CATÓLICA PORTO

BIOTECNOLOGIA

Food Risk Mitigation Tools and Strategies

Lima-Peru, 6th September, 2012

CBQF

Centro de Biotecnologia e Química Fina

CONCIA 2012

Congreso Nacional de Calidad e Inocuidad Alimentaria

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BIOTECNOLOGIA

Universidade Católica Portuguesa - Porto

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Portuguese Catholic University

Portuguese Catholic University

BRAGA
Theology
Philosophy
Social Sciences

Portuguese Catholic University

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Theology
Philosophy
Social Sciences

LISBON
Theology
Law
Business and Economics
Humanities
Health Sciences
Engineering

Portuguese Catholic University

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Philosophy
Social Sciences

BEIRAS
Health Sciences
Social Sciences

LISBON
Theology
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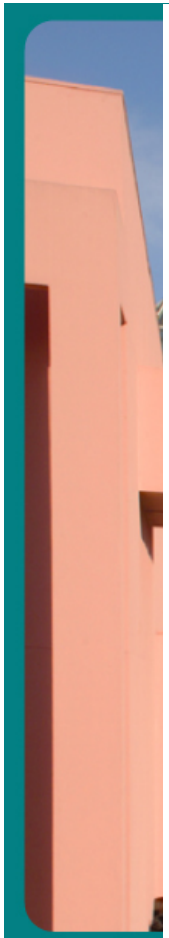
Business and Economics
Humanities
Health Sciences
Engineering



Portuguese Catholic University

BRAGA
Theology
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PORTO
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Education and Psychology
Arts
Biotechnology
Health Sciences

BEIRAS
Health Sciences
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LISBON
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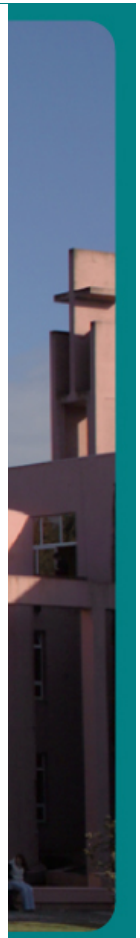


Portuguese Catholic University

- 45 years (1967)
- 1st non-state University in Portugal
- 20.000 graduates
- 2012: 12.000 students (including professional training)

PORTO

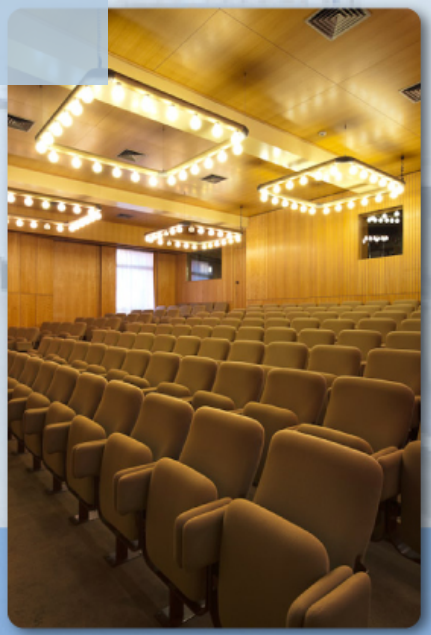
BRAGA

BEIRAS

LISBON

College of Biotechnology

Portuguese Catholic University



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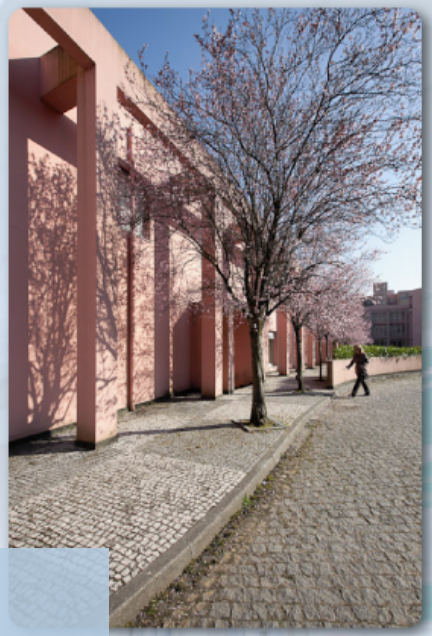
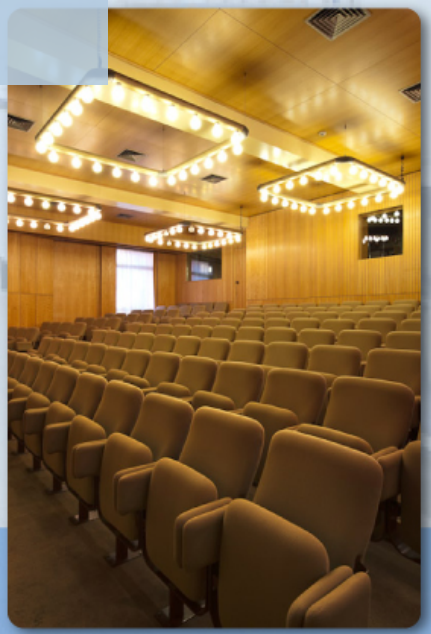
ESCOLA SUPERIOR DE BIOTECNOLOGIA



Research Center - CBQF



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e Química Fina

Registered as an Associate Laboratory since 2004.

CBQF occupies a single site and is functionally dependent on the Catholic University.

Currently has 47 PhDs listed as members.

Initial candidature focussed on environmental and food risk assessment, current scope slightly refined.

Statute renovated as from January 2011

Contract signed for 10 years, subject to availability of funds and 2-yearly evaluations.

At present fixed Associate Laboratory finance is awarded (i) based on 2-yearly "strategic projects" (known previously as plurianual) and (ii) salary funding for 5 senior researchers.

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Research Budget for 2011.

External Finance:
1.2 M Euros in researcher obtained finance*
0.3 M Euros in Strategic Project (FCT).
0.35M Euros in Associate Laboratory personnel.

* Not including significant non-research finance for entrepreneurship and networking.

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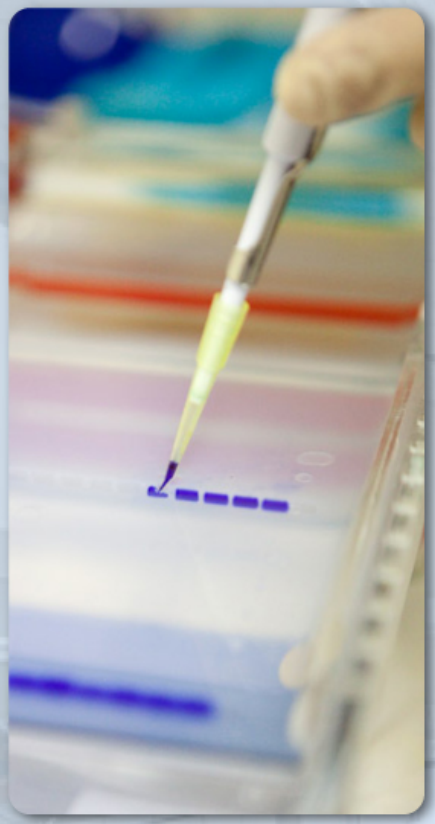
Intended contribution to society.

1. Preparation of high calibre professionals and academics by exposing them to a demanding but supportive research environment.

2. To generate and communicate new knowledge which addresses 2 major societal challenges.

    a. *The health and well being of the citizen* –
    Safety in the food chain and the protection of the environment

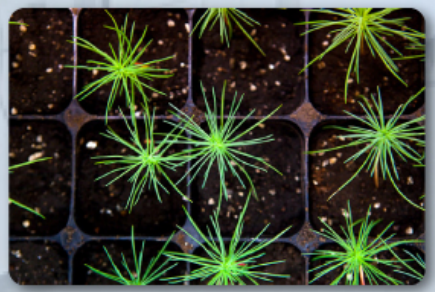
    b. The *competitiveness of the agricultural and food systems* –
    Underpinning health-, safety- and sensory- driven innovation in foods and supporting sustainable use of agricultural resources.



Research Centre - CBQF

Research Lines:

- **Food: safer, healthier, more nutritious and more competitive food**
  - Food risk assessment
  - Food risk mitigation tools and strategies
- **Environment: Innovative approach to environment and sustainability challenges**
  - Environmental diagnosis
  - Environmental mitigation tools and strategies

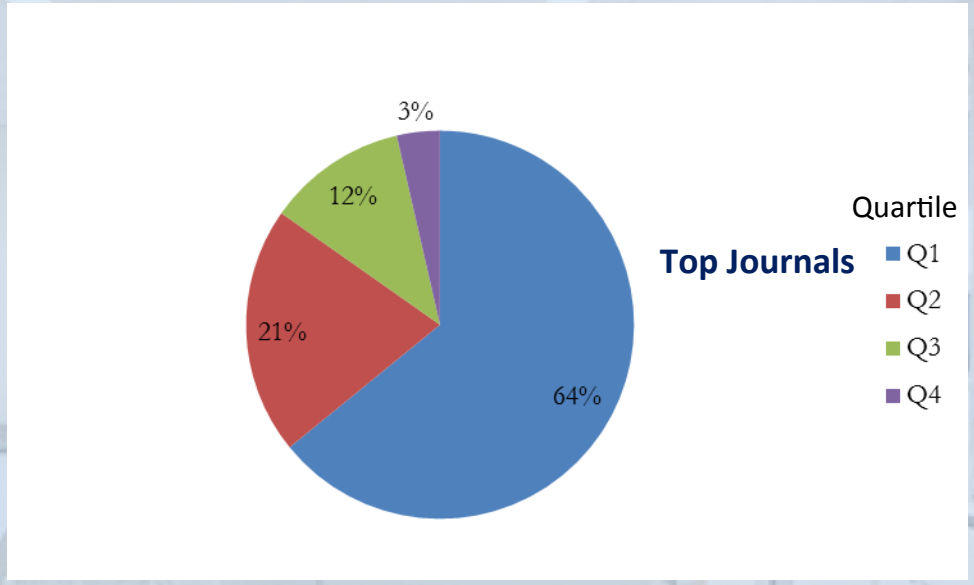


Research Centre - CBQF

- *Ca.* 110 researchers
  - 47 PhD researchers; 41 PhD students; 30 research fellowships
- Scientific production (2007-2011)
  - 300 international scientific papers (ISI)
  - 6 patents
- 35 ongoing projects / year
- 40 partnerships in national and international active networks

Research Centre – CBQF

Bibliometric Analysis 2008-2011



- **2011: 125 scientific publications, including 87 international scientific papers (ISI)**

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Research structure and plan 2011/2012.

The CBQF presents itself formally in the following Research Lines and Groups*

Food Line – Leader; Tim Hogg

- Food Risk Assessment Group – Leader; Paula Teixeira
- Food Risk Mitigation Group – Leader; Cristina Silva
  - Subgroup - Safety
  - Subgroup – Nutrition and Food Quality

Environmental Line – Leader; António Rangel

- Environmental Diagnostics Group – Leader; Célia Manaia
- Environmental Risk Mitigation Tools and Strategies Group – Leader; Paula Castro

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Food: safer, healthier and more nutritious and more competitive food.

Food Risk Assessment

- ☑ Data for exposure assessments
- ☑ Refining methods

Food Risk Mitigation Tools and Strategies

- ☑ Quality and safety interventions in specific product lines
- ☑ Safety interventions for the food chain in general.
- ☑ Quality and nutrition.

Food Risk Assessment

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Data for exposure assessments

- ✓ Microbiological and chemical hazards in traditional foods
- ✓Listeria abundance and modulation in relevant chains
- ✓ Staphylococci in cheese plants

Refining methodologies

- ✓Package usage in specific populations

Food risk mitigation tools and strategies

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Product line interventions in quality and safety

- ✓Wine quality – Dekkera, LAB, phenolics and chemiometrics.
- ✓ Fruits and vegetables – postharvest and minimal processsing.
- ✓ Dairy - authenticity of traditional cheeses
- ✓Charcuterie - authenticity

Food risk mitigation tools and strategies

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Safety interventions for the food chain.

- ✓ Modelling of inactivation and transport processes
- ✓Phages, bacteriocins and other natural antimicrobials
- ✓Molecular and microscopical methods for pathogens
- ✓Analytical methods for safety and quality parameters

Food risk mitigation tools and strategies

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Food Quality and Nutrition.

- ✓ New functional ingredients and new sources
- ✓ Matrix development and optimisation
- ✓ Applications – probiotic fish feed, functional edible films
- ✓ Genetics of nutrient uptake in key food plants

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Food Risk Mitigation Group

Subgroups:

Safety

Nutrition and Food Quality

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Quality and safety in specific food product lines

Wine

To study positive aspects of wine sensory quality and develop knowledge to underpin the achieving and protecting of this quality – grape quality determination on vine, fermentation and spoilage yeasts, LAB, and oxidative degradation.

Fruits and vegetables

To systematically study fruits and vegetables quality changes and its biochemical basis provoked by preservation processes and minimal processing and to develop technological strategies for the mitigation.

Dairy

Traditional Charcuterie

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Quality and safety in specific food product lines

Highlights 2011

Modulation of sulphur-containing volatiles in wine by the presence of nitrogen supplementation

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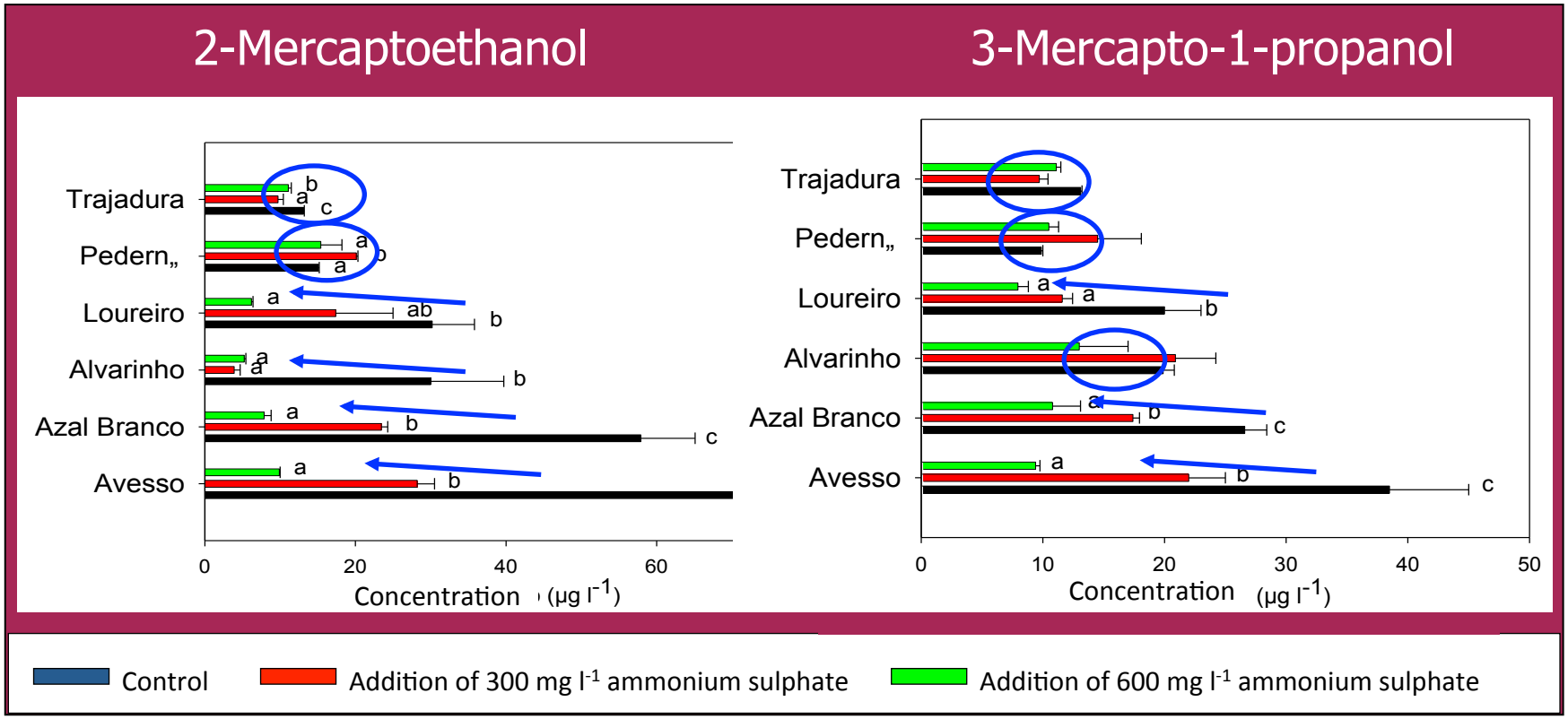

→ the repressive effect is variety sensitive due to differences in abundance of amino acids in the musts.

Refinement of chemometric methods

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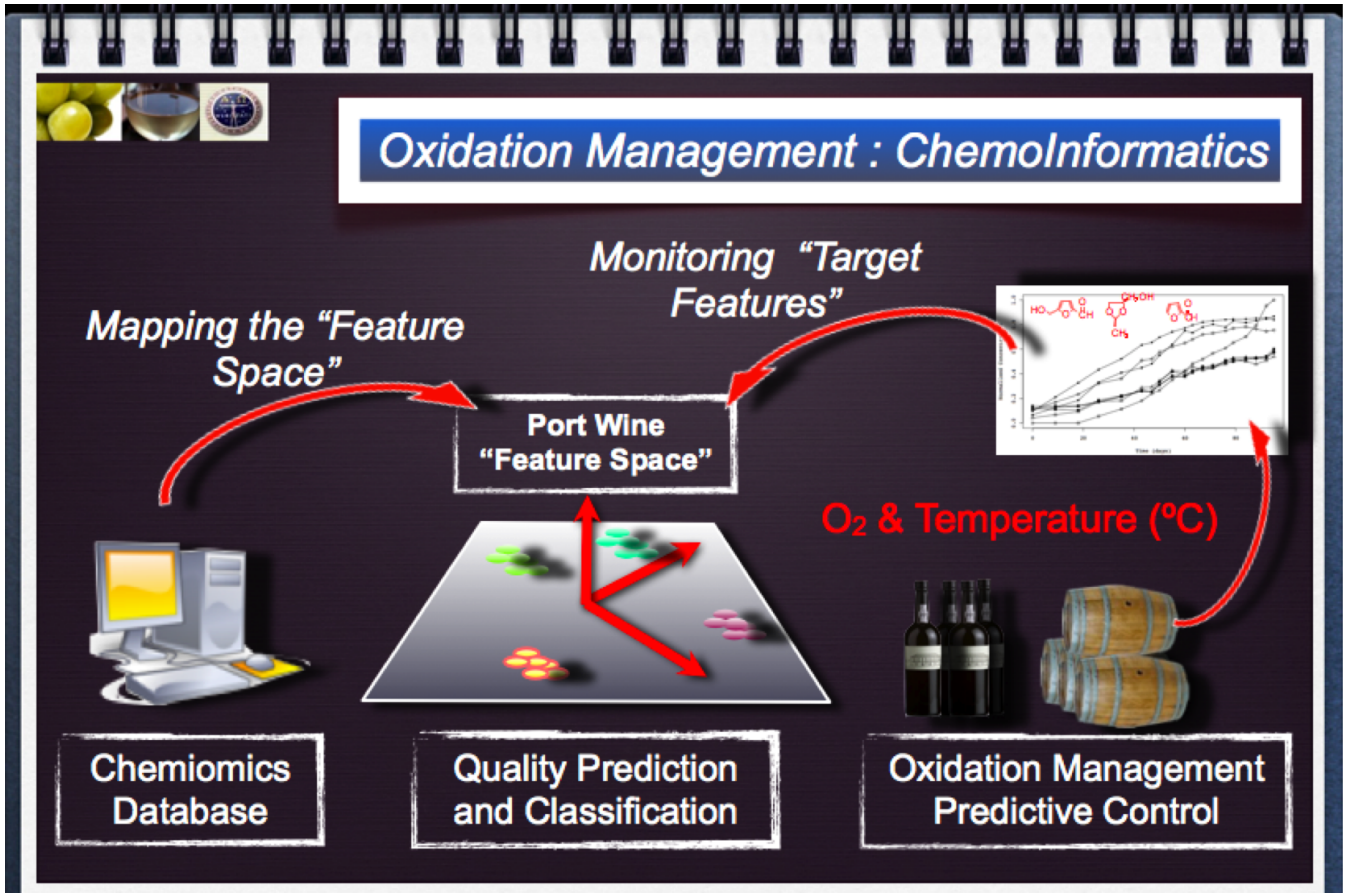

→ moving from the prediction of quality in tawny ports to the management of quality.

New functional dry food

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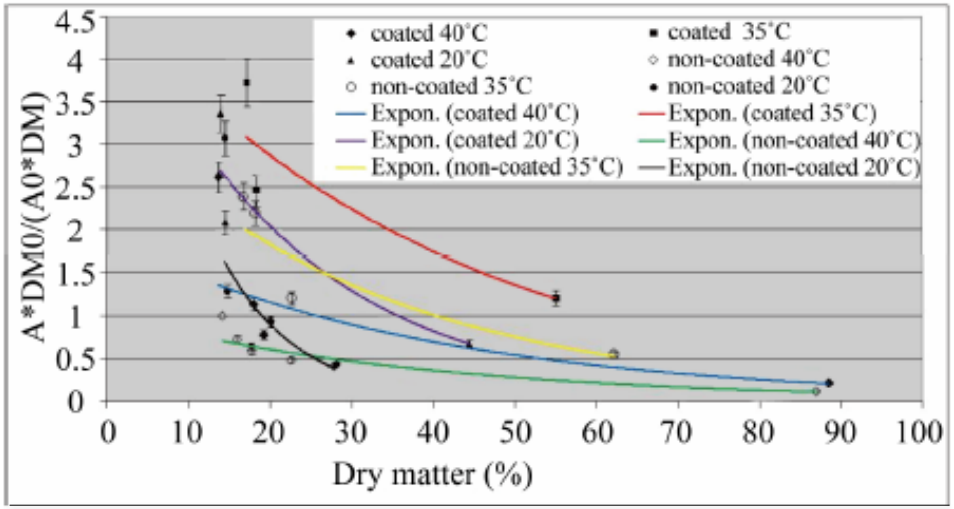

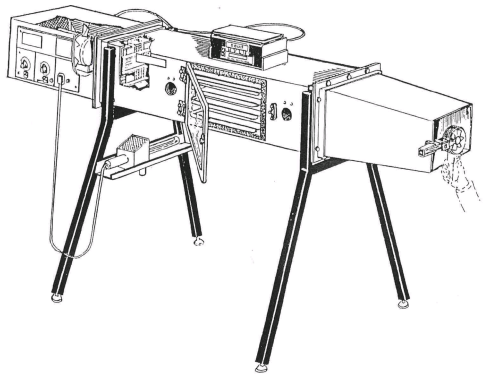

Interventions for the food chain and food ingredients

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Tools and strategies for pathogen and spoilage control

Knowledge and technologies for prediction, detection and control. Models of inactivation with novel technologies, bacteriocins, phages and natural antimicrobials.

Nutrition and ingredients

Novel functional ingredients and sources, matrix and delivery development, naturally, nutritionally-fortified plants - Probiotics, phenolics, CLA, Omega 3, bioactive peptides, microencapsularion, spray drying, phytate and minerals transport.

Detection and measurement

Automated wet-chemistry and spectroscopy, development of high-throughput, flow-based systems.

Upgrading of secondary streams

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Interventions for the food chain and food ingredients

Highlights 2011

Dynamic approach for assessing food processes

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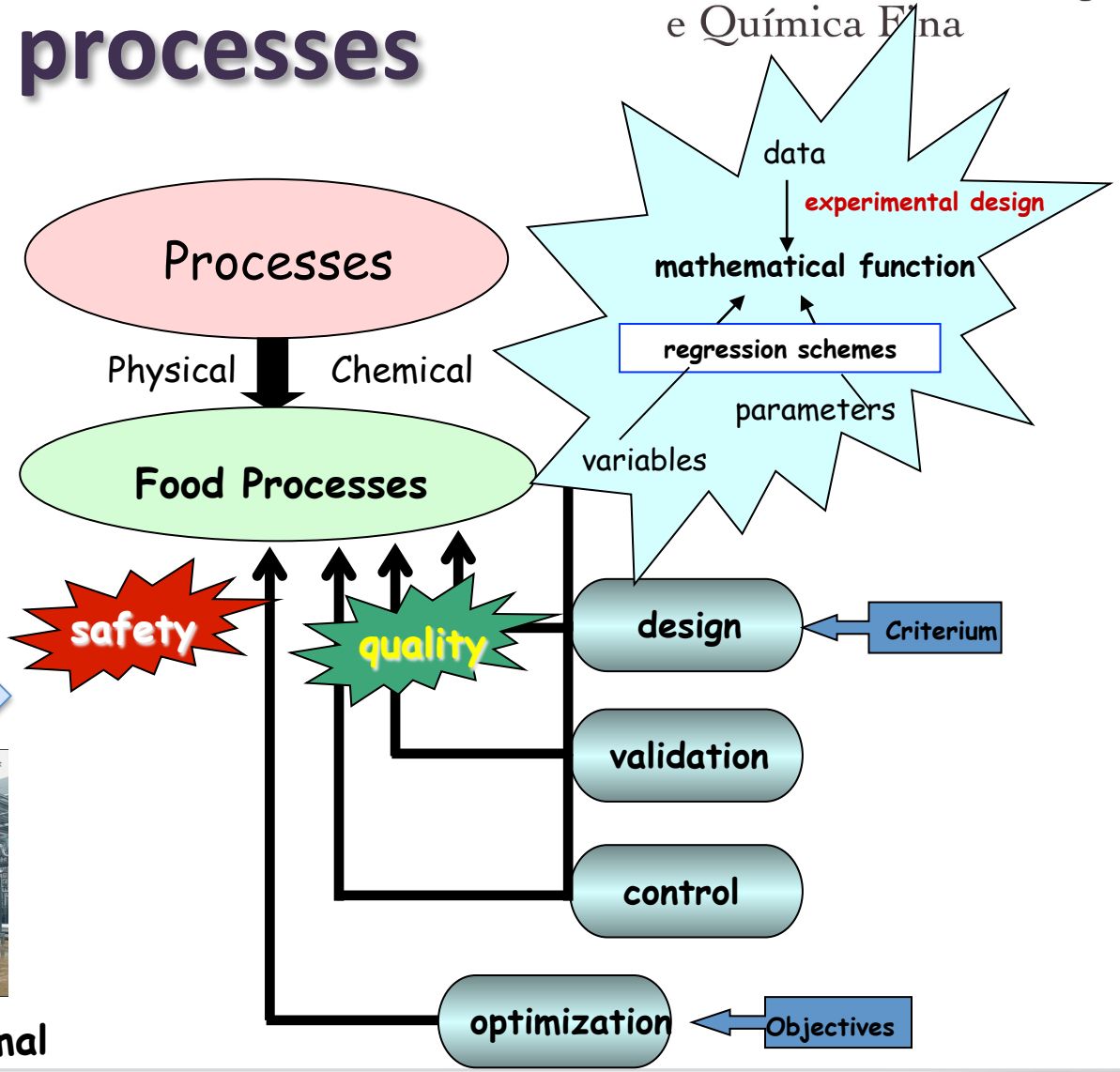
Modelling

Transport Phenomena
- heat
- mass
- *momentum*

Reaction kinetics

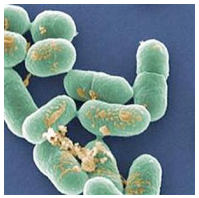
Properties



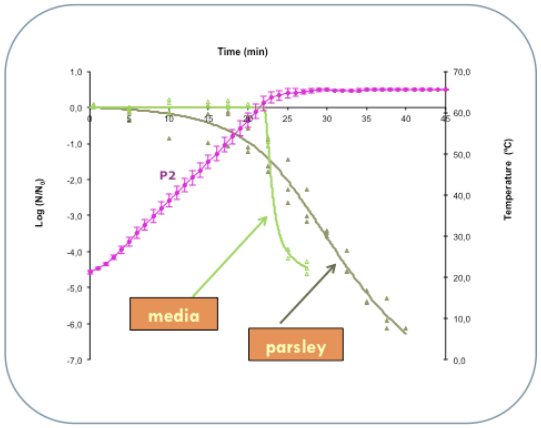
Transfer of results is compromised

Isothermal

Non-isothermal



Thermal inactivation of *Listeria* in culture media and foods



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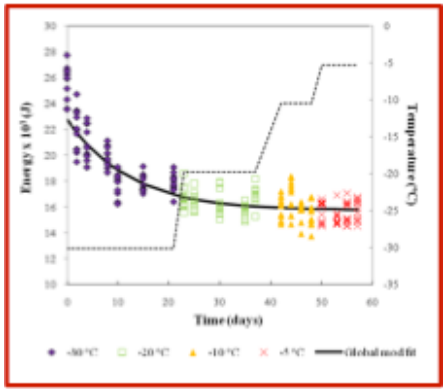
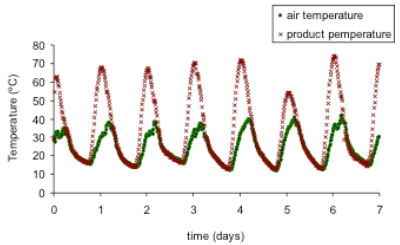
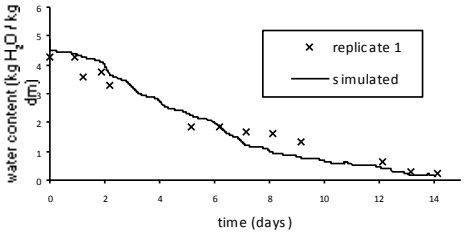


Frozen storage of vegetables

Solar drying of grapes





Functional Ingredients: Probiotics

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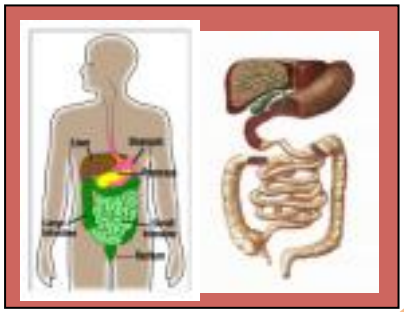
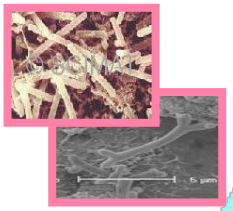


Protective effect of whey cheese matrix



- Microencapsulation in tailored carriers
- Spray-drying and aerodynamically assisted flow extrusion

Protection Technology

Functionality

Probiotics

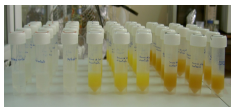
Food Matrix
Dairy
Non-dairy

Cell factory

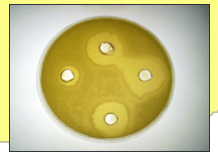
Oat matrices

Granola bars

Whey cheese



Fruit juices



ACE-inhibitory peptides
Probiotic cheese

c9,t11 CLA

t10,c12 C

	mg CLA/ mL skim milk	
Strain	Safflower	Linolei acid
L. lactis	23,053	45,513
B. animalis BLC	18,413	48,246
B. animalis Bb12	22,290	42,207
L. acidophilus Lac1	4,683	40,936
L. plantarum	21,527	51,678

Functional Ingredients: Biopeptides

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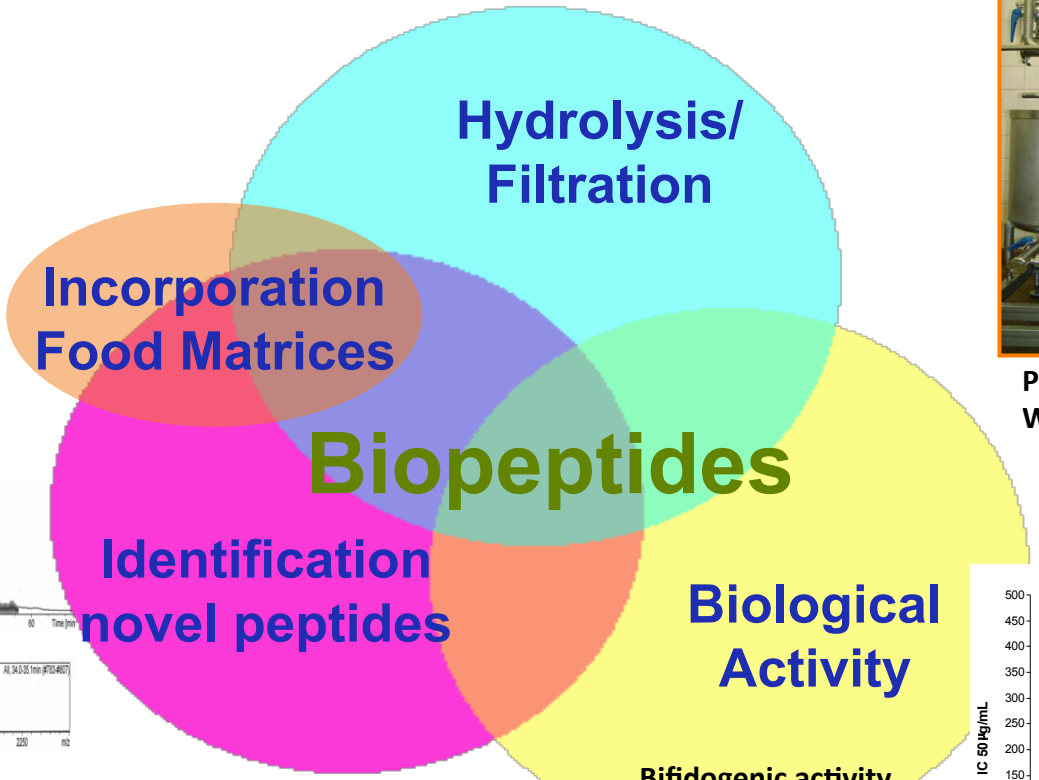
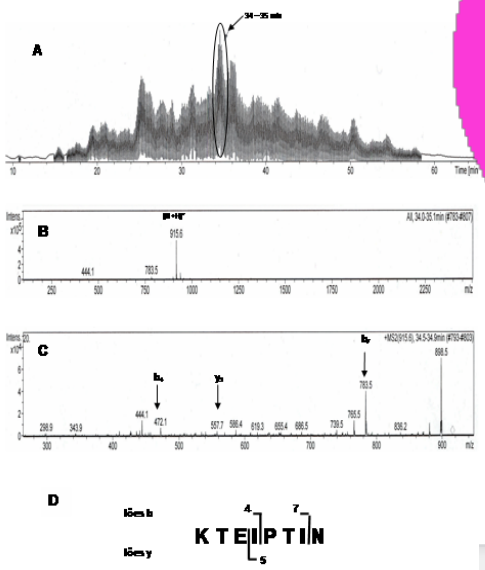
Fruit juices

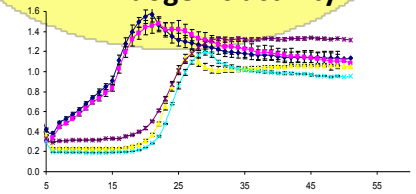
Whey cheese

Pilot scale preparation of bovine WPC and peptide extracts

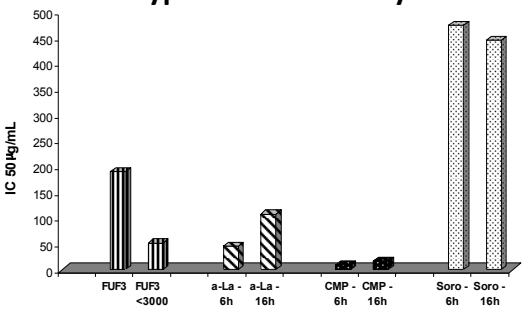




Bifidogenic activity



Antihypertensive activity *in vitro*



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Automation/miniaturization of different food chemical analysis

Enzymatic analysis

Ethanol

Peroxidase activity

Substrate quantification
Enzymatic activity
Enzyme inhibition studies

Solid phase spectrophotometry

Iron

Protein content

Minimization of interferences
Pre-concentration

Micro-Flow analysis as a tool

Micro-Flow system

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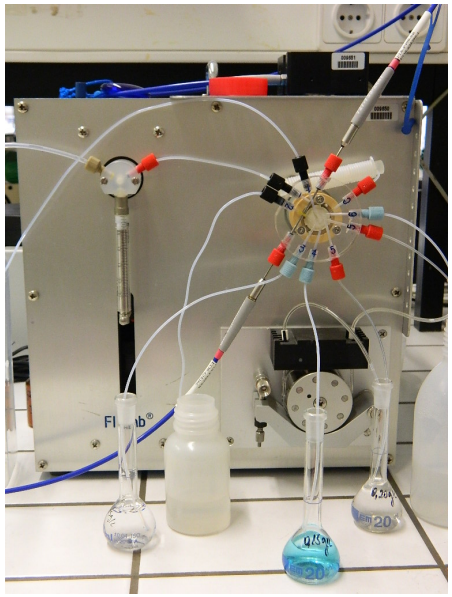
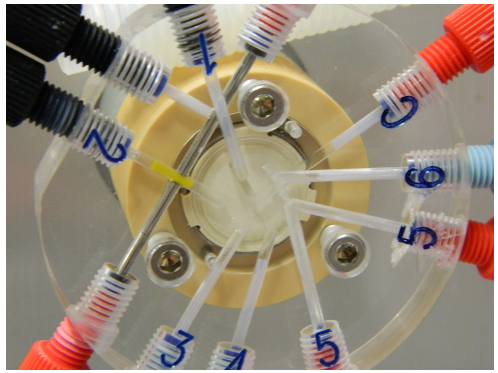
- Pre-concentration and detection directly on the solid-phase (micro-beads)
- Reagents/sample consumption μL; μg
- Integrated device for sample manipulation and detection (charge coupled device detector / fiber optics)
- Wine: determination of alcohol (enzymatic), iron and total protein content



- Vegetables: Enzymatic activity and inhibition studies



Caso prático – **Degradação nos alimentos** *(hortofrutícolas)*

UNIVERSIDADE CATÓLICA PORTUGUESA
Escola Superior de Biotecnologia

Enquadramento

Hortofrutícolas

Vitaminas
[Vit C,
pro-vitamina A]

Minerais
[K, Zn, Ca, Mg,
Cu...]

Fibras
[complantix]

Compostos protectores
[flavenóides..]

Aliados essenciais para a prática de hábitos alimentares saudáveis

Baixas calorias

Cores apelativas

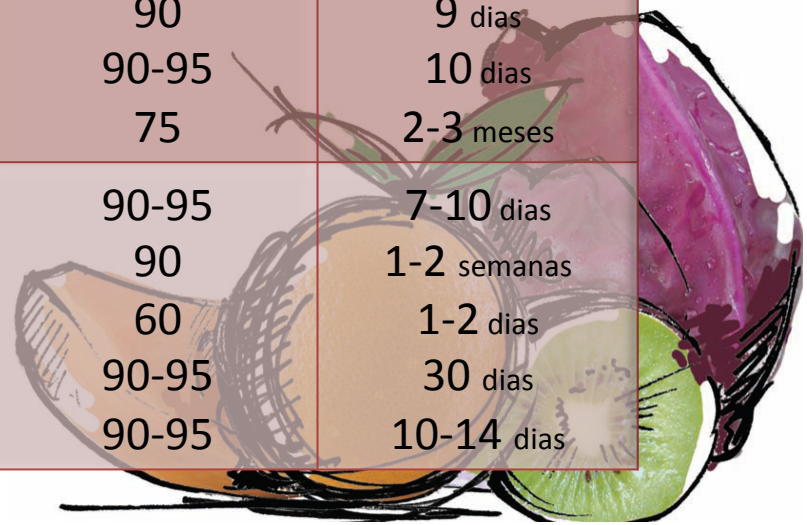
Sabores diversos

Enquadramento

Hortofrutícolas frescos

As características fisiológicas e de composição química, determinam a elevada perecibilidade

Produtos	Temperatura (ºC)	HR (%)	Tempo
Manga	12	85	2-3 semanas
Maçã	2	-	4 meses
Cereja	4	90	9 dias
Pepino	14	90-95	10 dias
Abóbora	10	75	2-3 meses
Feijão-verde	5-6	90-95	7-10 dias
Cenoura	2	90	1-2 semanas
Brócolos	0	60	1-2 dias
Couve-flor	2	90-95	30 dias
Beringela	8-10	90-95	10-14 dias



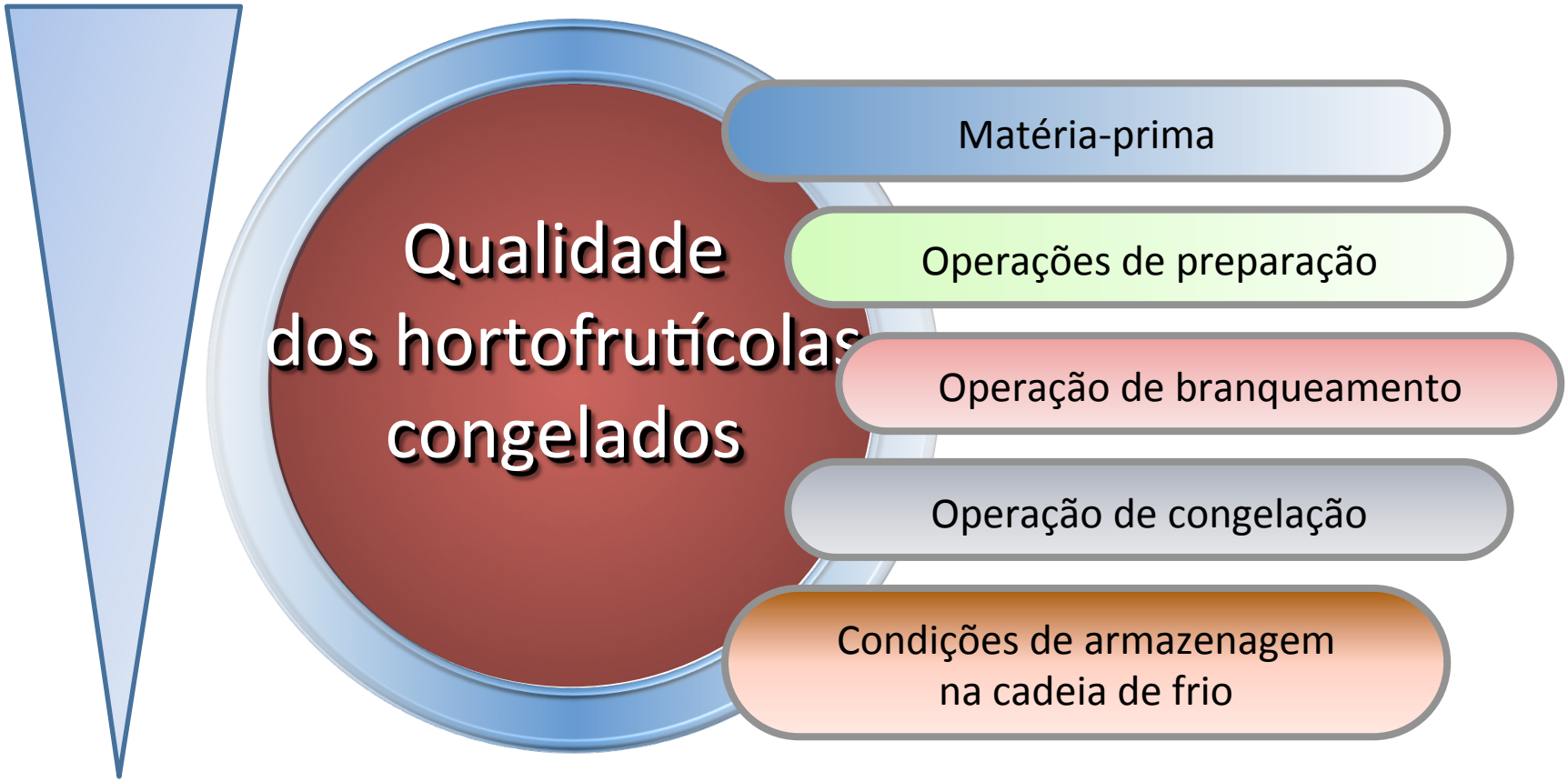
Enquadramento

Hortofrutícolas congelados

Enquadramento

A qualidade dos HF_Congelados

Qualidade



Qualidade

Enquadramento

Tratamento térmico de branqueamento (TT_B)

Objectivos:

Inactivar enzimas degradativas

- Referência: Peroxidase (POD)

E ainda:

Eliminar bactérias

E contaminantes químicos

...

No entanto ...

Afecta negativamente os factores da qualidade...

Físicas e organolépticas

- Textura
- Cor

Nutricionais

- Vitaminas
- Sais minerais
- Pigmentos

Operação de branqueamento

Obtenção das cinéticas de inactivação e de alteração da qualidade.

Obtenção da condição optimizada de branqueamento (90% de inactivação da POD e maximização da qualidade).

Branqueamento em água

- Cilindros - 50 mm diâmetro e 15 mm altura;
- T (ºC) : 75, 80, 85, 90 e 95
- t (min): 0-50

Peroxidase

- Bifani et al. (2002)
- Método espectofotométrico
- Abs min^{-1} $.g^{-1}$

Cor

- Colorímetro Minolta (iluminante C)
- CIE L*a*b*

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

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

Textura

- Texturómetro TA.HDI
- Célula 500N; sonda 5mm
- Firmeza (N) & Energia (J)

Tratamento de dados

- ANOVA

Abóbora *(Cucurbita maxima L.)*

Branqueamento em água

- Floretes 5 cm diâmetro
- T (ºC): 70, 75, 80, 85 e 90
- t (min):0-40

Peroxidase

- Bifani et al. (2002)
- Abs min^{-1} g^{-1}

Cor

- CIE Lab
- Parâmetros: -a*/b*; hue (tan^{-1} b*/a*)

Textura

- Célula Kramer
- Corte
- Firmeza (N)

Análise sensorial

- Cor e textura
- Escala não estruturada de 10 cm
- Nº de provadores: 10

Fenólicos Totais

- Singleton and Rossi (1965)
- mGAE

Brócolos (*Brassica oleracea L. ssp.*)

Branqueamento em água

- Rodelas 5 ± 1 mm
- T (ºC):70, 75, 80, 85 e 90
- t (min): 0-40

Peroxidase

- Bifani et al. (2002)
- Abs min^{-1} $.g^{-1}$

Cor

- CIE Lab
- Parâmetros: L*, a*, b*

Textura

- Texturómetro TA.HDI
- Célula 500N; sonda 5 mm
- Firmeza (N) & Energia (J)

Fenólicos Totais

- Singleton and Rossi (1965)
- mGAE

Cenoura *(Daucus carota L.)*

Modelar as condições de TT_B

Avaliar o efeito do tempo e temperatura de TT_B na velocidade de inactivação/ degradação dos atributos analisados

Modelos cinéticos

Ordem zero

$$C = C_0 - k_{(T)}t$$

1ª ordem

$$C = C_0\, e^{-k_{(T)}t}$$

Fraccionário

$$\frac{C - C_{eq}}{C_0 - C_{eq}} = e^{-kt}$$

Atributo no tempo t

Valor inicial

Tempo

Valor de equilíbrio

Velocidade de reacção

$$k_{(T)} = k_{ref} \exp\left[-\frac{Ea}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]$$

Energia de activação

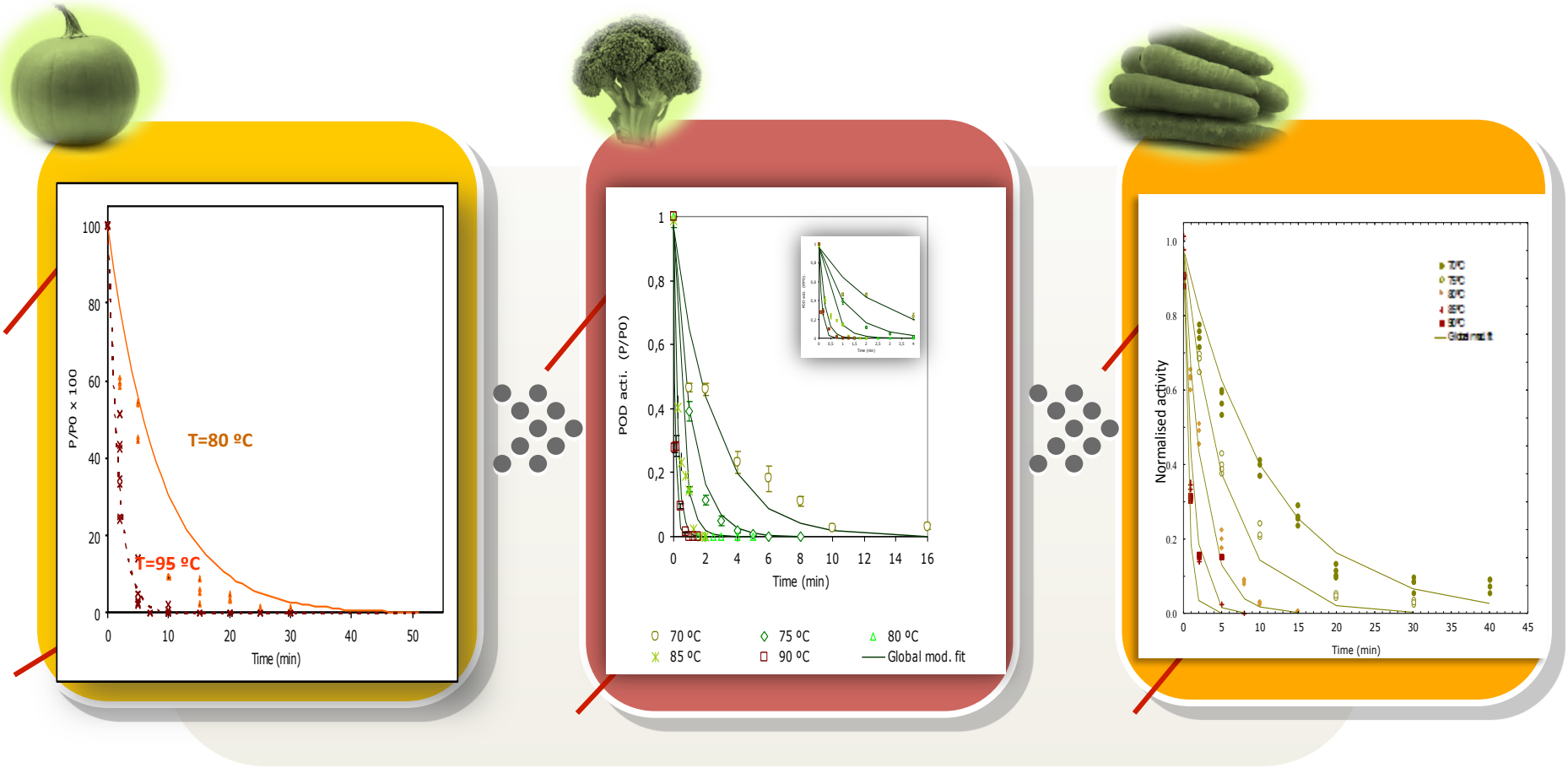
T. referência

Const. gases

Efeito da T em k

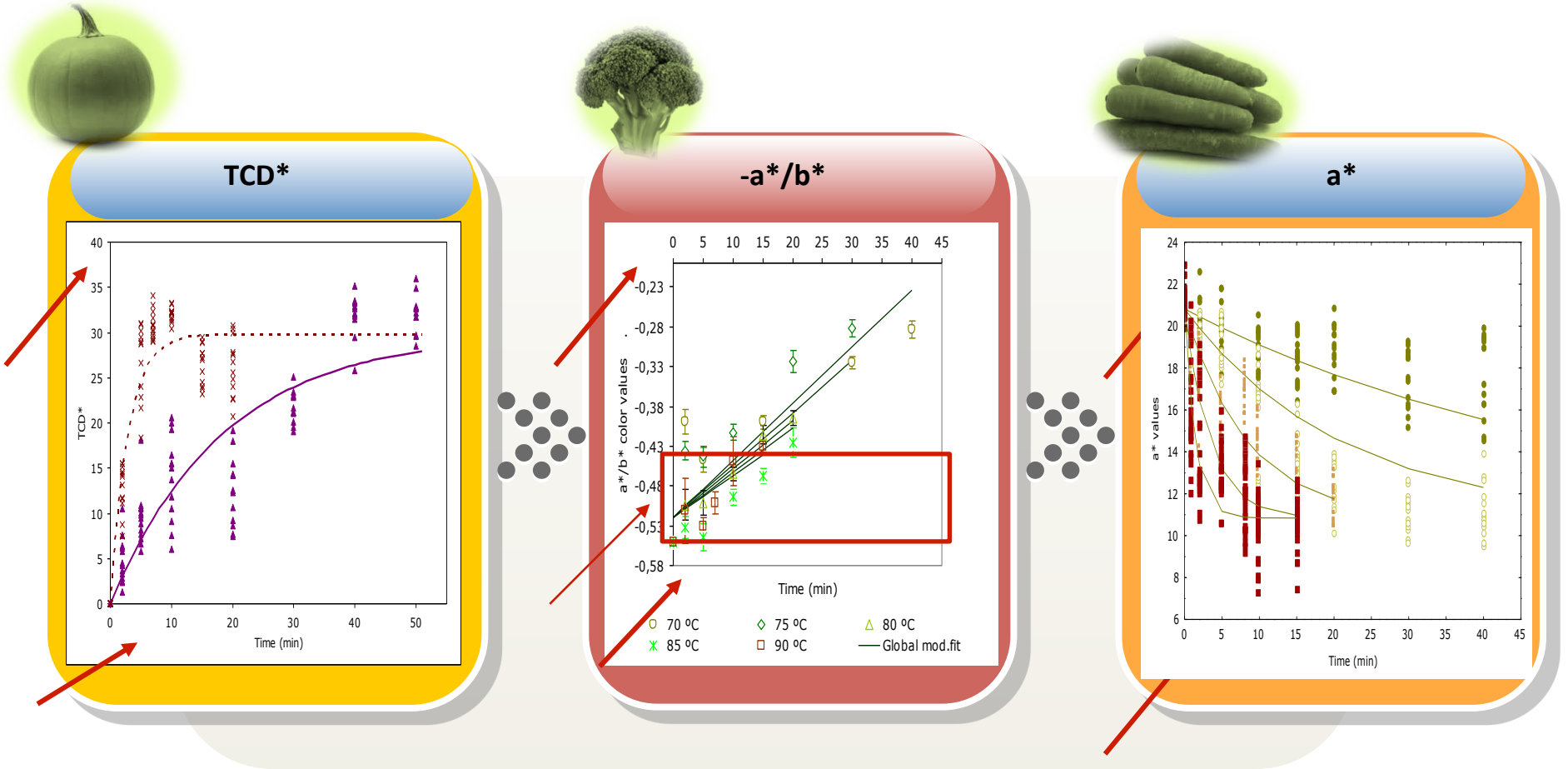
Lei de Arrhenius

Inactivação da POD

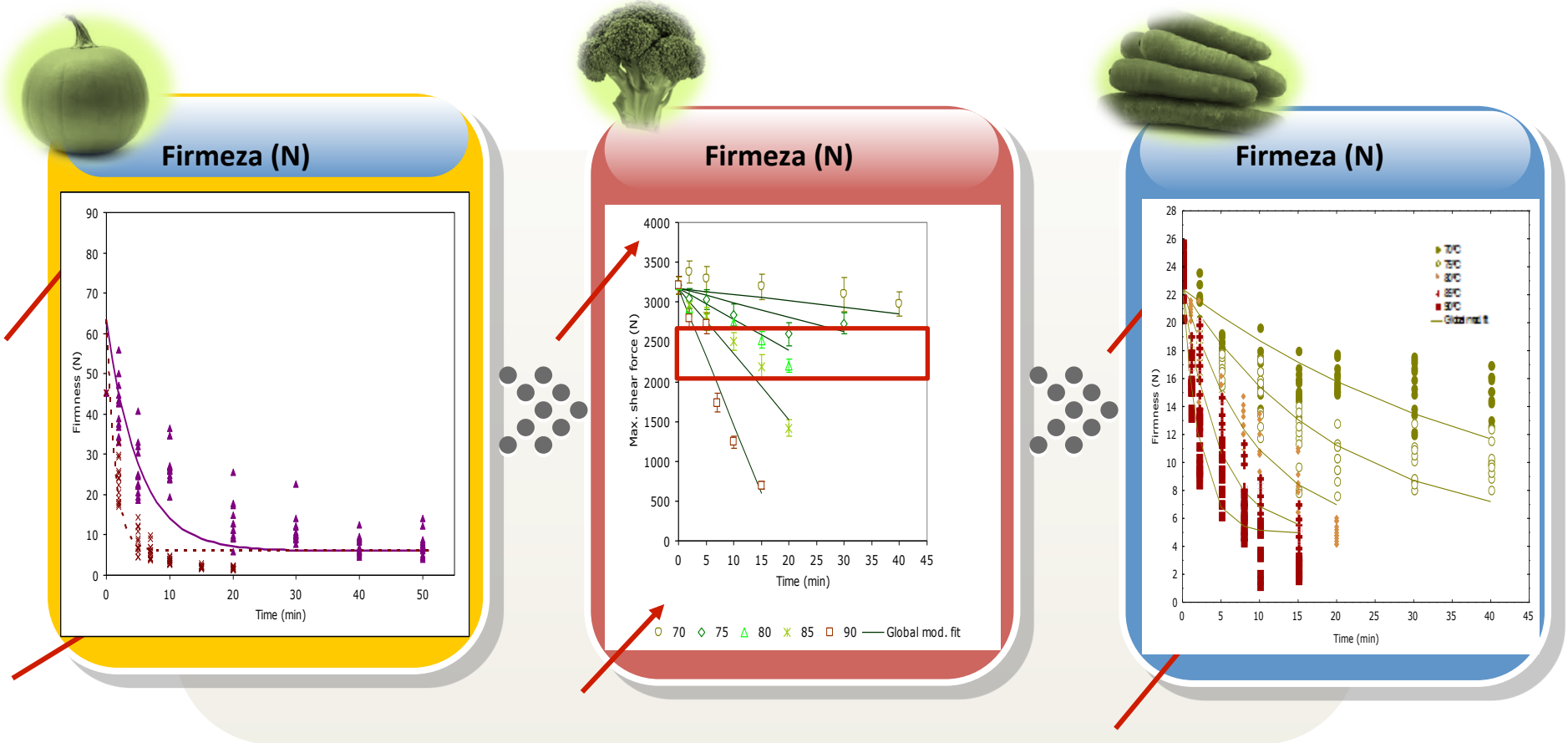


Resultados

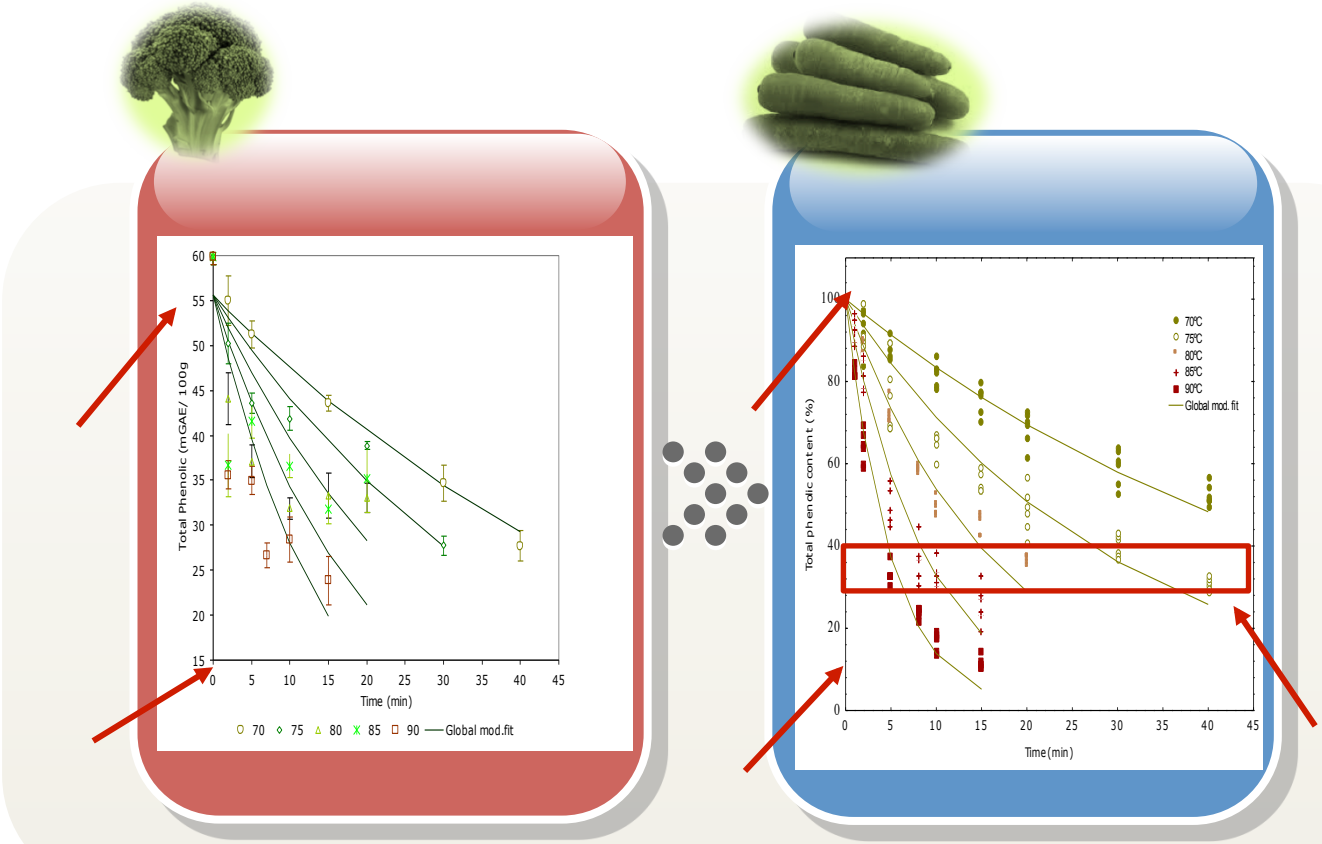
Cor CIE L*a*b*



Alterações de Firmeza

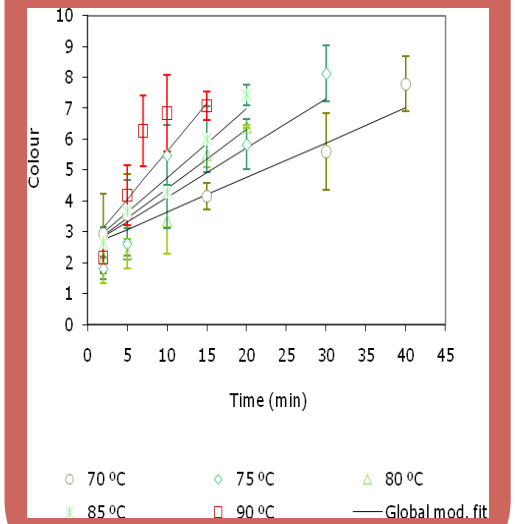


Conteúdo em fenólicos totais

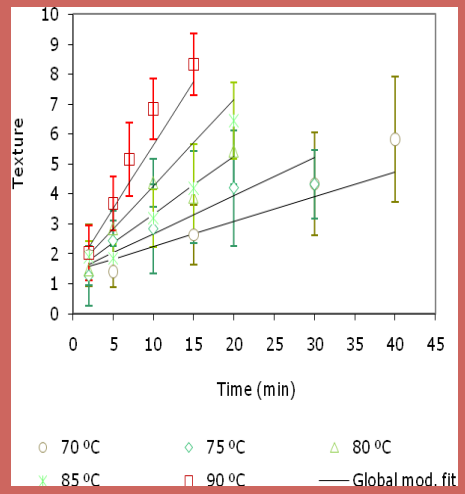


Análise sensorial

Cor



Textura



Boas correlações entre os atributos físicos e a análise sensorial

Condição optimizada de branqueamento

Condições de tempo-temperatura de branqueamento para atingir 90% de inactivação da POD e correspondente perda da qualidade dos diferentes atributos

Temperatura (ºC)	Tempo (min)	Perda da qualidade (%)				
		Fenólicos totais	Parâmetros de cor			Textura (N)
			L*	a*	b*	
70	25	36.5	2.5	21.2	10	35.4
75	12	33.4	3.0	24.5	13.9	36.2
80	6	31.1	3.8	28.2	18.5	37.5
85	2.8	26.8	4.3	30.6	22.2	36.5
90	1.4	24.1	5.1	33.3	25.8	36.7

& verificando a sensibilidade dos parâmetros cinéticos...

Abóbora

- **Textura** atributo mais sensível
- **Cor** atributo "indicador"
- 95 ºC – 3.9 min

Brócolos

- **Cor** atributo "indicador"
- 70 ºC – 6.5 min

Cenoura

- **Fenólicos** atributo "indicador"
- 80 ºC – 6.0 min

Armazenagem em congelação

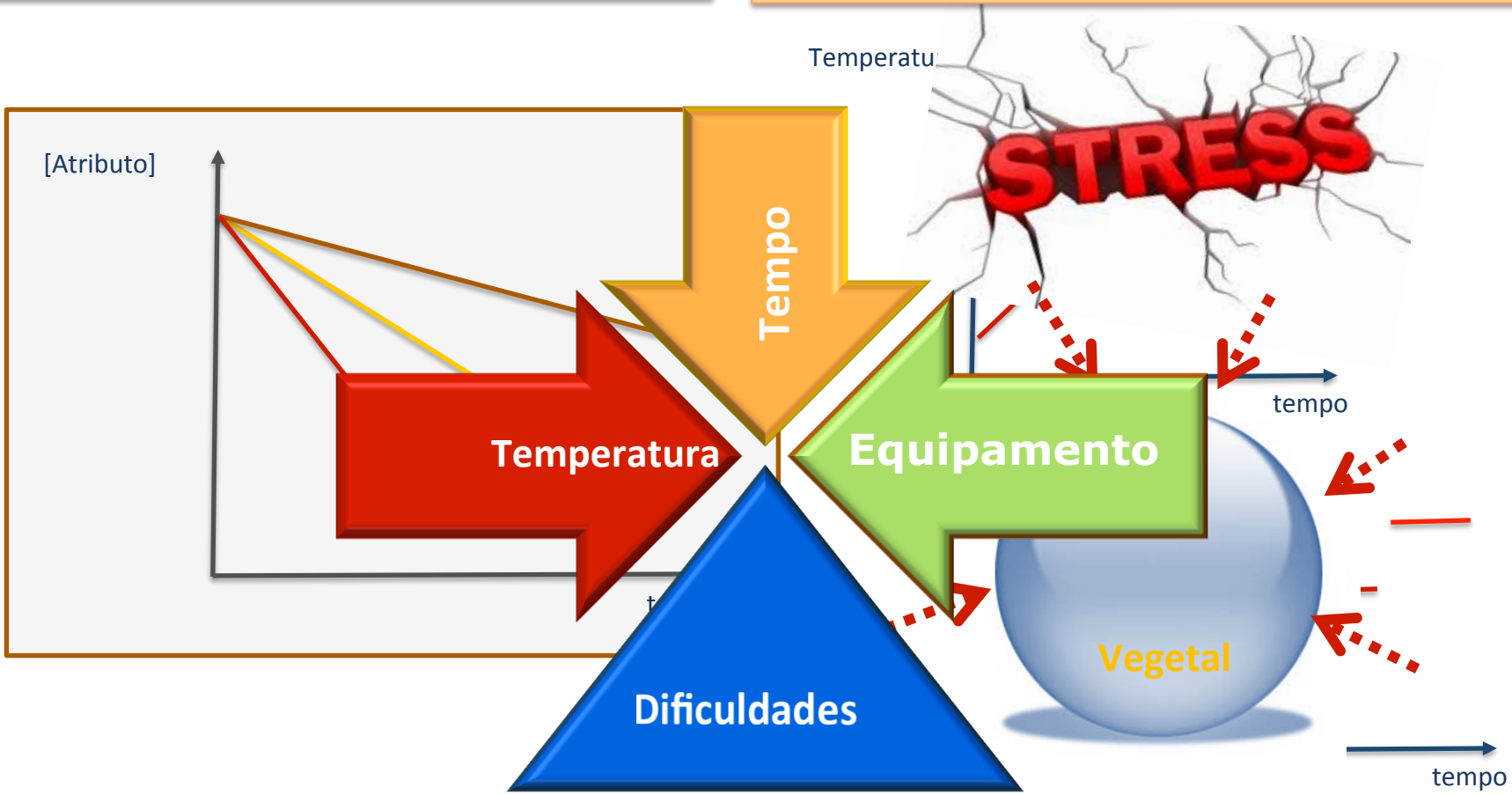
Obtenção das cinéticas de alteração nos regimes isotérmicos e não–isotérmicos.

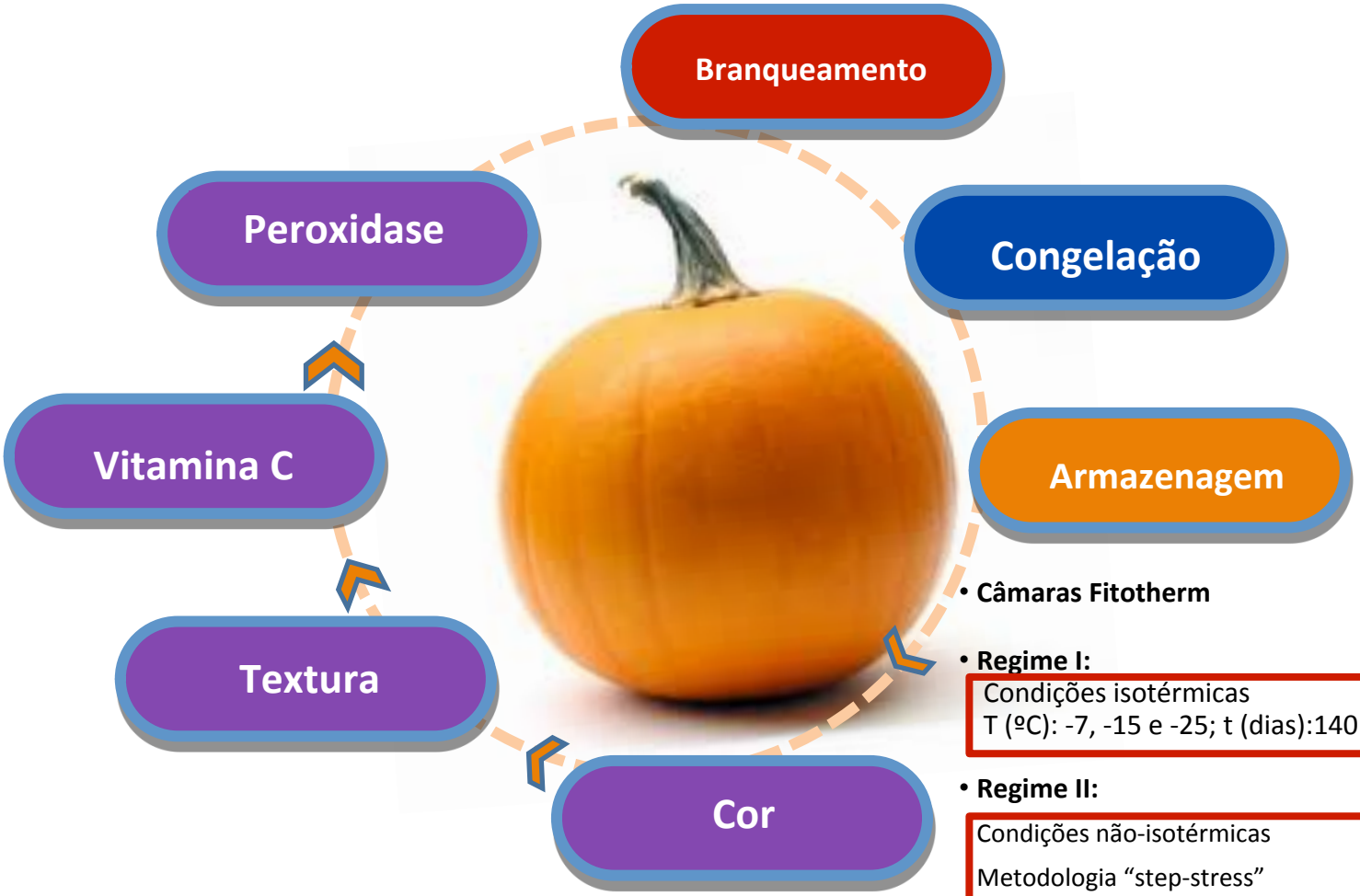
Obtenção do tempo de vida do produto à temperatura de -18ºC.

Condições de armazenagem

Estudos de Tolerância-Tempo-Temperatura (TTT)

Estudos Acelerados de Tempo de Vida Útil



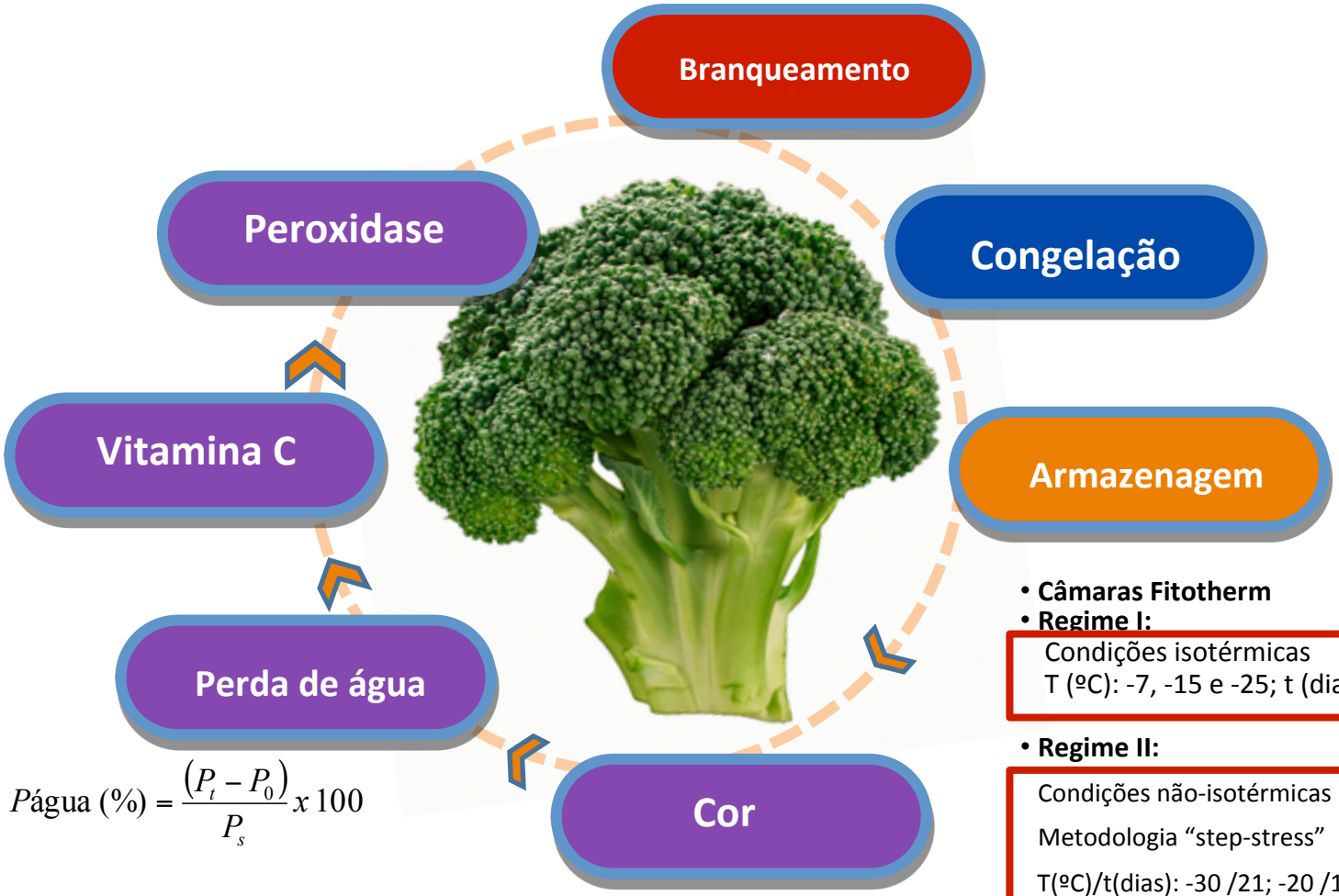


- **Câmaras Fitotherm**
- **Regime I:**

Condições isotérmicas
T (ºC): -7, -15 e -25; t (dias):140

- **Regime II:**

Condições não-isotérmicas
Metodologia “step-stress”
T(ºC)/t (dias): -30 /21; -20 /16;-10 /11 e -5 /9 ;
(total 57 dias)



$$P\text{água}\ (\%) = \frac{(P_t - P_0)}{P_s} x\ 100$$

- Parâmetros: a^*/a_0^*; hue ($\tan^{-1} b^*/a^*$)

- **Câmaras Fitotherm**
- **Regime I:**

Condições isotérmicas
T (ºC): -7, -15 e -25; t (dias):140

- **Regime II:**

Condições não-isotérmicas

Metodologia "step-stress"

T(ºC)/t(dias): -30 /21; -20 /16;-10 /11 e -5 /9; (total 57 dias)

Brócolos (*Brassica oleracea L. ssp.*)

- **Câmaras Fitotherm**
- **Regime I:**

Condições isotérmicas
T (ºC): -7, -15 e -25; t (dias):140

- **Regime II:**

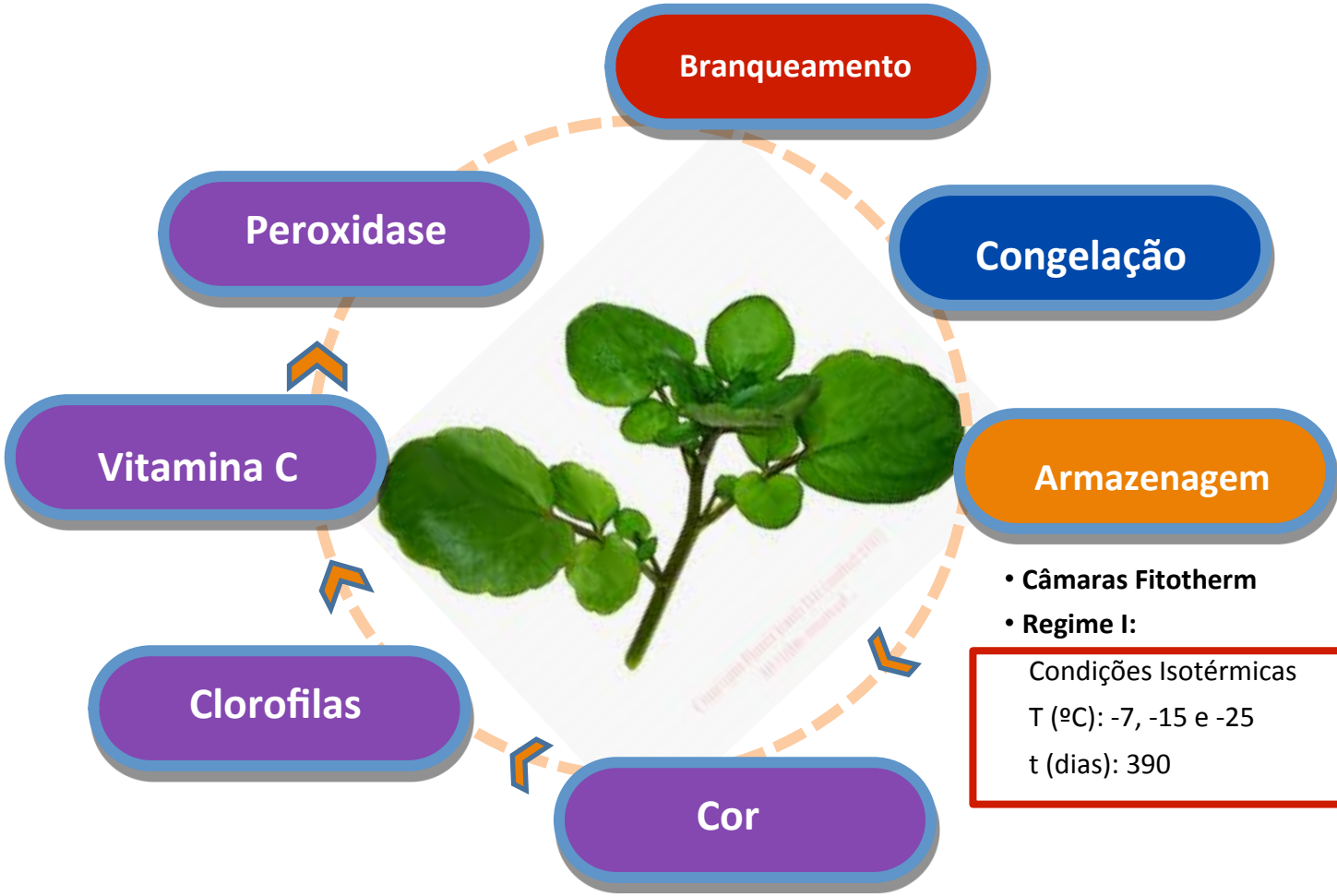
Condições não-isotérmicas

Metodologia "step-stress"

T(ºC)/t(dias): -30 /21; -20 /16;-10 /11 e -5 /9; (total 57 dias)

- Parâmetros:
  L*; hue (h*)

Material & métodos de análise



- **Câmaras Fitotherm**
- **Regime I:**

Condições Isotérmicas

T (ºC): -7, -15 e -25

t (dias): 390

Modelar as condições de armazenagem

Avaliar o efeito do tempo e temperatura de armazenagem na velocidade de degradação dos atributos analisados

Modelos cinéticos utilizados

Condições Isotérmicas

$$C = C_0 - \left(k_{ref}\exp\left(-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)t\right)$$

$$C = C_0\exp\left(-k_{ref}\exp\left(-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)t\right)$$

$$C = C_{eq} + (C_0 - C_{eq})\exp\left(-k_{ref}\exp\left(-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)t\right)$$

Condições Não-Isotérmicas

$$C = C_0 - \left[k_{ref}\int_0^t \exp\left[-\frac{E_a}{R}\left(\frac{1}{T(t)}-\frac{1}{T_{ref}}\right)\right]dt\right]$$

$$C = C_0\exp\left[-k_{ref}\int_0^t \exp\left[-\frac{E_a}{R}\left(\frac{1}{T(t)}-\frac{1}{T_{ref}}\right)\right]dt\right]$$

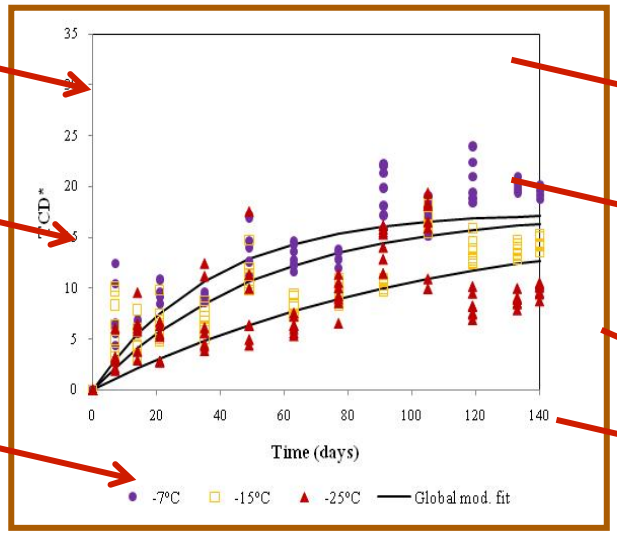
$$C = C_{eq} + (C_0 - C_{eq})\exp\left[-k_{ref}\int_0^t \exp\left[-\frac{E_a}{R}\left(\frac{1}{T(t)}-\frac{1}{T_{ref}}\right)\right]dt\right]$$

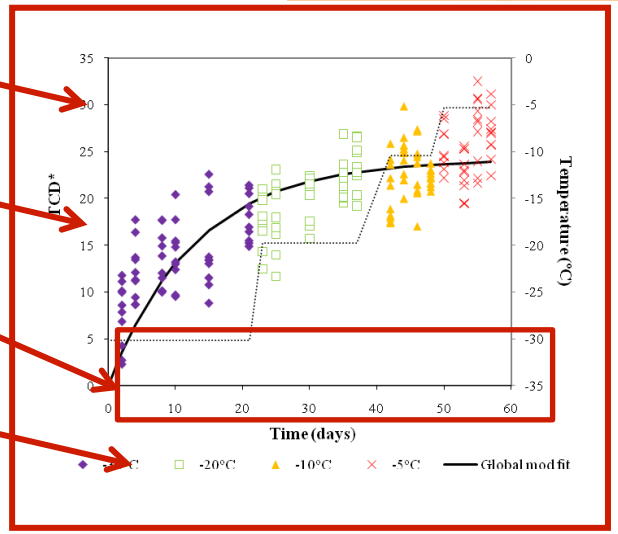
Integração das equações

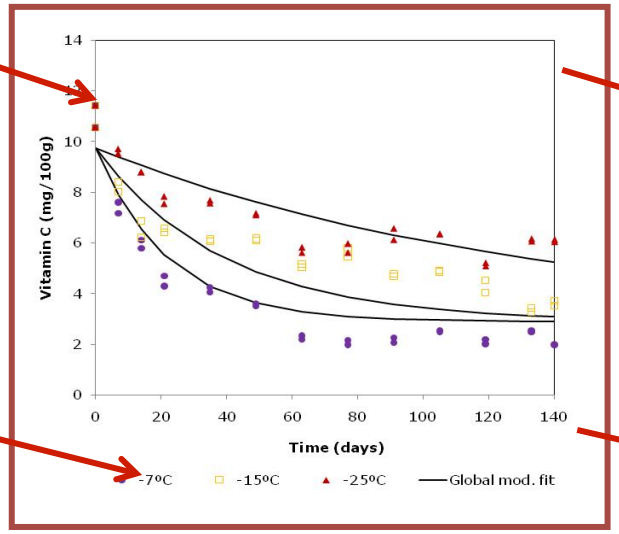
Abóbora *(Cucurbita maxima L.)*

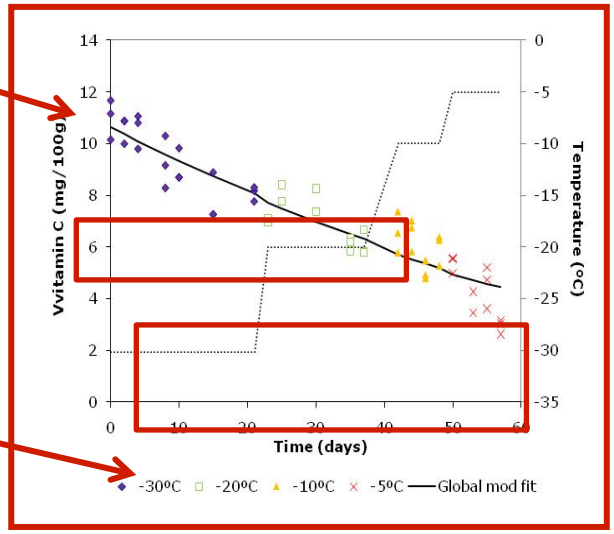
Vitamina C

Cor_TCD

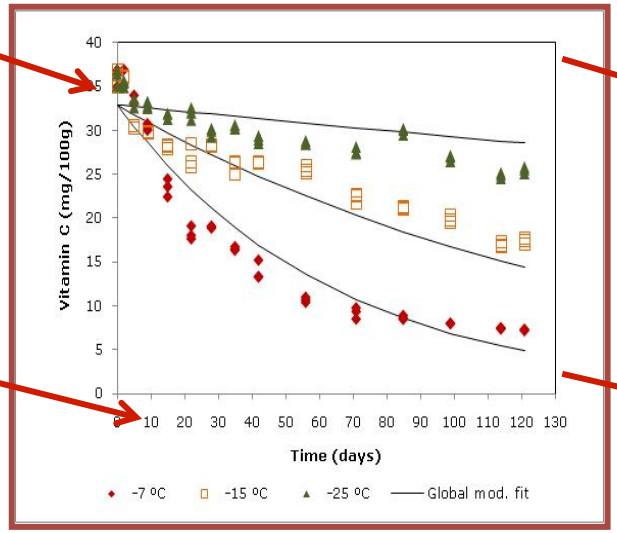


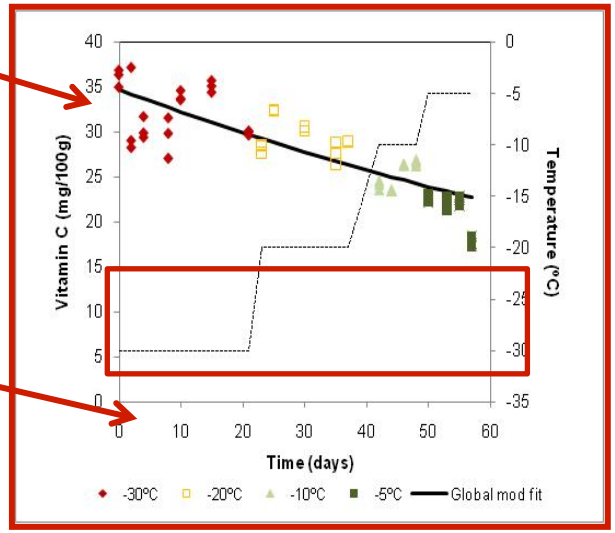




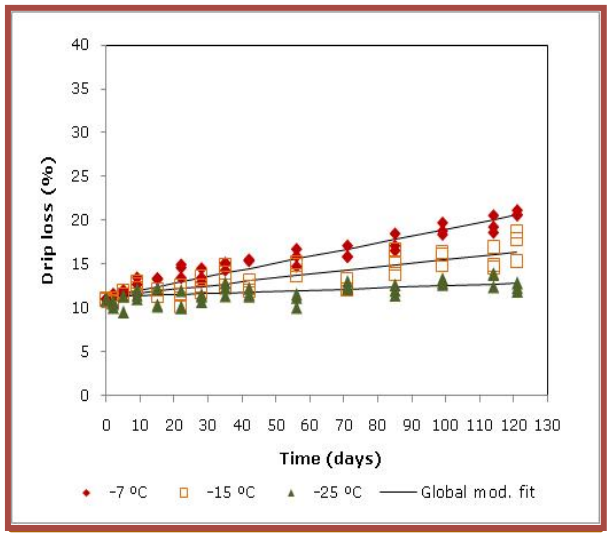


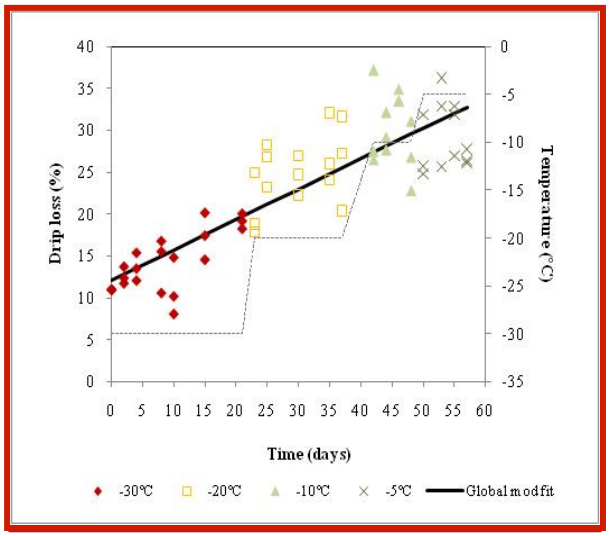
Brócolos (*Brassica oleracea L. ssp.*)





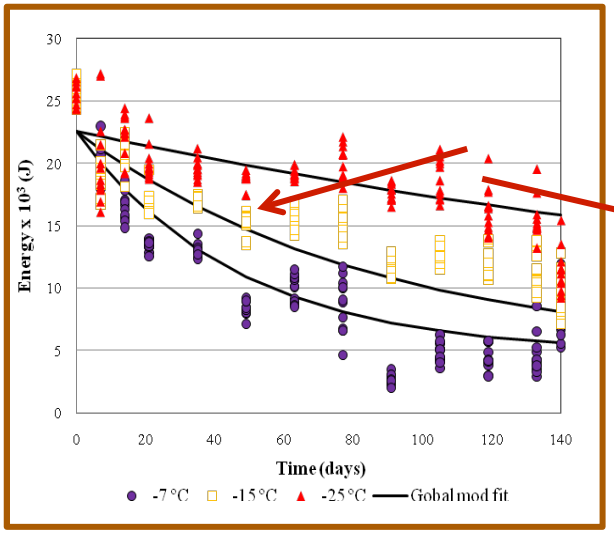
Vitamina C

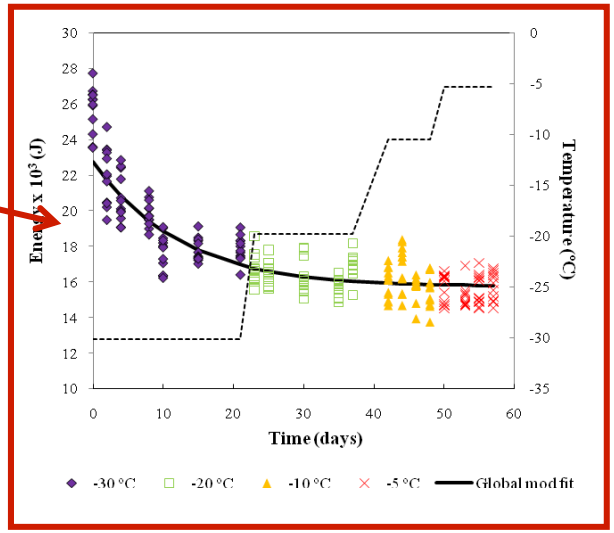




Perda de água

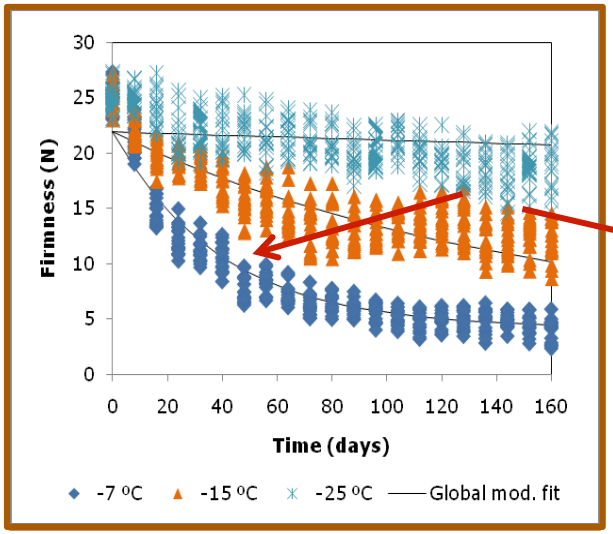
Textura

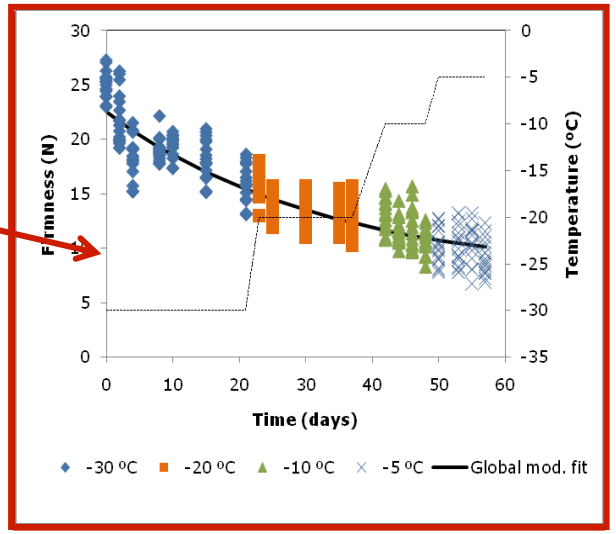




Abóbora

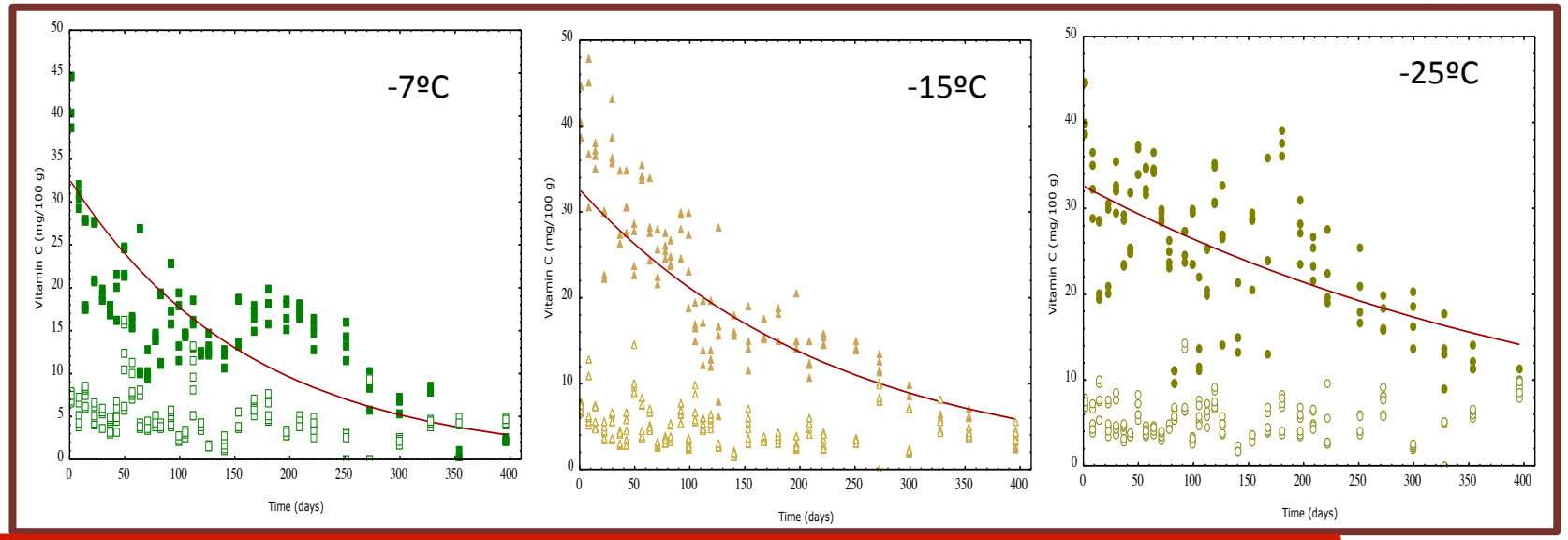
Cenoura

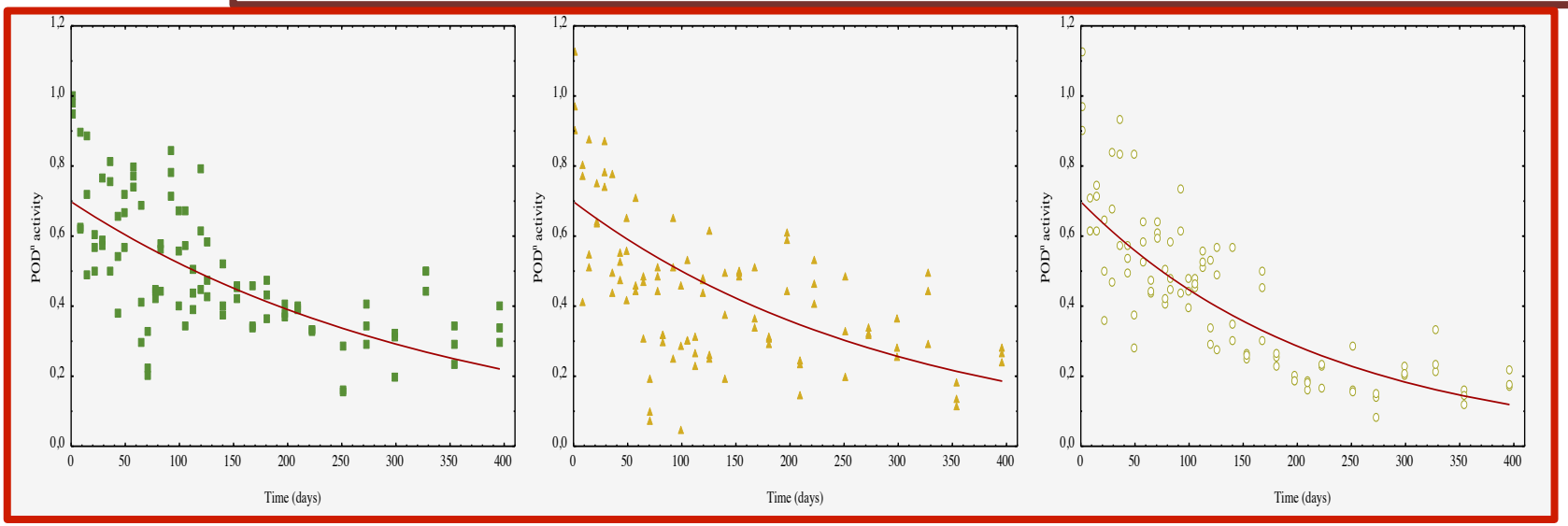




Agrião *(Nasturtium officinale R. Br.)*

Vitamina C AA +DHAA





POD

Tempo de Vida = Modelo cinético + Redução de 50% Vitamina C + -18ºC

Previsão

61 dias

125 dias

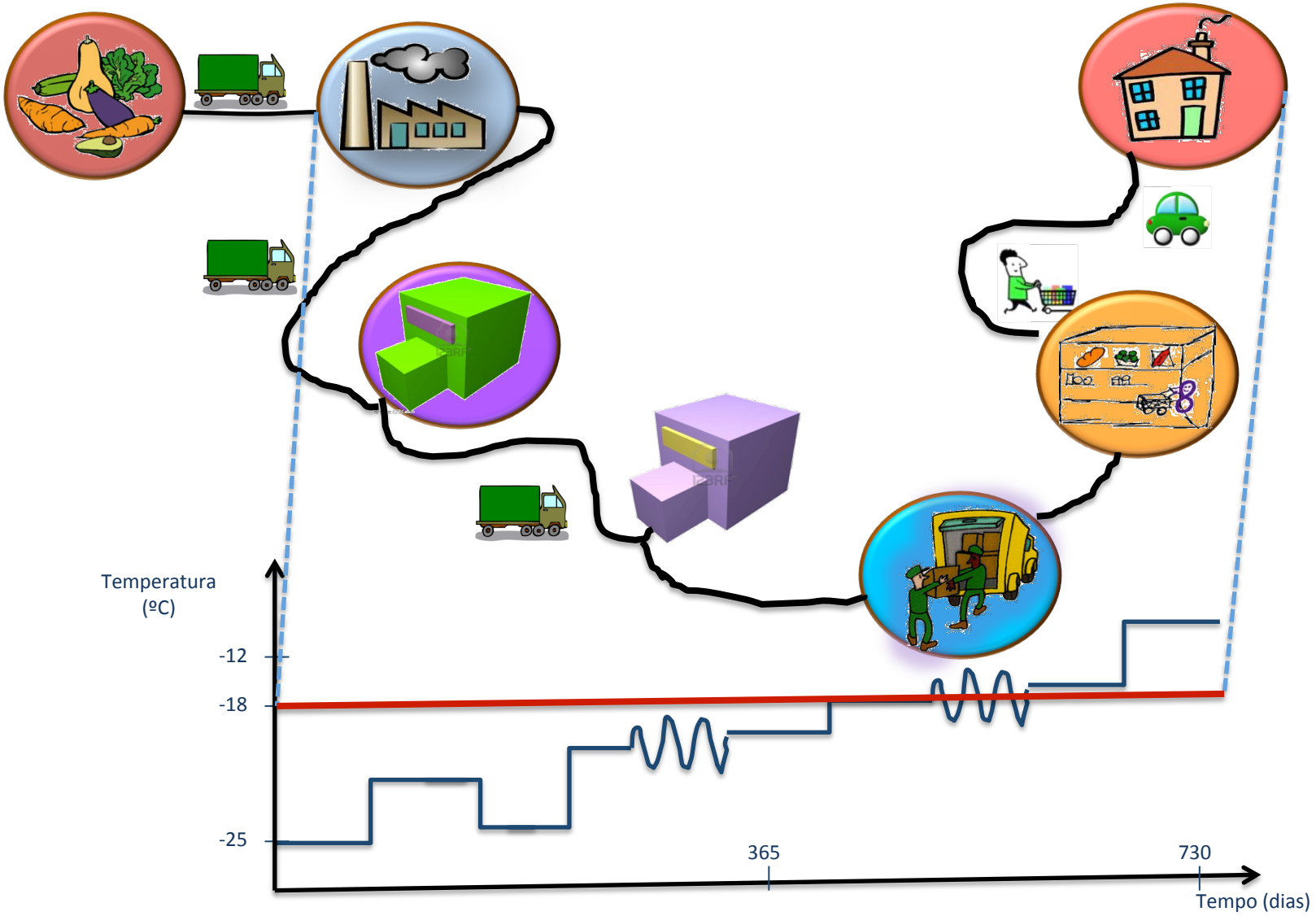
118 dias

6 meses

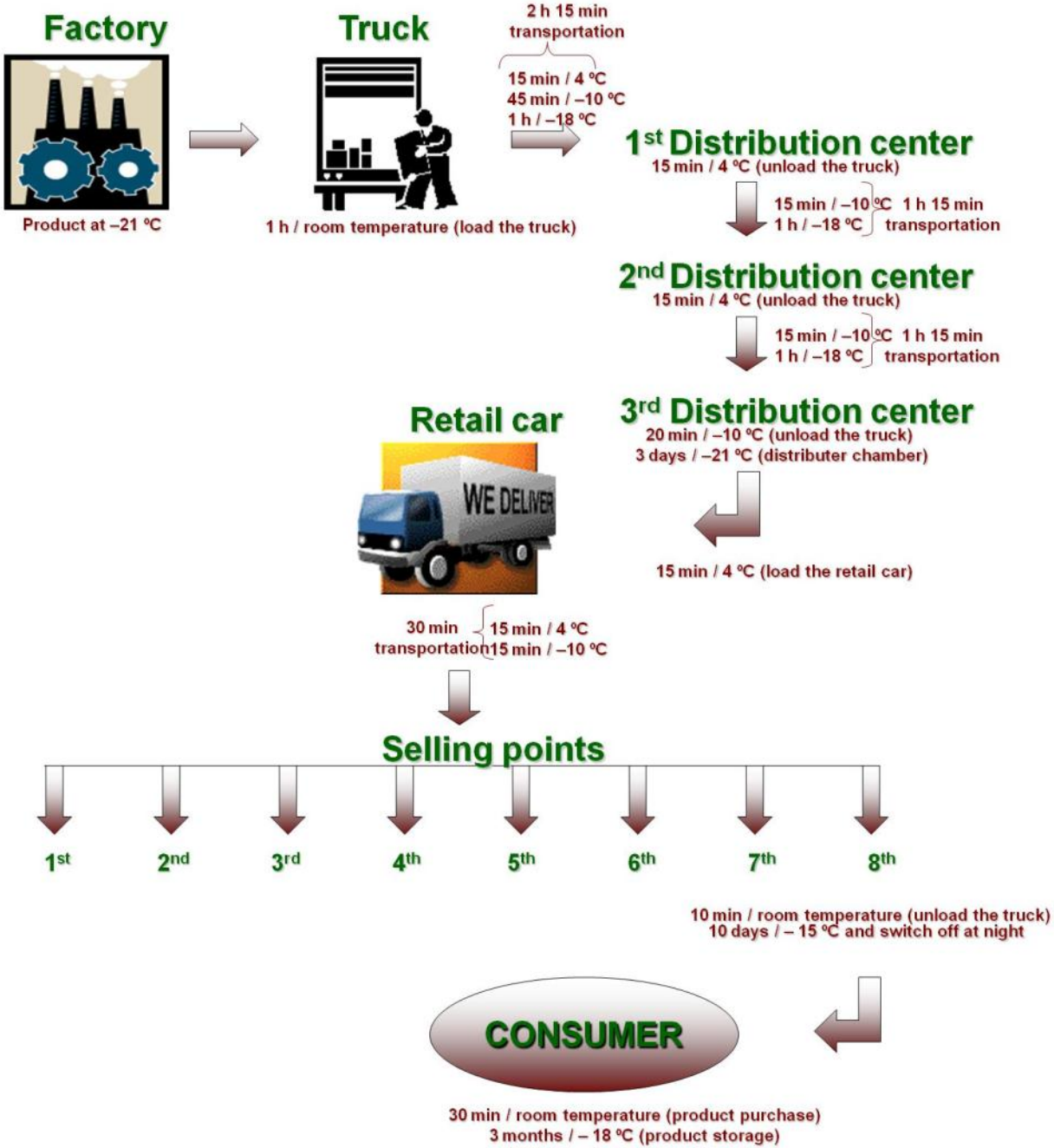
Armazenagem e Cadeia de distribuição

Enquadramento

Armazenagem e cadeia de distribuição



Plano de Abusos de Temperatura



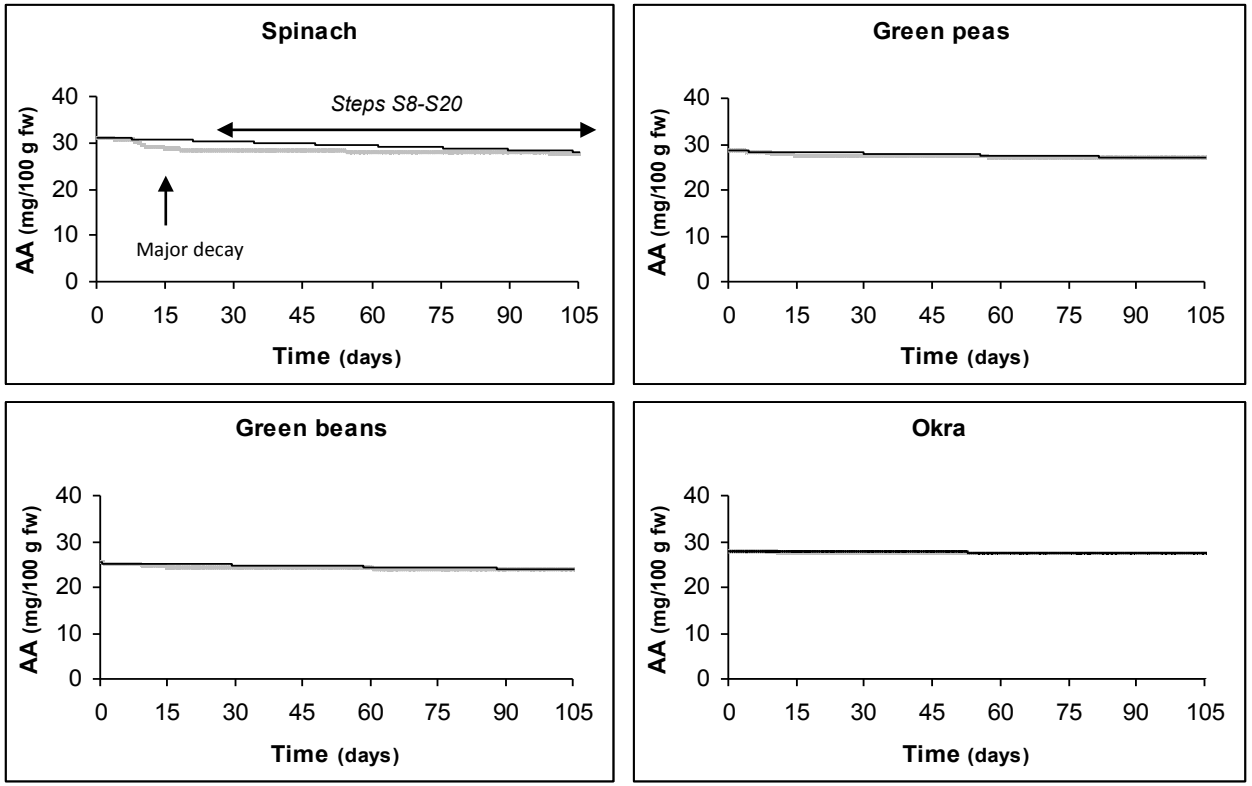


Figure 8- Spinach, green peas, green beans and okra ascorbic acid prediction models for constant temperature (-18 ºC; black line) and for temperature abuses following the storage plan (grey line).

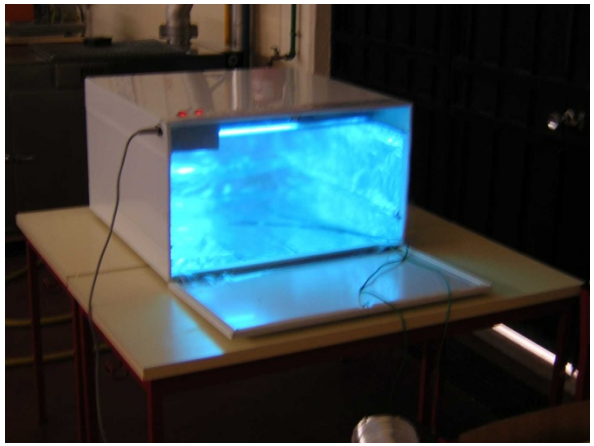
Novas Tecnologias

New Technologies

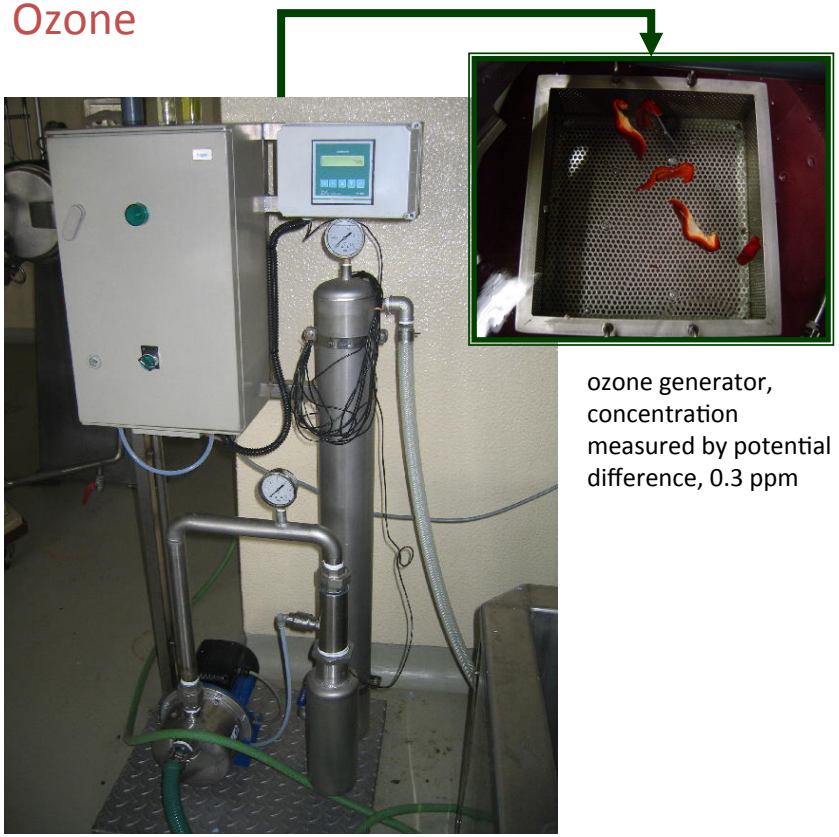
Combining Heat and other Non-Thermal Technologies to preserve foods

UV-C radiation

UV-C chamber (University of Algarve), 4 germicidal UV lamps (TUV G30T8, 16 W, Philips, peak emission at 254 nm), average intensity 12.36 W/m^2



Ozone



ozone generator, concentration measured by potential difference, 0.3 ppm

Ultrasonication / Thermosonication

ultrasound equipment (Bandelin Sonorex RK 100H) operating at 32 kHz

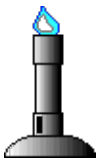
New Technologies

Combining Heat and other Non-Thermal Technologies to preserve foods

watercress

Nasturtium officinale

Types of combined treatments with ultrasound



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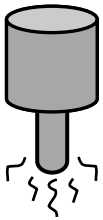


Heat + Ultrasound

Thermosonication

New Technologies

Combining Heat and other Non-Thermal Technologies to preserve foods

Quality

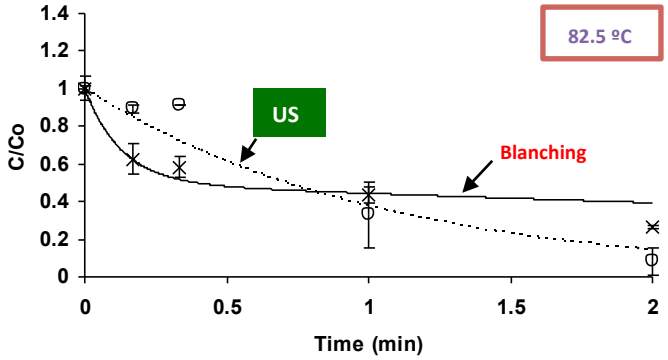
Vitamin C

Colour changes

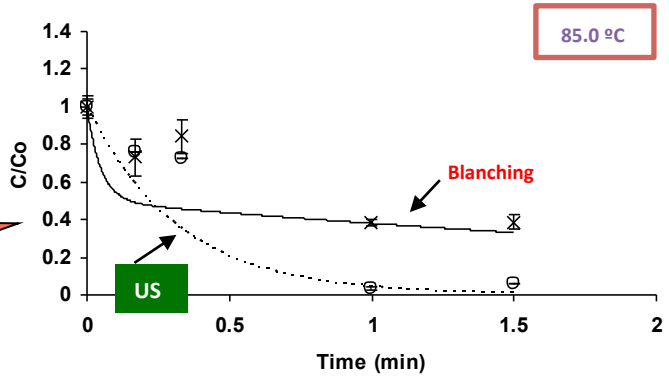
Peroxidase

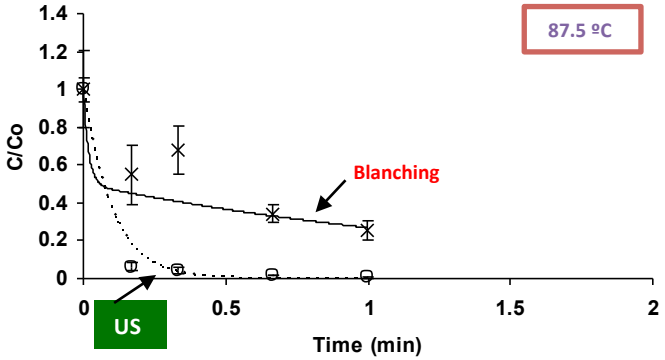
New Technologies

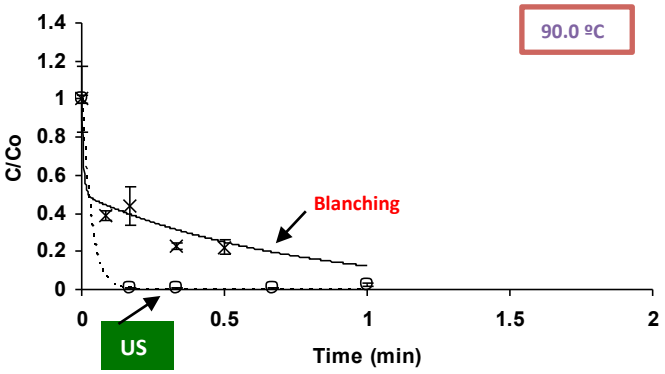
Combining Heat and other Non-Thermal Technologies to preserve foods



Peroxidase







New Technologies

Combining Heat and other Non-Thermal Technologies to preserve foods

The application of thermosonication

- temperatures above 85 ºC and for the same blanching times

led to higher enzyme inactivation when compared to heat blanching
ocesses

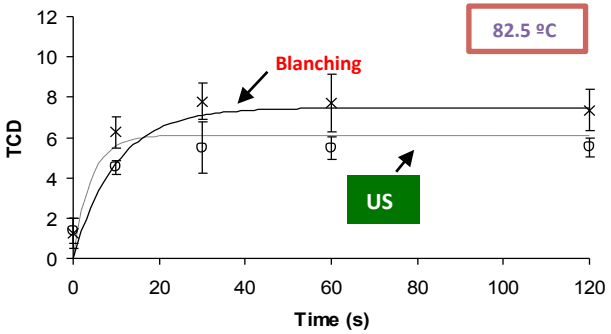
Peroxidase

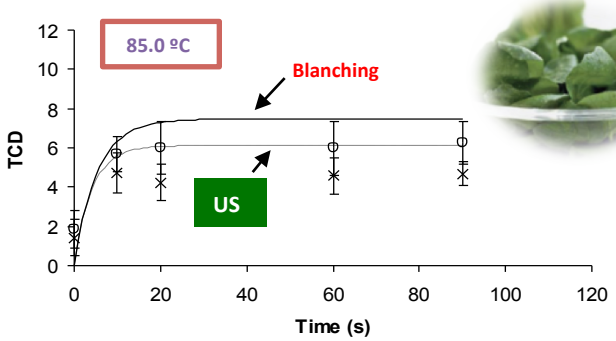
These results allow the application of shorter blanching times at this range of temperatures, leading to a product with a higher quality, or minimized processing

New Technologies

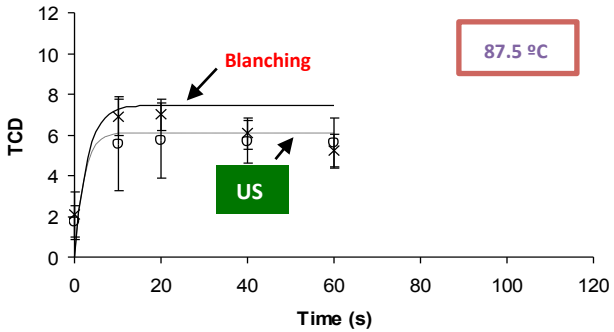
85 ºC

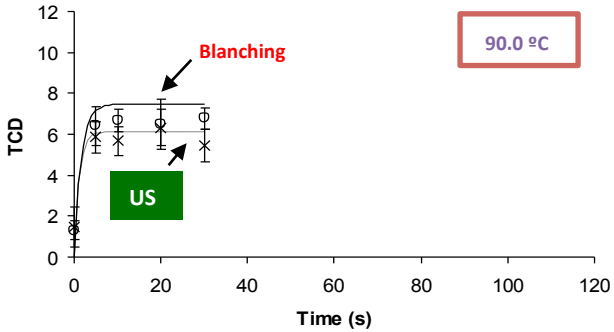
Combining Heat and other Non-Thermal Technologies to preserve foods

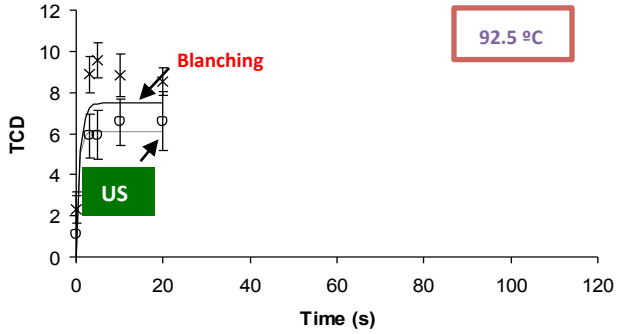




Colour







New Technologies

Combining Heat and other Non-Thermal Technologies to preserve foods

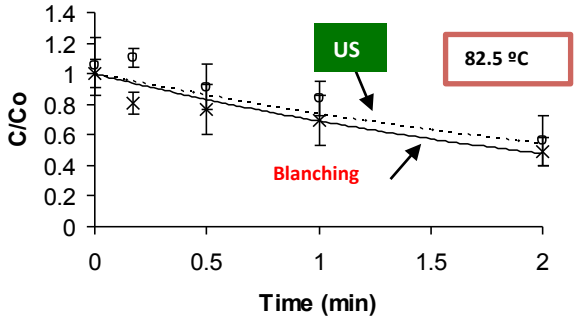
The application of thermosonication

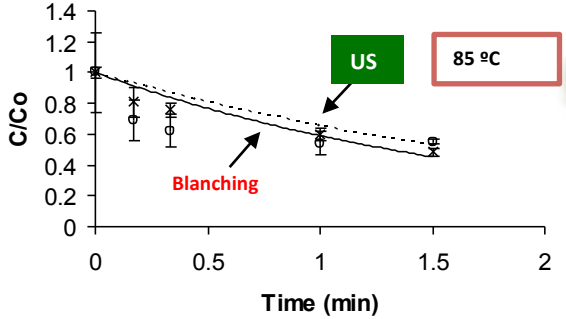
Colour

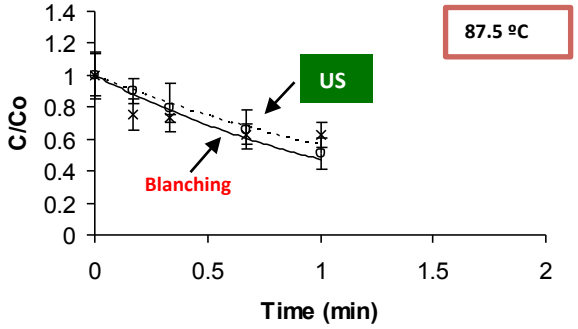
Reaction rates of watercress colour changes due to heat and thermosonication blanchings were not significantly different

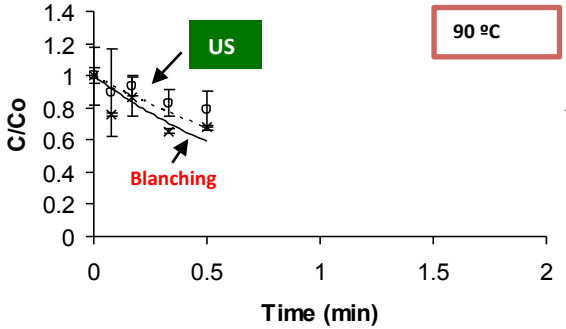
New Technologies

Combining Heat and other Non-Thermal Technologies to preserve foods

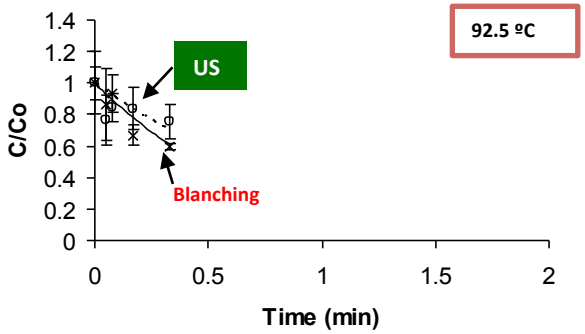








Vitamin C



New Technologies

Combining Heat and other Non-Thermal Technologies to preserve foods

The application of thermosonication

Vitamin C

Results showed no significant differences between heat and thermosonication treatments

The treatment will allow good vitamin C retention

New Technologies

Combining Heat and other Non-Thermal Technologies to preserve foods

The application of thermosonication

Quality

The thermosonication treatments can be a good alternative

to the traditional heat blanching processes,

since higher quality products are attained

Thank you for your attention!

Escola Superior de
BIOTECNOLOGIA
Universidade Católica Portuguesa - Porto

CBQF
Centro de Biotecnologia
e Química Fina