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STUDY OF HUMIDITY AND WATER ACTIVITY OF CORK SLABS DURING CORK STOPPER MANUFACTURING PROCESS - PRELIMINARY RESULTS

ESTUDO DA HUMIDADE E ACTIVIDADE DE ÁGUA DE PRANCHAS DE CORTIÇA DURANTE O PROCESSO DE FABRICO DE ROLHAS DE CORTIÇA – RESULTADOS PRELIMINARES

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SUMMARY

The main goal of this work was to make evidence of the applicability in the cork stopper industry of a control method along the cork slabs maturing stage after boiling, aiming to ensure the adequate conditions for *Chrysonilia sitophila* colonisation of cork slabs during that period, avoiding the development of other fungi. Cork humidity and water activity (wa) were used as indicators. Comparing the drying rate of the outer and the inner parts of the bark it could be observed, under the assay conditions, that to attain 0.9 wa, (the critical point for *C. sitophila* development), 39.8h were necessary in the case of outer bark while inner bark only need 20.4h. As the visible part of the cork slabs corresponds more to its outer part, the time of 40h can be taken as a good visible indicator to limit the cork slabs maturing period after boiling. Moreover it could be observed that under that period of time the evident prevailing fungus on the cork slabs was *C. sitophila* so probably assuring low probabilities of 'cork taint' occurrence, under the conditions propose on this study.

RESUMO

O principal objectivo deste trabalho foi verificar a aplicabilidade na indústria da rolha de cortiça, de um método de controlo, ao longo do processo de maturação das pranchas após cozedura, de modo a garantir condições adequadas para a colonização preferencial da cortiça por *Chrysonilia sitophila* durante aquele período, evitando o desenvolvimento de outros fungos. A humidade da cortiça e a sua actividade de água (wa) foram usados como indicadores. Comparando a taxa de secagem das partes externa e interna da prancha de cortiça, verificou-se, nas condições do ensaio, que, para atingir 0.9 wa, (o ponto crítico para o desenvolvimento de *C. sitophila*), eram necessaries 39.8h no caso da parte externa e 20.4h no caso da parte interna da cortiça. Uma vez que a parte mais facilmente observável da prancha é a sua parte externa, propõe-se um período de secagem após cozedura, não superior a 40h como um indicador de fácil aplicabilidade. Foi alias observado, como era esperado, que o fungo dominante nas pranchas durante este período de tempo foi *C. sitophila*, assegurando, em princípio uma baixa probabilidade de ocorrência do 'gosto a rolha' nas condições aqui propostas.

Key words: Cork, slabs maturing stage, humidity, water activity, 'cork taint'

Palavras Chave: Cortiça, período maturação das pranchas, humidade, actividade de água, 'gosto a rolha'

INTRODUCTION

Water activity as a physiochemical parameter has mainly been discussed in only two scientific disciplines: physical chemistry and food microbiology. In the former, it measures the thermodynamic free energy of water and in the latter it is used to define the lower limits of growth of food spoiling microorganisms. Microbiologists turned to water activity measurements upon discovering that microbial spoilage of food occurs at widely varying levels of water content because microorganisms can only use 'available' water, which differs considerably depending on the solute. At the same molecular concentration, salt lowers the water activity more than sugar (Fontana Jr, 2000; Mujundar and Devahastin, http://www.geocities.com/drying_guru/chapter1fundamentals.pdf)

Water activity should be regarded as an external parameter like pH or temperature. Under certain conditions, it will act synergistically with other environmental parameters. Under other conditions it will be the sole parameter determining the outcome of a certain process. Water activity's usefulness as a food quality and safety measurement was suggested when it was obvious that water content could not adequately account for microbial growth fluctuations

(Fontana Jr, 2000). Biological cells and tissues are not homogeneous solutions and water is not uniformly distributed.

The active part of moisture content and, therefore, water activity, provide better information than the total moisture content regarding the micro-biological, chemical and enzymatic stability of consumable products. Water that is not bound to the product molecules can support the growth of bacteria, yeast and fungi. The term water activity (wa) refers to this unbound water.

The microbes behavior in a defined environment depends on the water content of the medium but mainly on the form as the water present is (free water or bind water), so the concept of water activity is developed and wa can be defined as the accessible water in the system (http://en.wikipedia.org/wiki/Water_activity; http://www.wateractivity.com/aw_info.html). Water activity is a better index for microbial growth than water content. Water activity better predicts the growth of microbes because they can only use 'available' water, which differs considerably depending on the solute.

The relationship between water content and water activity (wa) is complex. An increase in wa is almost always accompanied by an increase in the water content, but in a non linear shape. This relationship, at a given temperature, is called the moisture sorption isotherm which can be defined as the relationship between the equilibrium moisture content of a material (expressed as mass of water per unit mass of dry matter) and water activity. For most materials sorption isotherms are sigmoidal in shape although foods that contain large amounts of sugar or small soluble molecules have a J-type isotherm curve shape. These curves are determined experimentally.

A critical wa also exists below which no microorganisms can grow (Beuchat 1981). For most foodstuffs, this is in the range of 0.6-0.7 wa. In general, dehydrated foods have wa 's less than 0.6; semi-moist foods, such as cereal grains, raisins, dates, syrups, and intermediate-moisture pet foods usually have a wa between 0.62 and 0.92. Thus, knowing the moisture sorption isotherm, it is possible to predict the maximum moisture that the product can be allowed to gain during storage (Stencle, 1999).

Water in porous systems may be bound in two ways: by lowering the energy state of the water in the system, and by reducing the rate of movement of water to interfaces. Forces of adhesion and cohesion (van der Waal-London forces) lower the energy state of adsorbed water compared to pure, free water. These two effects combine to lower the total free energy of the water. The lower energy (compared to pure, free water) of the water in the media binds it, meaning that work would need to be done on the water to remove it from the media. The energy per unit mass required to remove an infinitesimal quantity of water from the media and transport it to the pure, free reference state is called the water potential. Water potential describes the thermodynamic state of water in foods and other porous media, and is an equilibrium measure. A system is said to be in equilibrium when the water potential is the same at every location in the system. Food and other porous systems are often far from equilibrium, and this provides a second sense in which water can be bound http:/www.wateractivity.com/bound.html.

Cork, is a porous material, and it is obtained from the layer just below the bark of the cork oak tree (*Quercus suber* L.). Each nine-year interval the cork is separated from the tree as rectangular boards (cork planks). The inner layer of cork bark (the peridium) will continue to produce cork as long as it has not been injured by the stripper's hatchet. The operation of cork striping destroys the phellogen layer, and the death of the newly exposed inner bark tissues with a subsequent formation of a traumatic periderm that will constitute the outer part of the bark. This part of the cork barks cracks caused by an intense radial growth tensions. A new phellogen is formed in the inner bark and initiates its meristematic activity (reproduction cork) (Natividade, 1950). Inner bark is composed of layers of living, growing tissues, formed of independent tetradecahedron cells, 20 to 40 million per cubic centimetres, and so about 800 million cells in a single wine cork stopper. The cell walls remain attached to the tree after dying. These layers are the phloem, phelloderm, and cork cambium, or phellogen. The phloem in mature woody plants is produced by tissue called the cambium, which lies between the wood and the bark and consists of primarily of sieve tubes. As new phloem accumulates, it pushes the older phloem out and crushes it into the outer bark enlarging the phelloderm and the cork cambium until they break apart and die. New layers of phelloderm and cork cambium then develop to replace the dead tissues. The phelloderm is a layer of food storage cells; it is produced by the cork cambium, which acts similarly to the cambium in the production of new tissues. The regularly arranged walls of cork cells have thick walls that contain a waxy, waterproof substance (suberin) that is almost impermeable to water or gases. Gases enter and leave the stem through lenticels, which are round or oval blisters in the surface of the bark (Graça e Pereira, 2004).

Outer bark consists chiefly of cork, a hard, dead tissue produced by the cork cambium. Patches of dead phloem occur throughout the outer bark of mature trees and shrubs. This dead phloem has been pushed out by the growth of new phloem. The transfer of liquids and the diffusion of gases through the walls of these cells are extremely slow, to the extent that a thickness of a single millimetre of cork, consisting of about 30 layers of cells, is almost totally impermeable. The microscopic observation of the outer layer of the bark allows distinguishing large sclerotic phloemic beams and cellular irregular layers of dead cells whose thin walls have become thickened and waxy. This

structure confers to this part of the cork bark properties nearer of that of a woody tissue. The different structure of the two parts of the bark suggests a different behaviour concerning water (Gibson, 1981; Pereira, 1988)

As a consequence, the inner bark is responsible by the unique properties of cork from its structure of air-filled cells, which result in watertight, light and flexible structure. Cork has a high coefficient of friction and is almost impermeable to liquids. These qualities make cork valuable for bottle stoppers manufacturing, insulating materials, linoleum, and many household and industrial items.
The manufacturing process of cork stoppers includes a stabilisation period of the cork slabs, after boiling, during which slabs dry, attaining the humidity level allowing the subsequent phases of its processing, and fungi growth completely covers them. This process has been traditionally used for several decades, however due to the possibility of certain fungi isolated from cork to produce off flavour compounds, especially 2,4,6-trichloroanisole and 2,3,4,6-tetrachloroanisole, cork stoppers are recently being unsoundly targeted with the accusation of inducing 'cork taint' in wine (for a review see Silva Pereira et al, 2000a). The responsibility of 'cork taint' appearance in wine can not be attributed to all the fungi associated with cork during stopper manufacture. Silva Pereira et al. (2000b) reported that *Chrysonilia sitophila*, the dominant mould colonising cork slabs after boiling (Danesh et al., 1997) possess low potential for chloroanisole production and a higher growth rate than the other fungi also identified at this stage of the cork stopper processing. It was observed that the metabolic strategy used by *C. sitophila* to degrade pentachlorophenol (PCP) resulted in a very high level of degradation without production of 2,4,6-trichloroanisole (TCA).

Moreover, that mould can tolerate higher PCP concentration than other fungi. These results reinforced the importance of *C. sitophila* in the cork stoppers processing.

The water activity necessary to *C. sitophila* growth is around 0.9 while *Penicillium sp* and *Trichoderm*a sp can tolerate lower wa (Pitt and Hocking, 1997). The main goal of this work is to make evidence of the applicability in the cork stopper industry of a method of humidity control and monitorisation along the cork slabs maturing stage after boiling in order to ensure the adequate conditions for *C. sitophila* colonisation of cork slabs during that period, avoiding the development of other fungi. The particular structure of cork as a porous and waterproof material is considered.

MATERIAL AND METHODS

Samples preparation

• *Inner and outer bark pieces of cork slabs during their maturing stage after boiling*

In order to compare the humidity levels of those two components of the cork plank, during the plank maturing stage, several cork samples were obtained from different slabs, during cork maturing stage. From each sample, the inner part (inner bark) was separated from the external one (outer bark) using a band saw.

• *Inner and outer bark pieces of cork slabs to evaluate the cork drying rate:*

Twenty five pairs of cork pieces (6x3x2 cm) were prepared as follows: the inner part (inner bark) was separated from the external one (outer bark) using a band saw. To reproduce the industrial process of cork stopper manufacturing, all the cork pieces were completely immersed in water and 'boiled' during one hour. After boiling, a simple shaking eliminated the exceeding superficial water and each piece was weighted and put in an oven controlled to 29 ºC and 38 % relative humidity.

Cork humidity and water activity evaluation under controlled conditions:

Cork pieces were obtained, as described above, from a part of a cork slab (3-4 cm thick) boiled one hour in water and prepared in order to have pieces (outer and inner bark) with different humidity levels, by using the following empiric technique, established by doing several attempts:1) the cork pieces were put inside sealed bags and heated at 50ºC by 4 h; 2) the bags were opened and the cork pieces were dried at 100 ºC by previously established time periods; 3) the dried pieces were taken, put again in sealed bags and put at 50 ºC by 4h; 4) wa was now determined (as referred below), after a defined period of time in a temperature controlled room, where all the necessary equipment also was, in order to attain temperature stabilization. Inner and outer bark humidity was determined by drying the pieces of cork in an oven at 100 ºC, until constant weight was attained.

Water activity was performed in a temperature controlled room at 22ºC, using a PROTIMEMER DP989M, a chilled mirror dew point instrument, following the instructions of the supplier. Using this instrument, the sample is equilibrated within a sealed chamber containing a mirror, an optical sensor, and an infrared temperature sensor. At equilibrium, the relative humidity of the air in the chamber is the same as the water activity of the sample.

The cork drying rate was determined trough relative humidity evaluation by putting the inner and outer bark cork pieces obtained as described above, in an oven controlled to 29 ºC and 38 % humidity. The cork weight of each cork piece was determined at short interval, during 300h, and the respective dry weight was evaluated by drying them at 100 ºC until constant weight. The drying rate (R), expressed as $gH_2O/h\ cm^2$, was calculated:

$$R = -\frac{L_s}{A}\frac{\Delta X}{\Delta t}$$

L_S meaning the dry weight of each cork piece (g); A meaning the area of each cork piece (cm^2); ΔX meaning the difference between humidity and dry weight of each cork piece, e.g. humidity and the correspondent dry weight values along the time, of inner and outer bark, from boiling to dryness.

RESULTS AND DISCUSSION

Inner and outer bark humidity - Cork drying rate activity under controlled conditions (22ºC)

The results of inner and outer bark humidity of cork slabs, were determined in cork obtained from a cork stopper-manufacturing industry, and are expressed on Fig. 1. It is possible to observe higher % humidity of the outer bark pieces when compared to the correspondent inner parts.

This is an expected result considering the different composition of the two cork tissues. It was even expected a higher difference if the separation between the outer and inner parts were more complete.

The results show that the outer bark presents a 1.44g/h, drying rate during the first 12h. In the inner bark, the 2.49 g/h drying rate was constant during the first 4.8h. During the same period of time (7.5h) the outer bark lost 1.44 g water while the inner part lost 2.11g. At the end of the drying phase both materials present similar behaviour.

Water activity

The water activity represents the ratio of the vapor pressure of the material to the vapor pressure of pure water under the same conditions. Water affinity either for outer bark or inner bark was compared observing the relationship among humidity and wa in both materials and the sorption water isotherms are presented on (Fig 2).

It can be observed that aw of inner bark only points to a decrease when its humidity attained ca 12%, while the outer bark shows aw lower than 1 for humidity levels lower than 20%. These facts point to a low water/cork inner bark affinity when compared to the outer bark water affinity. The obtained results may be important when related to the fungi development on cork slabs after boiling, during the so called maturing stage. Water activity necessary for *C. sitophila* development is ca 0.9 (Pitt and Hocking, 1987) (vertical line in Fig 2), below this value many other fungi can grow. According to Silva Pereira et al (2000b), the ability of *C. sitophila* to transform PCP into TCA (the main compound responsible by the so called 'cork taint' in wine) is very low while *Penicillium spp* produce higher levels of TCA under

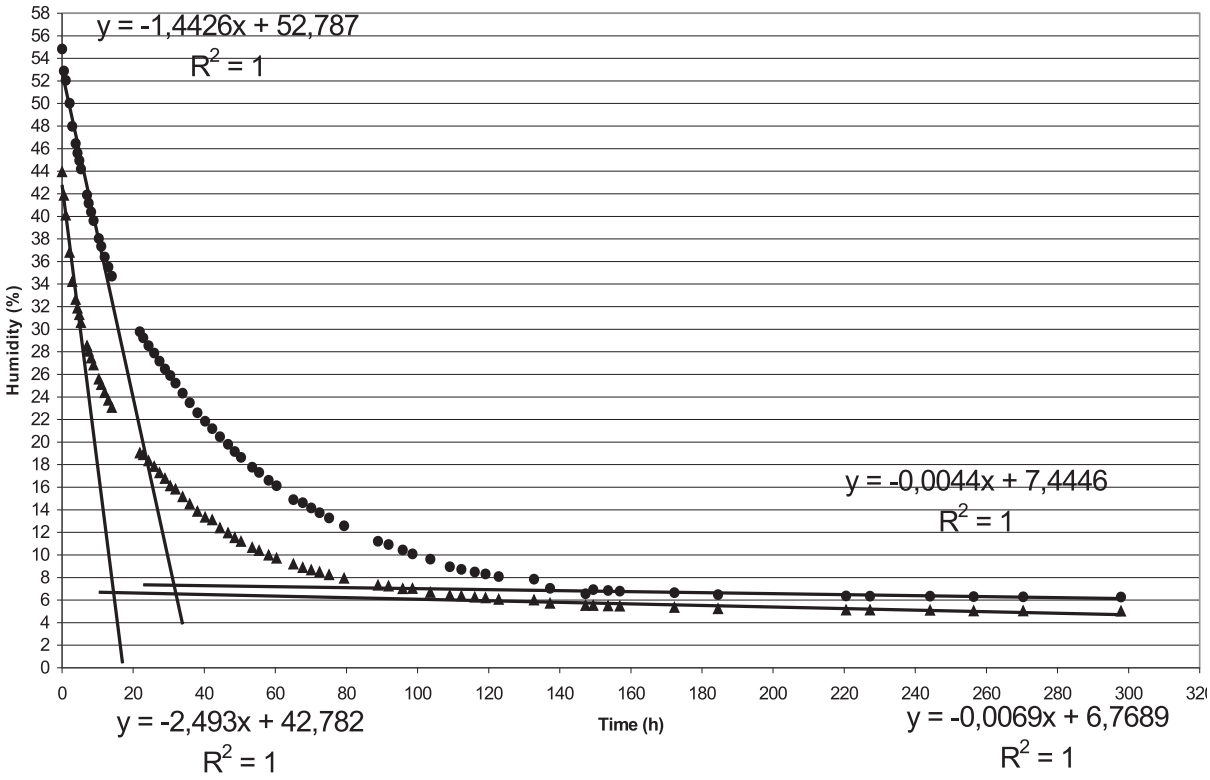

Fig 1. - % Humidity along time, from pieces of outer cork bark (•), and inner cork bark (▲), under controlled conditions
% de Humidade ao longo do tempo de porções da costa (•) e da parte interna de pranchas de cortiça (▲), em condições controladas

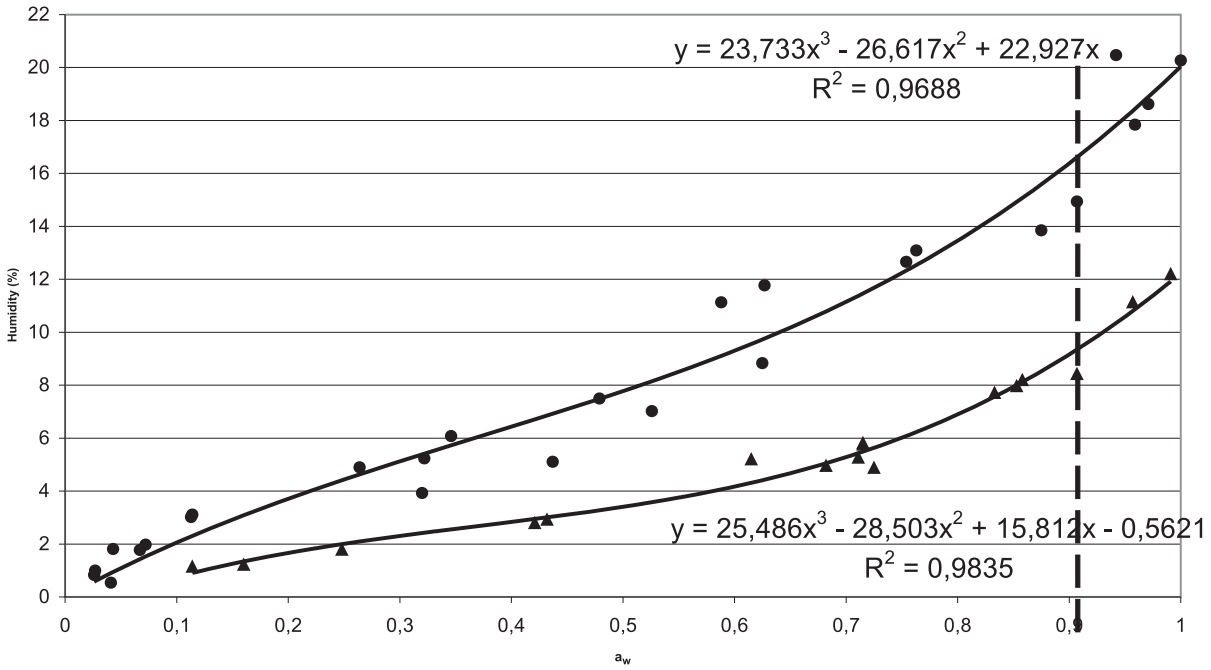

Fig. 2 - Isotherms from pieces of outer cork bark (•), and inner cork bark (▲), under controlled conditions (T= 22ºC)
Isotermas de absorção de porções da costa (•) e da parte interna de pranchas de cortiça (▲), em condições controladas (T= 22ºC)

the same assay conditions.

Comparing the results in Fig 1 and Fig 2 it can be concluded that, 0.9 wa in the outer part seems to correspond to 16% humidity while in the inner part corresponds to 9% humidity. Moreover, to attain 0.9 wa, under the assay conditions, 39.8h are necessary in the case of outer bark while inner bark only needs 20.4h. As the visible part of the cork slabs corresponds more to the outer bark, the time of 40h can be taken as a good indicator to limit the cork slabs maturing period after boiling. Furthermore it can be observed that, under this period of time, the evident prevailing fungus on the cork slabs is in fact *C. sitophila* (results not shown) so probably assuring low probabilities of 'cork taint' occurrence, under the conditions propose on this study.

However it may be taken into account that fungal spores inside cork can start to germinate before this time once 0.9 wa inside cork slabs is attained sooner (ca 20h), so the maturing stage should be reduced to one or two days, maximum, before the next stages of cork stoppers manufacturing, in order to reduce fungi development and the possibilities of the associated TCA occurrence.

This attempt is not easy for industry to attain, but having this knowledge in mind, some efforts can be done. The physical separation of the cork slabs boiling process, nowadays a current practice in many industry units, can be a valuable contribution to decrease TCA occurrence, once the possibilities of fungi cross contaminations also considerably decrease (Oliveira et al., 2003).

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