Symbios

"*The knowledge of nature*"

London; 19th May 2005

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CTCOR

Objectives

- ✓ Scientific and methodological frames
- ✓ Experiences and essays protocol
- ✓ Results obtained
- ✓ Technical validation of the process

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Scientific Approach

Problem: Formation of 2,4,6-trichloroanisol in cork

- ✓ Collection and critical analysis on the scientific and empirical knowledge referring to the problem;
- ✓ Frame the problem in the broad domain of the nature's biological processes. Avoid a specific and reducing approach of the problem;
- ✓ Address the target focus not for the compound itself, but for the mechanisms that conduct to its formation in cork material;

↳ sighting a *preventive solution*

Scientific Approach

In a broad perspective,

- ✓ The action of the microorganisms has a major influence in the world surrounding us;
- ✓ Its action is not only necessary but essential to the equilibrium and sustainability of the living systems;
- ✓ Being a biological organization, the microorganisms need nutrients in order to obtain the energy required for their cellular metabolism;
- ✓ For this purpose, the microorganisms make use of enzymes. Acting on the organic medium of support, the enzymes promote the synthesis of the nutrients required for the cellular activity of the microorganisms.

Scientific Approach

Specifically in cork substrate,

- ✓ The post-boiling stabilization period is the stage of major microbiological activity on cork material;
- ✓ The post-boiling stabilization period is characterized by the combination of several biotic and abiotic factors, that induce to the formation of chloroanisoles as intermediate products of biological reactions;
- ✓ The mechanisms, the species and the conditions that promote the formation of chloroanisoles in cork are sufficiently studied;

Scientific Approach

How to inhibit the formation of chloroanisoles in cork?

~~Hypothesis 1: Complete sterilization of the cork, aseptization of industrial plants and handling of the cork through asepsis conditions.~~

or

Hypothesis 2: Alteration of the characteristics of the medium, enabling the development of the microorganisms through controlled conditions and, simultaneously, arousing the denature of the enzymes;

Methodological approach

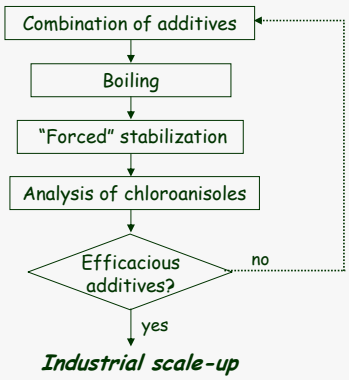
Hypothesis 2: Alteration of the cork medium characteristics.

How? Acting directly at the cork boiling stage.

Enrichment of the water with specific additives, which, by diffusion on an aqueous boiling medium, will be fixed to the interior walls of the pores and to the back of the cork planks (i.e.: structures rich in lignin-polysaccharides compounds).

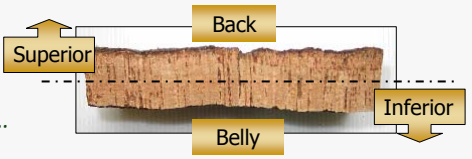
Methodological approach

It was planed an experimentation protocol at laboratory level:



Preliminary results

After several combinations of additives …
➡ formulation X



Determination of 2,4,6-TCA in cork

Fraction	Inferior	Superior
Boiling with additives X; "Forced" stabilization	n.d.	n.d.

Obs: n.d. – not detected; D.L.: 0,7 ng/l

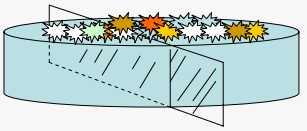
Preliminary results

Visual observation: All the specimens boiled with additives X display an *exuberant* microbial growth during post-boiling stabilization;

Way of fixing, profusion and species sporulation?

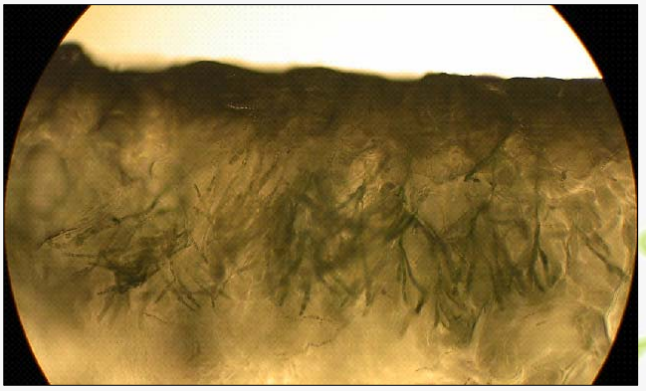
- Preparation of microbiological culture media (WLN) with and without additives X;
- Inoculation of microbiological species commonly found in cork;
- Incubation during 5 days at 27°C;
- Tangential cut of culture media; preparation of profiles for optical microscopy analysis.

Illustration of the cut:



Preliminary results

Tangential profiles of a culture medium without additives:
Optical microscopy observation (400x)



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Preliminary results

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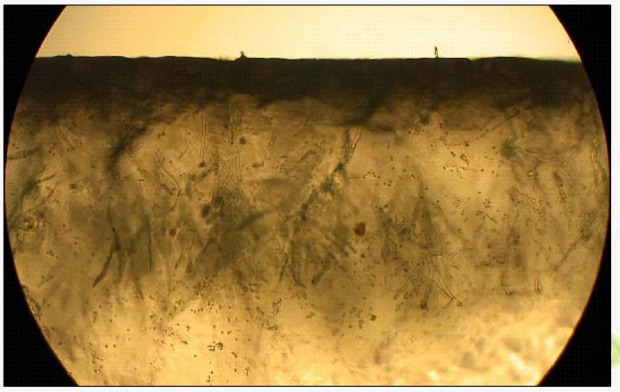

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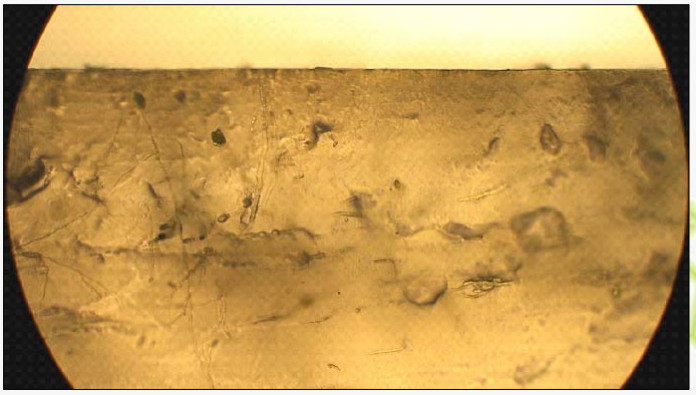

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Preliminary results

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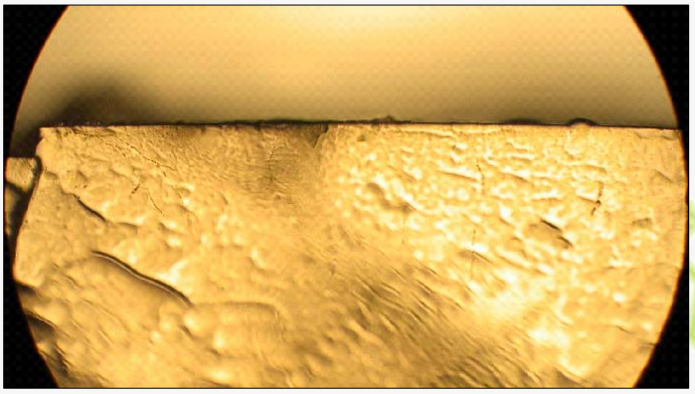

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Preliminary results

With the addition of formulation X…

- ✓ The activity of the species is markedly reduced into the supporting matrix;
- ✓ Inalterability of the chromatic and morphological characteristics of the culture medium, indicating that the enzymatic degradation effect was clearly reduced;
- ✓ Absence of sporulated forms in the interior of culture medium.

.

Preliminary results

With the addition of formulation X…

Barrier effect: Inexistence of hyphae in the interior of the culture medium, which indicates that the microorganisms (molds) find a "less attractive" internal media.

Symbiotic effect: With the availability of nutrients at the surface, the microorganisms tend to settle at the interface, exhibiting its visible vegetative structure (exterior mycelium) in an exuberant way. In this sense, the interface provides a "favourable ground" to the dissemination of microorganisms by superficial dispersion of their sporulated forms.

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Prompt action

Scale-up – Evaluate the efficacy of formulation X in real conditions of usage

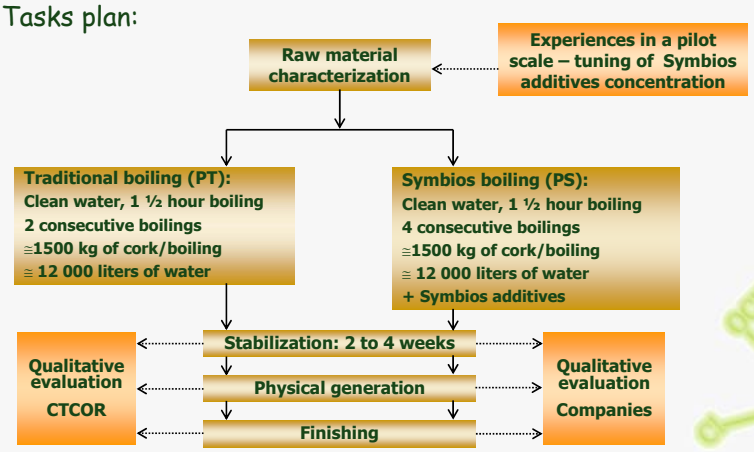
Symbios Process

Industrial Experience - Results

- ✓ Two cork manufacturing companies:
  Sercor – Sociedade Exportadora de Rolhas e Cortiça, S.A.
  Norcor – Indústria de Cortiça, S.A.
- ✓ Beginning of the industrial experience: August 2004;
- ✓ Batches of raw cork material : proceeding from 2003 harvest;
- ✓ Experiences done in a comparative basis, i.e.: *Symbios process* vs *Traditional process*.

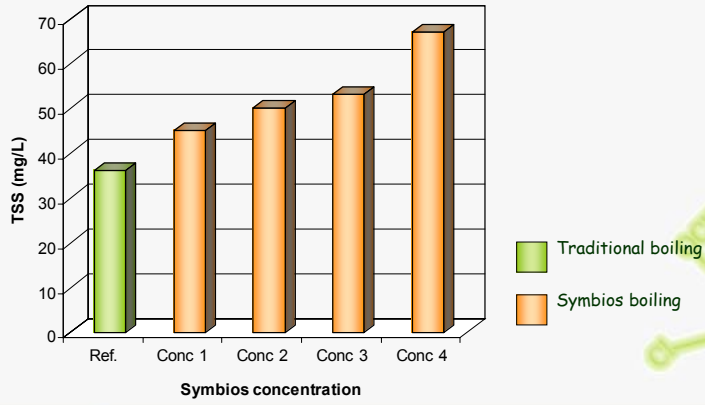
Industrial Experience - Results

Tasks plan:



Industrial Experience - Results

Symbios boiling:
Notable increase of the extractive capacity of boiling medium



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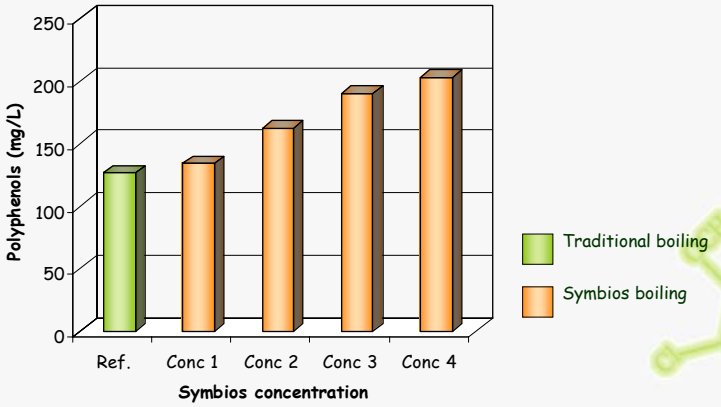
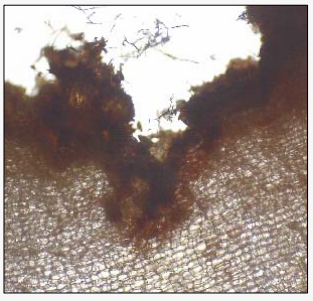
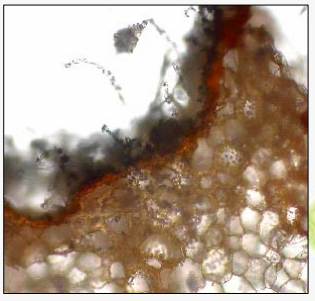


Industrial Experience - Results

Microbiological growth during post-boiling stabilization :
Traditional process vs Symbios process

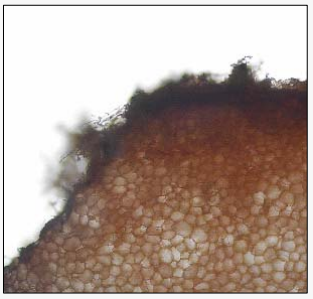




Traditional process

Industrial Experience - Results

Microbiological growth during post-boiling stabilization :
Traditional process vs Symbios process



Symbios process

Industrial Experience - Results

Mycological growth during post-boiling stabilization :
Traditional process (PT) vs Symbios process (PS)

Regulation restrictions

- Activity of filamentous fungi
- Potential of OTA mycotoxin synthesis?

Determination of Ochratoxin A (OTA) in cork planks after stabilization
(Tests carried out by the Biological Engineer Centre at Minho's University)

Sample	OTA ($\mu g_{OTA}/kg_{cork}$)
PT	0,376
PS	0,294

Industrial Experience - Results

Determination of Ochratoxin A (OTA) in cork planks after stabilization
(Tests carried out by the Biological Engineer Centre at Minho's University)

Critical analysis of the results:

✓ *Identical results between PT and PS samples*

✓ *Having regard to the proposed limit in wine: 2μg/L (…)*
(…) considering that each cork weights approximately 3,5 g (…)
(…) considering that the total amount of OTA eventually present in a cork could pass to the wine (!!!) (…)

The final concentration in the wine would be ≅ 0,0016 μg/L

Which represents 0,08 % (!!!) of the admissible maximum limit proposed for the wine

Industrial Experience - Results

Determination of releasable TCA in cork strips

Samples PT (ng/l)				
Useful cork tissue	1,8	4,1	3,1	2,8

Samples PS (ng/l)				
Useful cork tissue	n.d.	n.d.	n.q.	n.d.

DL: 0,7 ng/l; QL: 1,4 ng/l

Industrial Experience - Results

Sensory analysis of washed cork stoppers

On samples proceeding from both companies it was noted the following:

- ✓ The incidence of "moldy" on references PS occurs as traces (less than 1/1000) and, when occurs, its level of intensity is "slight";
- ✓ The samples PT registered a "moldy" incidence in 1 to 2% of corks analyzed, displaying levels of intensity from "slight" to "strong";
- ✓ "The samples PS exhibit a remarkable lowering of sensory basis line (i.e.: increase of sensory clearness), when compared with references PT":
- ✓ "The samples PS display a lesser variability of the sensory basis line, when compared with references PT".

Industrial Experience - Results

Sensory analysis of washed cork stoppers

Contact simulator: distilled water/odorless

(Note: Samples PT e PS form a single company)

Samples PT (ng/l)	
Absence of "moldy"	893
Detection of "moldy"	12
Total cork stoppers	905

Analytical confirmation

Quantification of "*releasable*" chloroanisoles in individual corks

29,1 ng /l
4,0 ng/l
8,9 ng/l
1,5 ng/l
28,4 ng /l
66,4 ng/l
5,0 ng/l
15,0 ng/l
21,2 ng/l
9,7 ng /l
75,8 ng/l
6,2 ng/l

2,4,6-TCA

Admitting that all of the releasable TCA migrates to the wine (?!)

Sensorial detection threshold in wine: 4 ng/l

"moldy" incidence rate = 11/905 corks = **1,2%**

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Industrial Experience - Results

Sensory analysis of washed cork stoppers

Contact simulator: distilled water/odorless
(Note: Samples PT e PS form a single company)

Samples PS (ng/l)	
Absence of "moldy"	1181
Detection of "moldy"	3
Total cork stoppers	1184

Analytical confirmation

Quantification of "*releasable*" chloroanisoles in individual corks

1,9 ng /l
1,6 ng/l
6,6 ng/l

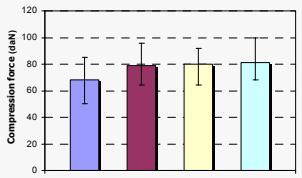
} 2,4,6-TCA

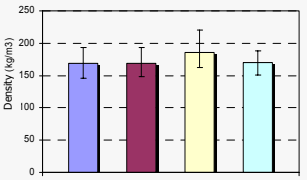
Admitting that all of the releasable TCA migrates to the wine (?!)
Sensorial detection threshold in wine: 4 ng/l

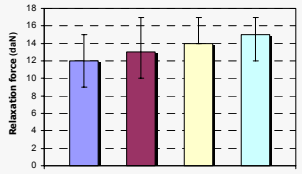
"moldy" incidence rate = 1/1184 corks = **0,08%** (!!!)

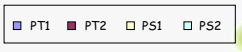
Industrial Experience - Results

Physical and mechanical behaviour of cork stoppers: Density; Compression and Relaxation









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Conclusions

- ✓ Symbios process is a preventive biological process, which promotes the development of benign microorganisms, and inhibits the development of species which are capable of producing undesirable metabolites;
- ✓ Symbios process promotes the inhibition of the biosyntheses of chloroanisoles throughout the preparation stage of cork;
- ✓ Boiling with Symbios assures a more efficient cleaning and extracting of removable substances present in cork (ex.: solid residues, polyphenols, ...);

Conclusions

- ✓ With the result of "cleaner" wastes of cork (physically and organoleptically), the advantages of the Symbios process are present throughout the manufacturing chain of technical corks;
- ✓ Symbios process is innocuous and complies with community regulations for food contact materials;
- ✓ Symbios process introduces no changes whatsoever to the physical and mechanical properties of the cork, thus the functionality of the product – cork stopper – is maintained.

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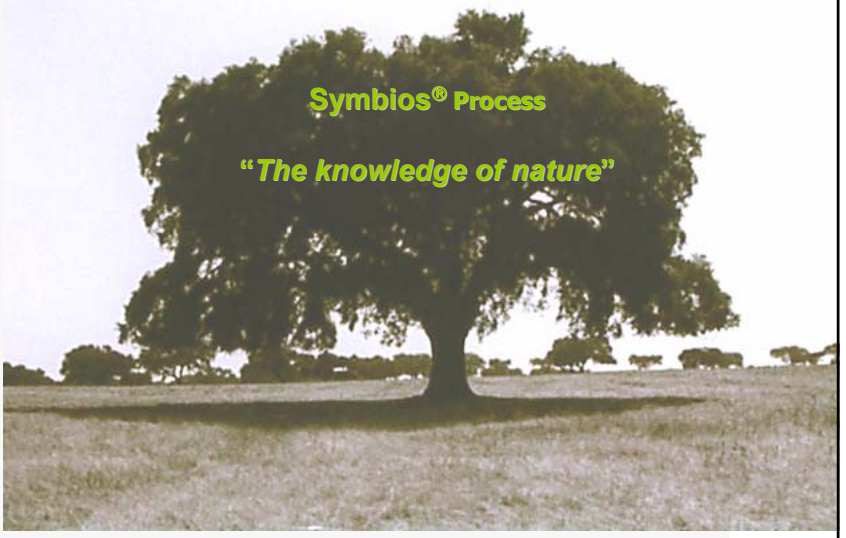

Symbios® Process

"The knowledge of nature"

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Thank You for Your Attention

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