Biochemical and fungal caracterization of dried pears and other fruits

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ABSTRACT

The drying of pears allows their conservation due principally to the inhibition of microbial growth, making the dried pear a safe food for consumers. The knowledge of microbial flora, as well as some biochemical factors, is very important for ensuring the consumers health. In this study samples of pears of the variety S. Bartolomeu were dried under different systems: traditional open air sun drying, a solar stove, a solar drier and a drying tunnel. It was intended to isolate yeasts and moulds from dried pears subject to different drying processes, and also to some other fruits dried industrially. In this way, the following biochemical characteristics of dried fruits were evaluated: water activity and moisture content. Furthermore, the morphological characteristics of the isolates were also analyzed. Finally, as previously stated, the results obtained for dried pears were compared with the results obtained for samples of prunes and raisins, dried by industrial methods.

The results of the biochemical characterization of the samples under study showed that all samples had a water activity lower than 0.60. After inoculated incubation it was only observed the grow of a mould in one sample of dried pears obtained by the traditional open air sun drying method, allowing to conclude that only this last method may induce the pears deterioration by microbial growth. The other systems tested for the drying of pears produce fruits that when dried can be conserved and consumed safely.

Keywords: dried pears, yeasts, moulds, water activity.

INTRODUCTION

The pears can be consumed fresh, or alternatively subjected to processing techniques, such as drying [1]. The food conservation by drying occurs principally due to inhibition of microbial growth, although the microorganisms are not necessarily eliminated [2,3]. In Portugal, pears of the variety S. Bartolomeu are traditionally dried by a direct sun-exposure method. The traditional solar drying method consists of five different phases: (1) the pears are peeled and left uncut to dry; (2) they are left at direct sun exposure for 5–6 days; (3) they are taken out of the sun at the hottest hour of the day and packed into barrels or baskets for 2 days (this procedure is supposed to increase elasticity, which will facilitate the following operation); (4) the pears are pressed and their form changes from spherical to flat; (5) finally the pears are left at direct sun exposure for 2–3 more days [4,5].

Presently, the open-air drying method is being substituted by other methods of drying. To encounter alternative drying methods, beyond the traditional drying, other drying processes were tested [6]: a solar stove in Higher Agrarian School of Viseu (ESAV), a solar drier in Higher Technology School of Viseu (ESTV) and a drying tunnel in University of Coimbra (UC). With these methods we can take advantage of the solar energy, and at the same time they allow the production of dried fruits with better quality, since contamination and infestation problems are minimized [7]. There is also a significant reduction in drying time, with important economical benefits.

The yeasts and moulds are included in the Fungi Kingdom, that are heterotrophic eukaryotes organisms, mostly saprophytic, obtaining nutrients from organic matter on decomposition [8]. The moulds are multicellular beings, consisting of filamentous cells, long and branched, called hyphae, which form a mycelium [9]. Physiologically, they can adapt themselves to more severe overloads than most microorganisms. They can grow on substrates with high concentration of sugar and acid, supporting pH variations between 2 and 9. The moulds can survive in dehydrated food, producing spores or entering in a state of latent life [10]. Those that can grow in conditions of water activity less than 0.85 are called xerophilous [11].

The yeasts are unicellular fungus that reproduce asexually, by budding, or binary fission, or sexually by forming spores [8]. Some yeast varieties can grow on high sugar concentrations, which restrict the moisture

supply, called osmophilic yeasts. The yeasts grow in a wide range of thermal variation, from 0 to 47ºC. In general, yeasts grow better in an acid environment, with pH between 3.5 and 3.8 [10]. The yeasts can be found largely on nature, particularly in fruits, vegetables and cereals. Their growth is limited to the outer surface of healthy and intact fruit, with no internal contamination [12].
The microorganisms' growth can be prevented by reducing the moisture content of the environment below the critical level. This critical level is determined by the microorganisms characteristics and the capacity of water to connect in food (water activity, aw), which reduces the free moisture [13]. The aw of most fresh foods is 0.99, while the dried fruits generally have aw values between 0.85 and 0.61 [11]. The xerophilous moulds and the osmophilic yeasts are the microorganisms which usually originate changes in the foods with those water activities. For aw values below 0.65, very few microorganisms are capable to grow, and deterioration is unlikely to occur before two years of conservation [14]. In general terms, food safety is achieved through the implementation of good manufacturing practice. However, by itself it doesn't assure that processed foods are free of pathogenic microorganisms [15].
This work's objectives are to isolate and characterize morphologically yeasts and moulds from samples analyzed by culture technique, pattern method reference according to the International Norm Organization for Standardization ISO- 21527- 2 from 2008 [16]. The physicochemical characterization of the 27 samples under study was based on determination of values of water activity (aw) and moisture.

MATERIALS & METHODS

Water activity determination

To express the degree of free water in foods the water activity (aw) was used. This parameter is very important because the available water is a major external factor that contributes to microbial growth. The values of aw change between 0 and 1 (for pure water). The aw of the different samples was determined with a Rotronic Hygrometer BTSR1. This equipment measures the relative moisture (HR) generated by the sample in an isolated chamber, at a desired temperature (e.g. 25 ° C), which must be stabilized. The relative moisture reading was done after value stabilization, and aw was calculated as aw = HR/100.

Moisture evaluation

Moisture evaluation was used to determine the amount of free water in the food, in this case in dried fruits. This was achieved by difference between the original mass sample and the final sample after heating until constant mass. For this determination a Halogen Moisture Analyser model HG53 Mettle Toledo was used. The operating conditions were the following: •Heat source: halogen lamp; •Drying temperature: 120ºC; •Drying velocity: 3 (medium).

Samples origin

To the realization of this work, 17 samples of dried pears subject to different drying methods were analyzed, together with 5 samples of dried prunes and 5 samples of raisins, obtained by industrial processes (Table 1). The samples of pears of the variety S. Bartolomeu were dried under different systems: traditional open air sun drying (TRADITIONAL), a solar stove in Higher Agrarian School of Viseu (ESAV), a solar drier in Higher Technology School of Viseu (ESTV) and a drying tunnel in University of Coimbra (UC). It was intended, as previously said, to isolate the largest possible number of yeasts and moulds from these samples, for their importance in the food deterioration.

Table 1. Plan of experimental analysis

Type of samples	**Number of samples**
Traditional	5
Stove ESAV	4
Stove ESTV	4
Drying tunnel UC	4
Prunes	5
Raisins	5
Total	27

Sample treatment

After the collection of samples, the treatment in the laboratory was made based on the International Norm Organization for Standardization ISO 2 of 2008 [16].
The solid samples can't be sown directly in plates. For this reason, a portion of 25g that is representative of the total sample should be used. Then the sample was homogenized using the stomacher. To the homogenate obtained was added strictly nine times of the weight, of the water sample peptone 0.1% (w/v) to obtain a dilution corresponding to 10^{-1} (mother suspension), according to the methodology described in ISO- 21527-2 [16]. The dilutions were carried out from mother suspension, until the dilution 10^{-6}.

Inoculation in a selective medium

One small portion of the appropriate dilutions was inoculated in a selective culture medium, the SDA (Sabouraud Agar), supplemented with clorophenicol, that after the sterilization was cooled until 50ºC and placed 15 ml of medium on each sterile petri plate. After medium solidification and drying, it was made the culture mean inoculation, with appropriated samples dilutions.
All tests were conducted in duplicated. The plates were examined from 2 to 2 days during the seven days of incubation, because the moulds have a very rapid growth, which can mask the growth of yeasts.

Isolation – culture technique

To the isolation of yeasts and moulds it was used the culture technique, in which the morphologic characteristics of the colonies of a kind (at least 3) were subcultured from the initial culture medium to the SDA medium supplemented with clorofenicol. Before carrying out identification tests in an optical microscope, is essential to assure that the growth in SDA medium is pure, doing the examination of the colonies morphology.

Macroscopic and microscopic observation

The naked eye observation of the plate with fungal growth can lead to the identification of some macroscopic characteristics.
To yeasts and moulds microscopic observation it was used the blade culture method, which enables the preparation and observation without disturbing the microorganism growth, according with the methodology of Levy [17].

RESULTS & DISCUSSION

Water activity determination

For all samples at study four measurements of water activity were made, at a stabilized temperature of 25 ºC. All samples show aw values less 0.57, as seen in the results shown in Table 2.

Table 2. Water activity for different samples.

Sample	Medium aw	Standard Deviation
Traditional	0.51	0.01
Stove ESAV	0.56	0.05
Stove ESTV	0.54	0.02
Drying tunnel UC	0.57	0.03
Prunes	0.56	0.01
Raisins	0.42	0.02

According to Silliker and others [18], it doesn't exist microbial growth for values of aw less than 0.60, which means that it is unlikely to exist fungal growth in all the analyzed samples.
The pears dried by the traditional method show the lowest value of aw, although all samples show very similar values.

Moisture evaluation

For all samples at study four measurements of moisture were made, at a constant temperature of 120ºC.

The dried prunes sample shows the higher moisture content, while raisins show the lowest moisture content. The pears show moisture values between 9 and 17 %, as seen in Table 3.

Table 3. Moisture percentage for different samples.

Sample	Medium	Standard deviation
Traditional	9.92	2.60
Stove ESAV	11.81	2.15
Stove ESTV	11.95	2.48
Drying tunnel UC	13.93	2.90
Prunes	25.66	5.42
Raisins	0.32	0.19

The dried fruits show normally moisture values between 15 and 20%, and of all the samples analysed, the only one who exceeds these values is prunes, with a moisture value of 25.66%. The pear samples have moisture contents lower than those commonly found in dried fruits, being in this way more protected against fungal attacks.

Isolates obtained

For yeasts and moulds detection in dried fruits, with a water activity less than 0.95, it was used the culture technique, based on the International Norm Organization for Standardization ISO 2 of 2008 [16]. In the fifth day of incubation the inoculated plate with traditional dryed pear dilution 10^{-1} shows one fungus (Figure 1). In all other plates there was not yet observed any fungal growth.

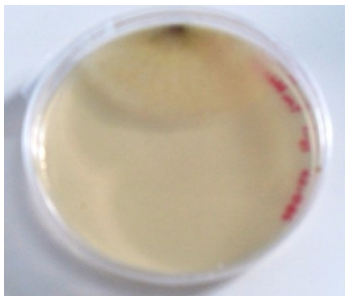

Figure 1. Fungus in dilution 10^{-1} in traditional pear sample at day 5.

In the seventh day of incubation the results remained the same. The fungus in the inoculated plate with traditional drying pear dilution 10^{-1} proliferated (Figure 2).

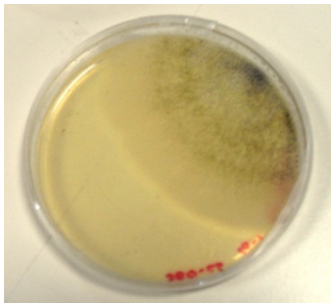

Figure 2. Fungus in dilution 10^{-1} in traditional pear sample at day 7.

In the remaining samples, Stove ESAV, Stove ESTV, Drying tunnel UC, prunes and raisins, there was not any fungal growth.

Macroscopic and microscopic observation

The observation of the macroscopic and microscopic characteristics of the fungus obtained in the pear sample of the traditional drying method with the dilution 10^{-1} was made. The macroscopic characteristics were evaluated as (see Table 4) [17]: the colony type, filamentous if it was mould or creamy if it was yeast; the colony colour; the colony texture that can be powdery, cottony and velvety; the colony size, invade all plate or limited; if spores are visible to the naked eye; if the verse is smooth or rough and the verse colour.

Table 4. Macroscopic characterization of the mould from the sample of the traditional dried pear at dilution 10^{-1}.

Macroscopic characteristics	
Colony type	Filamentous
Colony color	White and black
Colony texture	Cottony
Colony size	Limited (not invading the whole plate)
Spores	Visible to the naked eye
Verse	Smooth
Verse colour	White

After macroscopic visualization it was identified a mould due to filamentous colonies formation that showed a cottony texture. The observed microscopic characteristics are (see Table 5) [17]: the hyphae type, it is segmented or not; the spores type formation, it is formed in hyphae ends or inside hyphae; the spores form and ornamentation; the type of reproductive structure and the spores colour.

Table 5. Microscopic characterization of the mould from the sample of the traditional dried pear at dilution 10^{-1}.

Microscopic characteristics	
Hyphae type	Not segmented, coenocytic.
Spores formation	Hyphae end.
Spores form and ornamentation	Sporangiospores round and appear at the base of the sporangia.
Reproductive structure	Unbranched sporangia.
Spores colour	Greenish brown

The mould shows a hyphae coenocytic set at microscopic observation. In hyphae ends it is observed the growth of greenish brown round spores, which are formed in the base of sporangia that isn't branched. These characteristics are common in *Rhizopus* species.

CONCLUSION

According to the values obtained for water activity, the samples have very low values of aw, not propitious to microbial growth.

The prune is the sample with greater moisture percentage, which may eventually favour some fungal growth.

In the sample of pear obtained by traditional drying it was found a mould, only in the 10^{-1} dilution. Although it contains a water activity below 0.60, in this drying method there is an increased exposure to microbial agents because the fruits are dried by direct open-air sun exposure.

The samples of ESAV, ESTV, UC, prunes and raisins didn't have fungal growth because they have a water activity less than the minimum value for the microbial growth. In addition, those samples were dried by methods that take into account the hygienic conditions of the environment and tools. Furthermore, in these methods there was also the monitoring of drying humidity and temperature.

The prune sample dried by the industrial method proved the effectiveness of applying a drying method where the external contamination factors are controlled. This sample, although presenting the higher values of moisture, 30%, doesn't show fungal contamination.

The traditional drying method must be improved or replaced by one of the other methods analyzed, to provide longer product conservation and increase consumer safety.

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