Portuguese Research Priorities and Direction

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Introduction
Greek amphoras found in the Athenian agora show that cork was used as a sealing material as far back as the fifth century BC. More recently, cork has been used for fishing floats and ladies' winter shoes, which demonstrate two of its remarkable properties: buoyancy and thermal insulation. The first use of cork as a seal for bottled wine was in 1690, by Dom Perignon.

The growing of cork
Cork comes from the bark of the cork oak, Quercus suber. This tree grows widely in the southern part of Portugal and throughout the Mediterranean region. When the tree reaches maturity, the first harvest of the bark is made. This first layer of bark is called 'virgin cork', which cannot be used for the production of cork stoppers. The second harvest of cork, which takes place after another 9–10 years, is rarely used for the production of cork stoppers.

The bark is harvested in summer, when the rate of growth of the cork cells is at its highest. Following the harvest, a new protective layer is formed at an annual growth rate of 2–6 mm, depending on the tree and local conditions. Nine to 10 years after the stripping of the bark, a new layer covers the tree.

The cork produced in the third harvest is referred to as amadia. This cork is used for the manufacture of cork stoppers. Bark is harvested 12–15 times, until the tree succumbs to old age, after approximately 150 years.

The main characteristics of cork which confer its industrial value are:
- buoyancy;
- impermeability;
- compressibility and elasticity; and
- thermal, acoustic and kinetic (vibration) insulation.

Production of cork stoppers
Only the better amadia quality cork planks are used for the production of cork stoppers. The slabs of lower quality or of inappropriate dimensions are used for granulate and agglomerate cork products, as is the refuse from cork manufacturing. Even the cork dust is used. It is burned to produce the thermal energy required in heating boilers, or the manufacture of briquettes to be used in stoves for home heating. This is an industry that wastes nothing.

Preparation of the cork/boiling
After harvest, the planks of bark are aged for at least 6 months, during which time they lose sap and moisture. When ageing is complete, the cork planks are boiled in water. During this boiling process, the planks increase in both length and thickness until a maximum size is reached after 45 minutes. At the same time, there is a reduction of the width of the planks, and a marked improvement in visual quality.

Boiling results in the extraction of tannins and compounds with long carbon chains, along with some volatiles, polyphenols and sugars. The tannin content is reduced by more than 50% if clean water is used. A reduction in the pH of the slabs is accompanied by an increase in the concentration of polyphenolic acids in the water. The same water is used for boiling several batches of corks, the number of which depends on the factory, after which it is discharged and replaced by clean water.

Stabilisation
The boiled planks are stacked for approximately 15 days while they dry, and to allow the structure of the cork to stabilise. In order to prevent uncontrolled microbial growth, the stabilisation room must be well aerated and have a controlled humidity (RH < 70%).

The microflora of the cork slabs is largely destroyed during the boiling process, with only a few thermophilic species of microorganisms surviving. The slabs are contaminated by the microflora of the factory. From the second week, these microorganisms cover the slab. Figure 1 represents this growth and penetration of the slab by hyphae of the microorganisms.

Washing/drying
The goal of washing is to disinfect and bleach the cork. Various washing procedures are used, which can be carried out in tanks or in automatic machines.

The traditional method consists of a wash with chlorinated lime. A by-product of this process is sodium oxalate. In this case, there is no resultant crystalline deposit, and the exterior of the stopper is not likely to be as white as that of stoppers treated with the traditional
Table 1. Concentration of residues in corks subjected to different washing procedures

<table>
<thead>
<tr>
<th>Washing treatment</th>
<th>Oxidants (mg/cork)</th>
<th>Residues (mg/cork)</th>
<th>Chlorides (mg/cork)</th>
<th>Oxalates (mg/Cork)</th>
<th>Phenols (AU at 310 nm)</th>
<th>Pentachlorophenol (µg/kg)</th>
<th>2,4,6-trichloroanisole (mg/cork)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unwashed</td>
<td>0</td>
<td>1.6</td>
<td>0.5</td>
<td>0.8</td>
<td>0.14</td>
<td>&lt;2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tradional</td>
<td>0.008</td>
<td>1.6</td>
<td>1.6</td>
<td>&lt;0.1</td>
<td>0.11</td>
<td>&lt;2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Metabisulphite</td>
<td>0</td>
<td>0.9</td>
<td>&lt;0.1</td>
<td>0.6</td>
<td>0.11</td>
<td>&lt;2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Peroxide</td>
<td>0</td>
<td>0.5</td>
<td>0.6</td>
<td>0.6</td>
<td>0.08</td>
<td>&lt;2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

chlorinated lime wash. Alternatively, the corks may be bleached with peroxide. This is performed in an alkaline or acidic medium, and is followed by a citric acid rinse to neutralise any residual peroxide. Washing with metabisulphite involves submerging the corks in a solution of 1-2% w/v potassium metabisulphite. This process retains the natural colour of the cork, sometimes even darkening its appearance.

Research conducted at CTCOR into these bleaching procedures has shown that no residues of this process that would cause modification of wine flavour or aroma are detected in washed corks. Table 1 shows the concentration of residues in corks subjected to various washing treatments.

Scanning electron microscopy of washed corks reveals that the washing treatments cause no structural damage to the corks. It is of great importance that the various washing treatments be performed within strict chemical specifications. Similarly, the monitoring of residues of the washing process in corks is extremely important.

After washing, the cork should be dried immediately to a moisture content of 6-8%. Storage of corks with higher moisture content allows the uncontrolled growth of microorganisms, which can create 'mouldy' aromas.

Branding and surface treatment
Branding of the surface of the cork can be done either with ink or with hot metal implements. Surface treatments are applied to allow easy insertion and extraction of the cork within the neck of the bottle, and to improve the sealing capability of the cork. Normally, two types of products are used:

- paraffins, as a solid, oil or in emulsion; and/or
- silicone, as an oil, emulsion or an elastomer.

Paraffin increases the sealing capability, while the silicone reduces the friction between the cork and the bottle, thus facilitating insertion and extraction. The products used should be food grade (approved by the United States Food and Drug Administration, FDA), and should be selected according to the type of bottle, wine and cork.

Packaging

There are two main types of packaging, depending on whether the corks are sold as finished or semi-finished products. Semi-finished corks are generally packaged in bags made of raffia or jute with a capacity of 5000 to 20000 units. Branded and treated corks may be packed in airtight bags in quantities of 1000 or 1500, under vacuum and with sulphur dioxide, SO₂, to maintain aseptic conditions for several months. Finally, the bags are placed in cardboard boxes.

Transportation and storage in the cellar

It is well established that cork can become contaminated during transportation (due to contact with incompatible materials, such as pesticides or hydrocarbons), by exposure to inappropriate conditions such as high cork moisture content, or contamination with saline water. Documented cases exist in which the problem was obvious and the contamination was detected immediately on arrival. The possibility of less evident contamination, which is undetected at first, should not be ignored. Such contamination may cause modification of the aroma and flavour of wine which is difficult to diagnose.

Qualitative parameters

Standardisation
Within the European Union (EU), CTCOR is responsible for the development of Standard TC16 for cork, chairs Working Group 2 (Cork stoppers: assays) of the ISO TC 87 - cork standard, and has been involved in the CETIE committee for the standardisation of bottles since March 1994.

The Institute is represented on the following technical subcommittees of the European Committee for Standardisation:

- CEN TC 134 (Resilient floor covering): Chairman of Working Group 4 (cork);
- CEN TC 88 (Thermal insulating products): Chairman of Working Group 13 (cork);
- CEN TC 98 (Wall coverings): Chairman of Working Group 3 (cork);
- CEN TC 237 (Surfaces for sports areas);
- CEN TC 89 (Thermal performance of buildings and building components); and
- CEN TC 294 (Utensils in contact with food).

The process of setting specifications for agglomerate cork floor covering and thermal insulating products is almost complete. The preparation of the specific Directive for cork in contact with foodstuffs is ongoing. The current focus is on the assessment of the products used in the cork manufacturing process, and the study of migration of volatiles.

Analytical methods
CTCOR has a role in the development of methods for analysis of cork products. It is an 'Accredited Laboratory' (EN 45001) for cork assays. Table 2 lists tests applied to the characterisation and simulation of cork performance.

Research and development
CTCOR
CTCOR was created for research and development purposes, and is a non-profit trust. Its assets are largely private (70%), with the State's interests underwritten by INETI (National Institute of Industrial Engineering and Technology), IAP-MEI (Institute for the support of Small and Medium Enterprises and Investment) and IPO (Portuguese Institute for Quality).
The main private supporters are the Portuguese Cork Associations, ANIEC and AIEC. The Institute currently has more than 185 members, summarised in Table 3. The Institute’s headquarters are at Santa Maria de Lamas (near Oporto), with a branch at Montijo (near Lisbon).

Objectives of CTCOR

CTCOR seeks to serve the technology needs of the cork industry by:

- providing technical and technological services to companies in the sector, or in similar or complementary sectors;
- conducting applied research and development programs, and supporting the initiatives of other scientific institutions working to improve the quality of industrial products and processes; and
- providing technical and technological training, and the dissemination of technical information of interest to the sector, through the publication of a technical journal on cork research and development, published in Portuguese, English and French.

Since 1990, CTCOR has been responsible for the establishment of official standards for the cork industry. It has been granted the status of ‘Accredited Laboratory’ and will soon be the certification body for cork products. It is recognised by the Portuguese Ministry of Industry as a ‘Competence Centre’.

The Quercus project

The Quercus project comprises qualitative experiments to identify and eliminate the components responsible for undesirable sensory characteristics in beverages sealed with cork stoppers. It has several international participants:

- ADRIAC: Association pour le Developpement de la Recherche dans les Industries Agro-alimentaires et de Conditionnement de Champagne, France
- CIVC: Comité Interprofessionnel du Vin de Champagne, France
- CFDRA: Campden Food and Drink Research Association, UK
- CTCOR: Centro Tecnológico da Cortiça, Portugal
- FAG: Forschungsanstalt für Weinbau, Geisenheim, Germany
- LAEX: Laboratorio Agrario d’Extremadura, Spain
- LGAI: Laboratorio General d’Assais i Investigaciones, Spain
- SSDS: Stazione Sperimentale del Sughero, Italy

The project seeks to:

- identify the chemical and microbiological agents responsible for off-flavours in drinks due to contact with cork stoppers;
- identify the stages of manufacture and the factors involved in generating these agents;
- develop a strategy for cork stopper manufacture extending from harvest in the forest through to use of stoppers in bottles; and
- produce a standard protocol and control measures for taint-free cork stopper manufacture and use.

The project has six major aspects, shown in Figure 3.

Cork taint

Organoleptic changes in beverages are generically referred to as ‘cork taint’, even if the wine or other beverage does not have any contact with cork.

Three types of taints may arise from cork. These are:

- strong taste of natural cork (caused by poor extraction of compounds during cork processing);
- absorption of contaminants by cork or wine; and
- metabolites of fungal growth on corks.

A authentic ‘cork taint’ is very rare, and is characterised by a potent, rancid, ‘butyric’ taste which renders the wine undrinkable. The commonly accepted hypothesis is that it is derived from the ‘yellow stain’ of the cork, but this taint has not been reproduced in the laboratory. Poor cork production practices, such as insufficient extraction of phenolic compounds, high moisture content during storage and transportation, and poor sanitation may cause taints. The cork may be contaminated by extraneous compounds, such as hydrocarbons, styrene, acrylate, or pesticide residues, trans-
mission of adsorbants used in winemaking, or yeast-derived taint.

'Musty' characters represent approximately 90% of the taints found in wine. The compounds associated with it are principally the chloroanisoles, guaiacol, 1-octen-3-ol, 1-octen-3-one, 2-methylisoborneol and geosmin.

Chloroanisoles
The chloroanisoles are the most potent group of volatile compounds known, and can be detected as 'musty' taints in a variety of media at a concentration as low as 3 parts per trillion (ppt or ng/L).

Of these, 2,4,6-trichloroanisole (TCA) was first identified in wine in 1981. The source of the taint was attributed to chlorophenols formed due to the hypochlorite treatment of the cork during manufacture. Subsequent microbial methylation led to the formation of TCA. Other potential sources of this taint include chlorophenols, such as pentachlorophenol, which was used as a wood preservative in the 1950s and 1960s. Residues of this material still persist in the environment.

Another source of contamination is trichlorophenol, also used as a preservative of wood. Microbial growth following such treatment can lead to the formation of TCA, which is absorbed by the cork. The mechanisms of the formation of TCA are shown in Figure 4.

Guaiacol
Guaiacol has been detected as a volatile compound in tainted wines. Its threshold in wine is 20 μg/L. It is generally described as having a burnt, medicinal, smoky and woody aroma, and is formed from lignin (Figure 5).

1-octen-3-ol / 1-octen-3-one
These compounds are described as having a mushroom or metallic aroma. Penicillium spp., which are important microorganisms in the production of cheese, have been implicated in their formation (Figure 6).

2-Methylisoborneol (2-MIB) and geosmin
These two compounds are metabolites of soil bacteria, such as Actinomycetes spp. and the algae Cyanobacteria spp. They cause taints in water, fish and other aquatic and marine foods. Organisms such as Aspergillus spp., Penicillium spp.,
Trichoderma spp. and Rhizoctonia spp. may produce geosmin and 2-MIB (Figure 7) when grown on sterile, granulated cork. The most intense aroma is caused by 1-octen-3-one. 2-MIB is a metabolite of Streptomyces. Geosmin is rarely detected in wine and corks, due to its labile nature in an acidic medium.

Figure 8 summarizes the taint compounds found in cork, and lists their flavour threshold in wine.

These off-flavour compounds can arise from different sources, as summarized in Figure 9.

Practical advice for prevention of cork taint

The following procedures are proposed as a means of reducing the incidence of tainted corks:

- eliminate soil contact during the stabilisation process in the forest, which should be conducted for a minimum of six months;
- change the water used for boiling at least once a week;
- perform stabilisation of boiled slabs in a clean and ventilated space, with control of humidity, for a maximum of two weeks;
- avoid contact between corks and wood, by using polyethylene pallets;
- use germicidal lamps that emit UV-A radiation for the sanitation of factories and cellars;
- avoid chlorine bleaching;
- do not store or transport corks with a moisture content higher than 8%;
- ensure that storage and shipping areas are dry and clean;
- do not use chlorophenol biocides or washing products that contain chlorine; and
- regularly inspect and disinfect the corker jaws.

Leakage

The main causes of leakage are:

- an incorrect relationship between the diameter of the cork and neck of the bottle. The difference in diameter between the uncompressed cork and that of the narrowest part of the neck should be 6-9 mm;
- incorrect humidity of the cork, resulting in variable compression during insertion;
- cork deformation, caused by incorrect adjustment or maintenance of the jaws, or jaw defects;
over-compression (a compressed cork diameter of 1.5–2 mm less than the smallest internal neck diameter is recommended);
irregular bottle neck profile;
excessive fill level (headspace too small for thermal expansion);
inappropriate cork surface treatment for the particular bottling machine, type of wine or bottle;
structural and/or manufacturing defects in corks; and
immediate inversion of the bottle after corking.

A leakage problem might cause a cork taint problem, as mould growth on the top of the cork might produce undesirable compounds.

The cork industry is very concerned about cork quality, other than the visual appearance of the corks, and is working to solve these problems.

Optimisation of the production of corks
If developed and implemented, the following techniques may improve the quality of corks:

- a system for filtration of water used for boiling of the raw cork slabs;
- monitoring of the behaviour of cork slabs in a sterilised room after boiling;
- development of a washing and bleaching process without hazardous chemical compounds, that gives a high degree of disinfection;
- alteration of physical design and orientation of processing plants, to avoid microbial contamination;
- development of automated machines for punching and visual quality selection; and
- study of chemical parameters to enable the tracing of cork from the forest to the end of processing.

Certification to ISO 9000
Responding to current trends, individual cork companies are increasingly concerned with the quality of their products and services. At the moment, there are 4 cork factories certified under ISO 9000, and another 10 are expected to achieve certification in the next year.

CTCOR is developing a 'Procedures Manual' that will document good industry methods and processes. Companies are expected to adopt this Manual, thus demonstrating their commitment to improving their methods and products. Factories that follow these procedures will be 'qualified' by CTCOR. This will involve an agreement between the factory and CTCOR, which will require periodic inspections and audits of products and processes.