerem-se estudos que relacionem sistemas de embalagem ativa, com sistemas de embalagem inteligente (integradores tempo/temperatura; indicadores de maturação). O objetivo final será sempre promover tempos de armazenamento ainda mais alargados.

Para além disso, pretendem-se sistemas de embalagem ativa que interajam de forma rápida com os legumes, sobretudo na eliminação de exsudados que ocorrem, fundamentalmente, no final do tempo de vida dos produtos embalados. Serão indicados laminados de polímeros, otimizados por nanotecnologia, como materiais de embalagens plásticas.

A avaliação dos parâmetros microbiológicos deve ser aprofundada. Essa informação claramente completará o trabalho aqui apresentado, uma vez que apenas se usam contagens totais de microrganismos a 30 ºC, como indicadores do avanço da carga microbiana, e no ajuste das condições de prova e seleção de atributos a analisar nas sessões de análise sensorial.

APÊNDICES/ANEXOS

Ficha de prova de legumes crus

Provador: _____ Data: _____

Produto: _____ Código: _____

Sr(a) provador(a), por favor, primeiro aprecie o aspecto geral dos produtos, depois o seu cheiro e finalmente aprecie o sabor, seguindo esta lista tal como apresentada. Note, também, que o ponto [7] é atribuído ao produto fresco.

ASPECTO GERAL:			
humidade superficial	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
cor característica	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
acastanhamento	menos	1 2 3 4 5 6 **7** 8 9 10 11 12 13	mais
brilhante	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
da textura/forma	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
outro aspecto: _____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
TEXTURA APRECIADA COM OS TALHERES:			
comprime-se e recupera a forma	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
consistência (desfaz-se ao comprimir)	desfaz-se	1 2 3 4 5 6 **7** 8 9 10 11 12 13	mantém a forma
dureza ao corte	mole	1 2 3 4 5 6 **7** 8 9 10 11 12 13	duro
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
CHEIRO:			
intensidade	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
característico	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
TEXTURA NA MASTIGAÇÃO:			
firmeza na primeira dentada	mole	1 2 3 4 5 6 **7** 8 9 10 11 12 13	duro
crocância	crocante seco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	crocante húmido
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
SABOR:			
intensidade	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
característico	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
azedo (ácido)	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
amargo	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
doce	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito

Obrigada

Ficha de prova de legumes cozidos

Provador: _____ Data: _____

Produto: _____ Código: _____

Sr(a) provador(a), por favor, primeiro aprecie o aspecto geral dos produtos, depois o seu cheiro e finalmente aprecie o sabor, seguindo esta lista tal como apresentada. Note, também, que o ponto [7] é atribuído ao produto fresco.

ASPECTO GERAL:			
humidade superficial	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
cor característica	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
acastanhamento	menos	1 2 3 4 5 6 **7** 8 9 10 11 12 13	mais
brilhante	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
da textura/forma	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
outro aspecto: _____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito

TEXTURA APRECIADA COM OS TALHERES:			
comprime-se e recupera a forma	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
consistência (desfaz-se ao comprimir)	desfaz-se	1 2 3 4 5 6 **7** 8 9 10 11 12 13	mantém a forma
dureza ao corte	mole	1 2 3 4 5 6 **7** 8 9 10 11 12 13	duro
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito

CHEIRO:			
intensidade	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
característico	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito

TEXTURA NA MASTIGAÇÃO:			
firmeza na primeira dentada	mole	1 2 3 4 5 6 **7** 8 9 10 11 12 13	duro
durante a mastigação	desfaz-se	1 2 3 4 5 6 **7** 8 9 10 11 12 13	tipo borracha
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito

SABOR:			
intensidade	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
característico	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
azedo (ácido)	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
amargo	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
doce	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito
Outro aspecto:_____	pouco	1 2 3 4 5 6 **7** 8 9 10 11 12 13	muito

Obrigada