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PASTEURIZATION OF AGRICULTURAL SUBSTRATES FOR EDIBLE MUSHROOM PRODUCTION

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ABSTRACT

Pleurotus ostreatus (oyster mushrooms) are the second edible mushroom produced worldwide. They have great nutritional value, their production is sustainable and uses a variety of cheap lignocellulosic substrates, including agricultural and industrial wastes. This article aims to discuss the various methods of substrates disinfection for growing *Pleurotus ostreatus*, which is one of the most critical step in mushroom cultivation. The main characteristics of thermal pasteurization and other disinfection methods such as microwave and ultraviolet radiation have been compared and discussed.
In terms of the production of *Pleurotus*, the most common substrate disinfection methods have been compared with particular emphasis on the yield and the biological efficiency. The ratio time/Temperature in thermal pasteurization has also been focused because of its major influence on the degradation of substrate nutrients and the equipment used for pasteurization has also been described.
The main conclusion of this review is that thermal pasteurization is the best option for disinfection as it eliminates the major competitive microorganisms from the substrates, retain major nutrients, and allows some beneficial microorganisms to survive. Among several pasteurization methods, scalding at 80ºC for short times less than 30 min seems the best option, and substrate processing tunnels seems to be the most suitable equipment.

Keywords: *Pleurotus*, pasteurization, edible mushroom, substrates, scalding

CULTIVATION OF *PLEUROTUS* AND DISINFECTION OF SUBSTRATES

Edible mushrooms have been an important part of the human diet from several decades, and over 100 varieties have been cultivated for their potential benefits to human diet and health (**Royse *et al.*, 2017**). As a food source, edible mushrooms have great nutritional value. They are low in calories, fat, and sodium, and high in protein, essential amino acids, fiber, vitamins (B1, B2, B12, C and D) and minerals (Ca, K, Mg, Na, P, Cu, Fe, Mn and Se) (**Krittanawong *et al.*, 2021**). The positive impact on human health by consuming mushrooms are reflected in most of studies reported (**Cheung *et al.*, 2010; Sánchez, 2010; Roupas *et al.*, 2012; Chen *et al.*, 2015; Roncero-Ramos and Delgado-Andrade, 2017**). For instance, edible mushroom polysaccharides have been studied for their activity against obesity, inflammatory bowel disease, and cancer (**Ma *et al.*, 2021**).
The rapid changes in culinary practices across the globe and the growing demand from health-conscious customers for fat- and cholesterol-free products, on the one hand, and fast demographic growth, on the other, are contributing towards growth in the production of edible mushrooms globally. The increasing interest towards the low-cost protein sources as alternative to animal protein to meet the dietary requirement of the population is strongly witnessed (**González *et al.*, 2020**). The cultivation of the *Pleurotus* species ranks second – after *Lentinula edodes*, the shiitake – in terms of global edible mushroom production (19%), with versatility in terms of growth substrates and cultivation conditions (**Kumla *et al.*, 2020**).
The preferred substrates for the cultivation of *Pleurotus* spp. are the agricultural crop residues. The agricultural residues or agro-residues such as sugarcane bagasse, straw from cereals, sawdust, coffee pulp, but also cotton textile industry residues, among others (**Sánchez, 2010; Siqueira *et al.*, 2012**). These lignocellulosic residues as plant materials mainly composed of macromolecular substances such as lignin, cellulose, hemi-cellulose and a little amount of pectin, starch and other polysaccharides, and also some low molecular weight organic compounds such as amino acids and vitamins and inorganic compounds such as essential minerals. The fungal mycelia secrete enzymes which breakdown those macromolecular substances of the substrate. The smaller produced molecules, together with the other mentioned ones already present in the residues, are the nutrients consumed by the fungal hyphae, playing a vital role in mushroom growth. This nutrient composition is generally higher in cereals straw (**Gowda and Manvi, 2020**).
Mushroom cultivation can even be a cottage industry. Being a protected crop, it does not compete for surface area with any other crop because it is usually grown indoors and is therefore safe from the vagaries of nature. This versatility means that these mushrooms can be cultivated in lower cost substrates such as these agro-residues and under different climatic conditions. However, it is critical that the substrates be appropriately disinfected to prevent the risk of contamination, and this can be a limiting factor for small producers in tropical countries, given the limited investment in infrastructure (**Siqueira *et al.*, 2012**). In particular, the disinfection of the agro-residues in the substrate is a complex task that is determined by the hygienic conditions (**Gowda and Manvi, 2019**). Substrates can be disinfected by steam or chemical sterilization. However, sterilization is not an ideal method because by definition it eliminates all microorganisms, both harmful and beneficial. As it is understood, pasteurization is a ideal disinfection method over sterilization because it is a mild treatment than sterilization, which eliminate most harmful and competitive microorganisms only. Thus it allows beneficial organisms to grow and survive during cooling of substrate (**Kurtzman, 2010; Sánchez, 2010**).
The information available on substrate disinfection is extensive and varied, but at the same time few review articles systematically summarize their methods. **Kurtzman (2010)** conducted a review of pasteurization methods to reduce the amount of fuel used and to demonstrate how method affects performance and biological efficiency during mushroom cultivation. On the other hand, the aim of the review by **Gowda and Manvi (2019)** was to update the data available on both chemical and non-chemical disinfection methods and their application in the growth of *P. ostreatus*. Our current article aims to update the particularities of the pasteurization of mushroom substrate as a disinfection operation.

PASTEURIZATION AND OTHER METHODS OF SUBSTRATE DISINFECTION

Pasteurization is a process in which foods or other materials or substrates for microbial growth, are treated with mild heat, usually less than 100ºC, to eliminate most microbes, mainly pathogens. In the case of foods, pasteurization extends their shelf life (**Fellows, 2017**). The process was named in honor of the French microbiologist Louis Pasteur, whose research in the 1860s demonstrated that thermal processing would deactivate unwanted microorganisms in wine (**Tewari and Juneja, 2007; Peng *et al.*, 2017**).
The process is intended to destroy or deactivate microorganisms and enzymes that contribute to spoilage or risk of disease. In the case of substrates for microbial growth or for biotechnological applications, pasteurization helps to create an appropriate environment that is clean of contaminating microorganisms, in which

the microbial agent can grow or provide a product. Pasteurization's mild heat is consistent with minimal chemical, physical and organoleptic changes in the product and media (**Wibey, 2014; Peng *et al.*, 2017**).

Pasteurization and other disinfection and sanitization methods are partial sterilization methods because they reduce rather than eliminate all forms of life and biological agents present. The method that eliminates all microbes is sterilization, after which an object is referred to as being sterile or aseptic. It eliminates all microorganisms, including bacterial endospores, characteristic of genera *Bacillus* and *Clostridium*, and viruses (**Peng *et al.*, 2017**).

Thermal sterilization or pasteurization technologies have been widely used in food and biotechnological industry since they are well developed, and the cost of investment is low. Recently, increasing attention has been paid to non-thermal sterilization technologies since they have the potential to provide products that require lower energy to operate. These non-thermal treatments are among the most focused research areas in the food sector due to consumer demands for safe and nutritious food free from microbes (**Picart-Palmade *et al.*, 2019; Jadhav *et al.*, 2021**). Non-thermal methods are highly diverse, and they include Ultraviolet (UV) irradiation, ultrasounds (**Bhargava *et al.*, 2021**), pascalization or high-pressure processing (HPP), pulsed electric field (PEF) (**Li and Farid, 2016**), and chemical treatments. However, few of these technologies have been commercialized, mainly for HPP such as Avure Technologies (Middletown, OH USA) or Hiperbaric (Burgos, Spain), and some for UV irradiation such as Dinies GmbH (Villingendorf, Germany).

Chemical treatments have the disadvantage that, afterwards, the chemicals remaining may be toxic to the microorganisms in the process or to consumers, if the product is a food. For instance, ethylene oxide or hydrogen peroxide are used in some disposable laboratory plastic materials and food packaging materials, and these chemicals are usually then displaced by sterile air (**Ansari and Datta, 2003**).

Since pasteurization is only partial sterilization, alternative methods of sterilization with microbial elimination can also be used but with less drastic conditions. Strictly speaking, pasteurization should only be used to refer to the treatment carried out with mild heat (that is to say, thermal pasteurization). Microwave pasteurization can be included in this definition since it is also heating. Moreover, it offers possibilities of shorter processing time and better heating uniformity than conventional thermal processing with steam or hot water. Thus, it has the potential to deliver safe and higher quality foods (**Tang *et al.*, 2018**).

Nevertheless, the term "pasteurization" is used for other non-thermal techniques that have the same aim of eliminating most microbes, such as "UV pasteurization" (**Gouma *et al.*, 2020**), "ultrasound pasteurization" (**Alongi *et al.*, 2019**), "pulsed electric field pasteurization" (**Saldaña *et al.*, 2011**) or "high-pressure pasteurization" (**Mújica-Paz *et al.*, 2011**).

EFFECTS OF HEAT TREATMENTS ON MICROORGANISMS

As discussed, the most common method for destroying microorganisms is a simple heat treatment. At high temperatures, virtually all macromolecules lose their structure and functional capacity. This effect of temperature is more pronounced in the presence of water (that is, it is more pronounced with humid heat than with dry heat). The process of macromolecule denaturation begins at around 50ºC. The high temperature affects proteins, nucleic acids, and cell membranes, leading to cell death in most microbes, except some thermophiles which can resist up to 100ºC, and spores, which can resist almost 120ºC (**Reineke and Mathys, 2020**). Obviously, the decrease in the number of viable cells in a microbial population subjected to a sterilizing thermal treatment is exponential and is more pronounced when the temperature is higher.

The thermal destruction of microorganisms is a set of reactions that behave as a single first-order reaction in which the Arrhenius equation can be applied:

$$k = A \cdot e^{(-E/RT)}$$

where k is the specific mortality rate of the possible contaminating microorganism, A is the pre-exponential or collision frequency factor of molecules, E is the activation energy, R is the gas constant (8.3 J / mol ºK), and T is the temperature in ºK.

Obviously, k is greater when T increases (**Stanbury *et al.*, 1995; Peng *et al.*, 2017**). Since k = D/t, where D is the quantification of microbial destruction at a time t, the above expression can be written as:

$$D/t = A \cdot e^{(-E/RT)}$$

rearranging above equation:

$$t = D/A \cdot e^{(E/RT)} \quad (1)$$

where, time (t) and temperature (T) are inversely correlated when microbial destruction D needs to be the same. A, E and R have been defined above.

As well as their lethal effect on microorganisms, heat treatments also have effects on the other components of the media or the food. They can be degraded by reactions, which lead to a loss of nutritional quality for subsequent fermentation or food quality. The longer the sterilizing treatment lasts, the greater this negative effect is. Loss of nutrient quality is mainly due to interactions between environmental components, such as Maillard-type reactions between reducing sugars and protein amino acids, and to degradation of labile components, such as vitamins and amino acids. The difference between this destruction of nutrients and that of microorganisms is that the activation energies (E) calculated experimentally are lower than those of the microorganisms: while the E of destruction of sporulated microorganisms is 250 - 300 kJ / mol, the corresponding E of nutrient destruction is 50 - 150 kJ/mol (**Richards, 1968**). Considering the same expression mentioned above (1) for nutrient destruction, its lower activation energy results in a lower slope of the relationships between time and inverse temperature, which shows that moist heat treatments at higher temperature and shorter time are more efficient for conserving nutrients and are therefore applied in both fermentation media and food (for instance, UHT milk). These are HTST (high temperature - short time) sterilizations or pasteurizations (**Cappozzo *et al.*, 2015; Peng *et al.*, 2017**).

CONSIDERATIONS ON APPLYING PASTEURIZATION IN FOOD PROCESSES

Since pasteurization is a partial sterilization, bacterial spores are not destroyed, but most of the vegetative cells of many microorganisms are. Because the main common pathogens of many foods (for instance, *Listeria*, *Brucella*, *Mycobacterium tuberculosis*, *Campylobacter*, *Salmonella*, *E. coli* O157: H7) are not sporulated, they can be removed by temperatures below 100ºC. Pasteurization also eliminates food spoiling microorganisms (**Peng *et al.*, 2017; Madigan *et al.*, 2018**).

All pasteurization methods must be based on the premise that products cannot be exposed for long times to high temperatures since they lose important nutritional properties. Other important factors that determine the choice of a pasteurization method are energy consumption, cultural factors that determine the consumption of the product (**Moreno Galindo, 2013**) and the protection of the environment.

THERMAL PASTEURIZATION METHODS IN FOOD PROCESSES

Scalding

Scalding is the preheating of products by immersion in hot water or steam, commonly used as a pretreatment before continuing to process many food products (canning, freezing, dehydration, etc.). Typical temperatures and scalding times can range from 88°C to 100°C for 1 to 10 minutes (**Barrett, 1994**). The main purpose of scalding is that it inactivates enzymes in the food that cause it to lose physical qualities such as texture and flavor. It can also influence its nutritional qualities (**Palou *et al.*, 2007**).

Microwaves

Microwaves heat due to polarization effects at a selected wave frequency in the range 300 MHz to 300 GHz. When an oscillating electric field hits dipole molecules like water, they try to realign themselves in the direction of the electric field. This realignment occurs at a million times per second, causing internal friction between the molecules, which causes the volumetric heat of the material **(Datta and Davidson, 2000**). This thermal effect of microwave irradiation is the main one causing the microbial death and in consequence the disinfection of substrates. The death rates of microorganisms by microwaves are similar to conventional heating (**Fung and Cunningham, 1980**). Similarly, bacteria are more resistant to microwaves than yeasts and molds, and bacterial spores are more resistant than vegetative cells (**Datta and Davidson, 2000**).

NON-THERMAL PASTEURIZATION METHODS IN FOOD PROCESSES

High pressure processing (HPP)

High pressure processing is also as called high hydrostatic pressure processing. It is a modern method of food pasteurization used commercially in many countries (**Silva and Evelyn, 2020; Yu *et al.*, 2020**), which consists of a high-pressure vessel and its closure, a pressure generation system, a temperature control device, and a material handling system. Air is removed from the container by means of a low-pressure quick fill and drain pump, in combination with an automatic vent valve. Then high hydrostatic pressure is generated by direct or indirect compression or by heating the pressure medium (**Palou *et al.*, 2007**). High pressure affects most microbial growth killing cells, but some mould spores can be very resistant (**Silva and Evelyn, 2020**), and some pressure-resistant subpopulations of vegetative microorganisms can revive after the HPP treatment. In those cases, the combined application of HPP with other treatments —high or low temperature, low pH or natural antimicrobials— is recommended (**Yang *et al.*, 2021**).

Ultraviolet radiation

Ultraviolet radiation exhibits germicidal properties in the UV-C region (200–280 nm), with microbial inactivation being maximum in the range 254 to 264 nm

(**Choudary and Bandla, 2012**). Several studies suggest that microorganisms are destroyed by UV-C light penetrating into cells, which causes tremendous DNA damage due to the formation of thymine dimers and eventually leads to cell death. UV disinfection treatments are effective against all microbes in short times of few minutes. UV irradiation is being commercially applied in the food processing industry to tenderize or age meat, cure, and wrap cheeses, prevent mold from growing on the surface of bakery products, and purify air in bottling and food processing establishments, and in pickle vats. Relative humidity affects the death rate of airborne bacteria, particularly at values above 0.50, when an increase in relative humidity results in a decrease in the mortality rate (**Siddhant *et al*., 2014; Gouma *et al.*, 2020)**.

Table 1 Comparison of different pasteurization methods in food processes

Method	*Advantages*	*Disadvantages*	*References*
Scalding	Removes air bubbles and reduces the initiation of microbial growth. Cleans raw food, which makes preliminary activities like peeling easier.	If temperature is not controlled, the texture, color and flavor can change. It has impacts on the environment, due to the large amounts of water and fuels or energy sources used for heating.	**Barrett, 1994 Palou *et al.*, 2007 Mejía and Alberto, 2013 Atila, 2016 Rózsa *et al.*, 2016 Shrestha *et al.*, 2021**
Microwaves	They penetrate the food or substrate, so cooking takes place throughout the internal volume. They preserve nutritional properties and sensory characteristics. Heating efficiency is high (80% or higher), and cost is low. The heating is silent and does not generate exhaust gases.	There are variations in the temperature distribution of the product during microwave heating. Kinetics of nutrient quality degradation depends on such factors as substrate products or oven designs. The dielectric properties of the product vary significantly during thermal processing.	**Datta and Davidson, 2000 Tang *et al.*, 2018 Boshkova *et al.*, 2020**
High pressure processing (HPP)	It is more sustainable and has less impact on human health and the environment since the effect on food nutrients and sensory properties is negligible or minimal. It is faster than thermal processes and independent of the size and geometry of the product.	Due to the high investment cost, the price of HPP processed foods is high. Some microorganisms can be resistant to HPP, and frequently it is combined with other treatments.	**Palou *et al.*, 2007 Silva and Evelyn, 2020 Yu *et al.*, 2020**
UV radiation	It is safe and environmentally friendly. It does not produce toxic by-products or affect the organoleptic characteristics of natural products. Energy cost is very low, and implementation is easy. The cleaning and disinfection of production lines is simpler and more efficient, which saves cleaning time and costs.	When used in high doses, there is a marked tendency for taste and odor to deteriorate before food is satisfactorily pasteurized. Special ultraviolet light emitting lamps are needed. Penetration of radiation in substrates is low, so it is only effective for surfaces.	**Choudary and Bandla, 2012 Siddhant *et al.*, 2014 Gouma *et al.*, 2020**

METHODS FOR DISINFECTING SUBSTRATES FOR EDIBLE MUSHROOM PRODUCTION

Substrates for the cultivation of fungi must be treated previously to reduce the harmful microbiota they contain and prevent microorganisms from competing for spaces and nutrients with the fungus to be cultivated. The main contaminant microorganisms are molds as *Trichoderma*, *Penicillium*, *Aspergillus*, *Neurospora* and *Coprinus*, and some bacteria causing diseases in the mushroom, such as *Pseudomonas* (**Gaitán-Hernández *et al.*, 2006**). The main methods currently used in mushroom cultivation are those of the following subsections.

Immersion method in hot water

The immersion method consists of preparing a water bath (60-80ºC) in which the substrate must be immersed from 30 to 60 minutes. The disadvantages of the method are the significant consumption of water it requires and the contaminated wastewater it leaves behind (**Mejía and Alberto, 2013**).

Table 2 Comparison of different substrate disinfection methods in the production of *Pleurotus ostreatus* in terms of yield and biological efficiency (BE).

Disinfection method	**Process conditions**	**Yield** (g/kg)	**BE** (%)	**Substrate**	**References**
UV irradiation	45 min	610	61	Paddy straw	**Siddhant *et al.*, 2014**
Chemical (Formalin 40%)	18 h	271	92	Poplar sawdust and wheat straw	**Atila, 2016**
	18 h	720	72	Paddy straw	**Siddhant *et al.*, 2014**
	18 h	322	107	Paddy straw	**Mejía and Alberto, 2013**
	18 h	210	86	Paddy straw	**Rózsa *et al.*, 2016**
	18 h	-	80	Paddy straw	**Shrestha *et al.*, 2021**
Steam	20 min	353	101	Paddy straw	**Shrestha *et al.*, 2021**
	120 min		69.4	maize cobs	**Jongman et al., 2013**
Scalding in hot water	60ºC – 60 min	136	47	Poplar sawdust and wheat straw	**Atila, 2016**
	65ºC – 60 min	290	119	Paddy straw	**Rózsa *et al.*, 2016**
Scalding in hot water	80ºC – 30 min	-	90	Paddy straw	**Shrestha *et al.*, 2021**
	80ºC – 60 min	263	91	Poplar sawdust and wheat straw	**Atila, 2016**
	80ºC - 90 min	228	76	Paddy straw	**Mejía and Alberto, 2013**
Scalding in hot water	100ºC - 60 min	223	77	Poplar sawdust and wheat straw	**Atila, 2016**
	100ºC - 60 min	680	68	Paddy straw	**Siddhant *et al.*, 2014**
Sterilization	121ºC - 60 min	670	67	Paddy straw	**Shrestha *et al.*, 2021**
Sterilization	121ºC - 90 min	240	80	Poplar sawdust and wheat straw	**Atila, 2016**
	121ºC- 120 min	282	95	Paddy straw	**Mejía and Alberto, 2013**
	121ºC - 90 min	230	95	Paddy straw	**Rózsa *et al.*, 2016**

Pasteurization method

The pasteurization method is a typical industrial-scale procedure, with treatment of materials inside a parallelepiped chamber capable of recirculation of the air with steam, maintaining a temperature of 60-80ºC for 6-12 h (**Sánchez, 2010**). The rooms where this procedure occurs are called pasteurization tunnels. It is particularly useful when large quantities of substrates (not less than 1 ton) need to be prepared.
The various substrate disinfection methods used in the production of *Pleurotus ostreatus* (Table 2) can be effective in terms of yield and biological efficiency (BE), which is calculated as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of the cultivation substrate.

FACTORS THAT INFLUENCE PASTEURIZATION EFFICIENCY

Role of time / Temperature during pasteurization

The duration of heat treatment to achieve pasteurization depends on temperature and type of pathogen to be killed in the substrate (**Gowda *et al.*, 2021**). Temperatures below 55ºC are usually insufficient for some microorganisms and it seems to be the lower limit during the process (**Ardón López, 2007**). Although high pasteurization temperatures (80-90ºC) can eliminate competing microorganisms, the temperature should not exceed 100ºC, as this would cause the death of all microorganisms (**Gowda *et al.*, 2014**).
Contamination of the substrate by competing organisms can have a considerable impact on the yields of mushroom cultivation. Several authors (**Moda *et al.*, 2005; Oseni *et al.*, 2012**) point out that these setbacks are caused by an inadequate t / T combination during the pasteurization stage, and that the literature is not clear about which t / T combination is appropriate.

T / t combination for elimination of competitors

When microorganisms and/or spores are subjected to a heating process such as pasteurization, these microbes are not killed instantly, some are destroyed in a given period of time while the rest survive. If the survivors are subjected to a similar process (equal time and temperature), the same proportion of microorganisms will die.
Mignucci *et al.* (2000) found that yields were best with two pasteurization cycles of 6 hour each over a period of 48 hours. The substrate obtained was also high quality and free of thermophilic microorganisms. Those competitors that reside in the substrate were activated during the first cycle, and subsequently eliminated during the second.
Choi (2004) stated that pasteurization of the substrate at 65°C for 6 to 8 hours eliminates mesophilic microorganisms, but clarified that mold spores are stable at 65°C and can die at temperatures above 80°C. The moulds *Trichoderma*, *Coprinus*, *Penicillium* and *Aspergillus* are some of the most prominent *Pleurotus* competitors and can appear even after disinfection with hot water at 80°C for 2 hours. These competitive molds will grow due to insufficient substrate disinfection and cause destructive green mould disease which hampers the growth mycelium (**Bisaria *et al.*, 1997; Gowda and Manvi, 2019**).

T / t ratio of elimination of beneficial microbes for mushroom

Kurtzman (2010) reported the existence of a group of what he called beneficial or helper microorganisms, because they assist the growth of the mushroom by acting in symbiosis with it. If the pasteurization is carried out properly, the competitors must die, leaving these beneficial microorganisms active.
Carrasco and Preston (2020) stated that the bacteria *Bacillus*, *Pseudoxanthomonas* and *Thermobispora* play a fundamental role in degrading complex carbon sources within the wheat straw-based substrate. They also pointed out that several *Thermus*, *Bacillus*, and *Actinobacteria* are responsible for the production of antibiotics that create a selective environment for *Pleurotus*.
It has been postulated that the synergy between bacteria and fungi within the shell material cause native bacteria to consume volatile organic components and, therefore, stimulate fruiting (**Chen *et al.*, 2013; Zhang *et al.*, 2016**).
Carrasco and Preston (2020) also clarified that high temperatures are not recommended during pasteurization since they have been associated with lower bacterial diversity measured in terms of α diversity (mean diversity of species in different sites or habitats on a local scale), understood as the mean richness of microbial species within an ecosystem.

PASTEURIZATION EQUIPMENT

A low-cost pasteurization equipment needs to be developed if mushroom growers are to solve the most critical problems encountered in mushroom cultivation (**Gowda and Manvi, 2019**).
Generally, in small-scale mushroom firms boiling water or hot steam are preferred to pasteurize substrates at a temperature above 80°C, but these methods are difficult to use on a large scale. Therefore, growers prefer to use chemicals, which are easy and cost-effective, but aggressive for the environment. Steam pasteurization of the substrate can be carried out in containers (boxes or bags), where steam is supplied. The disadvantage of this method is that there is a big difference between the temperature of the air in the chamber and the substrate due to the heat-insulating effect of the material of the container. Air or air-vapor mixture cannot freely pass through the substrate, so heat transport is too slow. For this treatment option, it is better to choose long exposure at a low temperature (for example, 60-65°C for 24-48 hours). After pasteurization and cooling, the substrate can be inoculated and packed into bags with perforations.

Immersion in hot water

An inexpensive method for pasteurizing substrate by using an ordinary steel drum has been developed (**Kurtzman, 2010**). This method involved heating the water inside the drums to 60°C and immersing the straw in hot water using a wire basket with a holding time of 30–60 minutes (Fig. 1-A). This method can pasteurize one ton of dry substrate per batch in 8–10 hours.
Most small-scale mushroom growers use normal oil drums as boiling equipment with little or no modifications. Substrate can also by pasteurized by being placed in gunny bags or polyethylene bags, which are arranged in layers inside pasteurizer drum with enough space for steam to circulate (**Taurachand, 2004**).
All these methods are inexpensive but have several drawbacks: among other things, they require a lot of water, they are time consuming, their thermal efficiency is low due to heat loss, they have difficulty in handling hot pasteurized straw and draining out hot water, and there is more chance of contamination due to unhygienic management (**Gowda *et al.*, 2014**).
To solve these issues, a paddy straw pasteurizer – with a dry straw capacity of 25 kg – was developed (**Gowda *et al.*, 2014; Gowda and Kumaran, 2014**), which consisted of a stainless-steel pasteurizing drum, temperature-time control with a temperature sensor, a drum tilting mechanism for loading and unloading straw and a straw compressing mechanism (Fig. 1-B). When it was used to compress paddy straw and hold it at the maximum compressed level, it required 210 L of water for soaking and pasteurizing whereas conventional methods required 360 L. The total time needed for pasteurization at 80 ºC was 5h 30 min, which was 2 h less than conventional methods (**Gowda *et al.*, 2014**).

Steam pasteurization devices

A prototype steam pasteurization system for producing oyster mushrooms has been described that uses heat from the production of rice husk biochar to steam pasteurize mushroom fruiting bags (MFB) (**Orge and Leal, 2018**). Briefly, the pasteurizing chamber is a hollow cubic chamber (Fig. 1 C). On its concrete floor, a two-prong assembly with 28-mm diameter GI pipes and fittings is installed which is then linked to the pipes and fittings outside the chamber leading to the steam generating vessel. Each prong has 32 holes (5 mm diameter) to distribute the steam inside the pasteurizing chamber uniformly. This system can pasteurize up to 560 MFBs per batch consuming an average amount of 11.7 kg rice husk per hour of operation with a biochar recovery of 35.03% and a thermal efficiency of 12.17%. The system provides optimum temperature (60°C) and exposure (60 min) thus resulting in a low percentage of contamination (an average of only 1.92%).

Pasteurization tunnels

Tunnels for pasteurizing the substrate for oyster mushroom production are currently the technology that saves most resources **(Royse and Beelman, 2014).** It ensures stable substrate blocks and a high mushroom yield. The raw material to produce the substate is humidified after grinding and stored at a site or in a silo. After rehandling, this mixture is placed in a tunnel for further processing. The tunnel itself is a thermally insulated chamber where raw material is steam pasteurized. After the biomass is cooled, it is ready for inoculation by adding mycelium to substrate bags.
The continuous substrate chamber (Fig. 1-D) is a room with two gates on opposite sides, one for loading and one for unloading materials. The walls, the ceiling and the floor must be insulated with plastic foam or polyurethane foam. Steam is supplied to the chamber into which the raw material has been loaded, and is circulated by the ventilation system, with fresh air and a discharge valve. To eliminate the human factor and improve processing consistency, an automated process control system is used in the steam chamber. The steam generator, powered by gas or solid fuel, is used to produce steam. The tunnel can be constructed in different sizes and capacities.

Microwave devices

Microwave is an emerging technology to treat different materials that may include agricultural wastes. In general, microwave devices operate on the principle that material in the working chamber absorb microwave (MW) energy (Fig. 1-E). The material moves as a continuous stream in a radio-transparent channel that passes through the work chamber. The driving node rotates the shaft of the screw conveyor (screw), which moves the product from the load tank to the product pipeline and then pushes it through the product pipeline and the outlet pipeline to the outside of the device. Microwave preparation of the substrate consumes much

less energy than traditional technologies. At the same time, the yield of mushrooms increases by 11 %, due to the improvement in the nutritional properties of the straw substrate (**Boshkova *et al.*, 2020**).

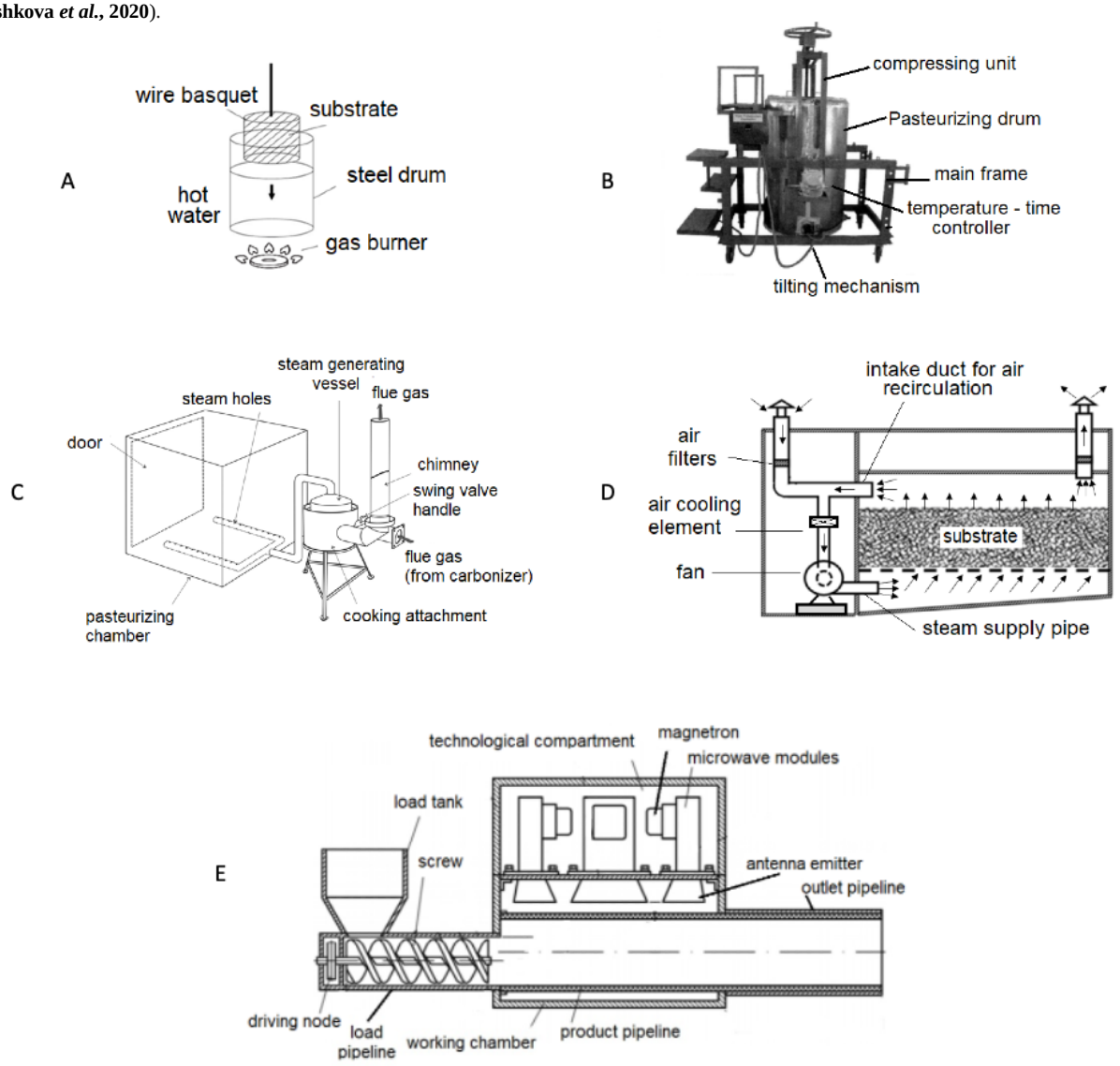

Figure 1 Pasteurization equipments. A) Hot water immersion pasteurizer. B) Paddy straw pasteurizer (figure adapted from **Gowda *et al.* (2014)**). C) Steam pasteurization device (figure adapted from (**Orge and Leal (2018)**). D) Pasteurization tunnel. E) Microwave pasteurizer.

CONCLUSIONS

The cultivation of *Pleurotus*, like other mushrooms, requires substrates to be appropriately disinfected. Given the limited investments, this can be an economically limiting factor for small producers in non-rich countries. The main conclusion of our review of this subject is that thermal pasteurization is the best option. Indeed, pasteurization helps create an appropriate environment that is free of contaminating microorganisms in the substrate by eliminating the main common pathogens, bacteria and fungi, and at the same time it does not destroy most vitamins and other nutrients, unlike complete sterilization. Moreover, thermal pasteurization allows some other microbes –mainly sporulates and other heat-resistant bacteria– to survive, which can help the mushrooms degrade complex carbon sources, produce antibiotics, and create a more selective environment. Another conclusion is that it is necessary to consider the time/Temperature ratio of pasteurization since it influences the degradation of substrate nutrients. The most suitable treatments are short and carried out at high temperature. Of the various pasteurization and other disinfection methods, pasteurization by scalding in hot water at 80ºC for short times seems to be the best. The conclusion is that substrate processing tunnels are the best equipment for pasteurization, since they save resources, and ensure the quality of the substrate and a high mushroom yield.

REFERENCES

Alongi, M., Verardo, G., Gorassini, A., Lemos, M.A., Hungerford, G., Cortella, G., & Anese, M. (2019). Phenolic content and potential bioactivity of apple juice as affected by thermal and ultrasound pasteurization. *Food and Function*, 10, 7366-7377. https://doi.org/10.1039/C9FO01762C

Ansari, I.A., & Datta, A.K. (2003). An Overview of Sterilization Methods for Packaging Materials Used in Aseptic Packaging Systems. *Food and Bioproducts Processing*, 81, 57-65. https://doi.org/10.1205/096030803765208670

Ardón López, C.E. (2007). The Production of Edible Mushrooms. Master's degree in University teaching with a specialty in Educational Evaluation, *University of San Carlos de Guatemala*, Postgraduate Department.

Atila, F. (2016). Effect of Different substrate Disinfection Methods on the Production of *Pleurotus ostreatus*. *Journal of Agricultural Studies*, 4, 1-14. https://doi.org/10.5296/jas.v4i4.10051

Barrett, D.M. (1994). Vegetable blanching—How long is long enough? *American Vegetable Grower*, 42, 46–47.

Bhargava, N., Mor, R.S., Kumar, K., & Sharanagat, V.S. (2021). Advances in application of ultrasound in food processing, A review. *Ultrasonics Sonochemistry*, 70, 105293. https://doi.org/10.1016/j.ultsonch.2020.105293

Bisaria, R., Madan, M., & Vasudevan, P. (1997). Utilization of agroresidues as animal feed through bioconversion. *Bioresource Technology*, 59, 5-8. https://doi.org/10.1016/S0960-8524(96)00140-X

Boshkova, I., Volgusheva, N., Boshkov, L., Potapov, M., Kolesnuchenko, N., Demiachuk, B., & Lapkin, O. (2020). Testing a microwave device for the treatment of plant materials by various technologies. *Eastern-European Journal of Enterprise Technologies*, 2/5, 104. https://doi.org/10.15587/1729-4061.2020.199816

Cappozzo, J.C., Koutchma, T., & Barnes, G. (2015). Chemical characterization of milk after treatment with thermal (HTST and UHT) and nonthermal (turbulent flow ultraviolet) processing technologies. *Journal of Dairy Science*, 98, 5068-5079. https://doi.org/10.3168/jds.2014-9190

Carrasco, J., & Preston, G.M. (2020). Growing edible mushrooms, a conversation between bacteria and fungi. *Environmental Microbiology*, 22, 858-872. https://doi.org/10.1111/1462-2920.14765

Chen, S., Qiu, C., Huang, T., Zhou, W., Qi, Y., Gao, Y., *et al.* (2013). Effect of 1-aminocyclopropane-1-carboxylic acid deaminase producing bacteria on the hyphal growth and primordium initiation of *Agaricus bisporus*. *Fungal Ecology*, 6, 110–118. https://doi.org/10.1016/j.funeco.2012.08.003

Chen, S.Y., Yu, H.T., Kao, J.P., Yang, C.C., Chiang, S.S., Mishchuk, D.O., *et al.* (2015). Consumption of vitamin D_2 enhanced mushrooms is associated with improved bone health. *Journal of Nutrition Biochemistry*, 26, 696-703. https://doi.org/10.1016/j.funeco.2012.08.003

Cheung, P.C.K. (2010). The nutritional and health benefits of mushrooms. *Nutrition Bulletin*, 35, 292-299. https://doi.org/10.1111/j.1467-3010.2010.01859.x

Choi, K.W. (2004). Cultivation modes, Shelf cultivation of oyster mushroom. In Kwon H and Kim BS (Eds.). *Mushroom Grower's Handbook-1*. Korea, Mushworld, 153-165.

Choudhary, R., & Bandla, S. (2012). Ultraviolet Pasteurization for Food Industry. *International Journal of Food Science and Nutrition Engineering*, 2, 12-15. https://doi.org/10.5923/j.food.20120201.03

Datta, A.K., & Davidson, P.M. (2000). Microwave and radio frequency processing. *Journal of Food Science*, 65, 32–41. https://doi.org/10.1111/j.1750-3841.2000.tb00616.x

Fellows, P.J. (2017) *Food Processing Technology Principles and Practice*. Woodhead Publishing Series in Food Science, Technology and Nutrition. Amsterdam, Elsevier, pp. 563–578.

Fung, D.Y.C., & Cunningham, F.E. (1980). Effects of microwaves on microorganisms in foods. *Journal of Food Protection*, 43, 8, 641-650. https://doi.org/10.4315/0362-028X-43.8.641

Gaitán-Hernández, R., Salones, D., Pérez Merlo, R., & Mata, G. (2006) *Manual práctico del cultivo de setas: aislamiento, siembra y producción* (1st ed.). Xalapa, Veracruz, México, Instituto de Ecología.

González, A., Cruz, M., Losoya, C., Nobre, C., Loredo, A., Rodríguez, R., *et al.* (2020). Edible mushrooms as a novel protein source for functional foods. *Food and Function*, 11, 7400-7414. http://dx.doi.org/10.1039/D0FO01746A

Gouma, M., Álvarez, I., Condón, S., & Gayán, E. (2020). Pasteurization of carrot juice by combining UV-C and mild heat, Impact on shelf-life and quality compared to conventional thermal treatment. *Innovative Food Science & Emerging Technologies*, 64, 102362. https://doi.org/10.1016/j.ifset.2020.102362

Gowda, N.A.N., Gurikar, C., & Hanumantharaju K.N. (2021). Disinfection methods for mushroom substrate preparation. In Kulshreshtha S. Recent Advances in Mushroom Cultivation Technology and its Applications. Vol-1, pp. 29-48. Bright Sky Publications, New Delhi, India. https://doi.org/10.22271/bs.book.20

Gowda, N.A.N., & Kumaran, G.S. (2014). Design and development of a hot water paddy straw pasteurizer for mushroom cultivation. *Agricultural Mechanization in Asia, Africa and Latin America*, 45, 2, 11-18.

Gowda, N.A.N., Kumaran, G.S., & Pandey, M. (2014). Performance Evaluation of Paddy Straw Pasteurizer for Mushroom Cultivation. *Agricultural Mechanization in Asia, Africa and Latin America*, 45, 3, 28-36.

Gowda, N.A.N., & Manvi, D. (2019). Agro-residues disinfection methods for mushroom cultivation, a review. *Agricultural Reviews*, 40, 93-103.

Gowda, N.A.N., & Manvi, D. (2020). Agriculture crop residues disinfection methods and their effects on mushroom growth. *Proceedings of the Indian National Science Academy*, 86, 3, 1177-1190. https://doi.org/10.16943/ptinsa/2020/154396

Jadhav, H.B., Annapure, U.S., & Deshmukh, R.R. (2021). Non-thermal technologies for food processing. Frontiers in Nutrition, 8, 657090. https://doi.org/10.3389/fnut.2021.657090

Jongman, M., Khare, K.B., & Khonga, E.B. (2013). Effect of different grain spawns and substrate sterilization methods on yield of oyster mushroom in Botswana. *International Journal of Bioassays*, 2, 10, 1308-1311.

Krittanawong, C., Isath, A., Hahn, J., Wang, Z., Fogg, S.E., Bandyopadhyay, D., *et al.* (2021). Mushroom Consumption and Cardiovascular Health, A Systematic Review. *The American Journal of Medicine*, 134, 637-642. https://doi.org/10.1016/j.amjmed.2020.10.035

Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K, *et al.* (2020). Cultivation of Mushrooms and Their Lignocellulolytic Enzyme Production Through the Utilization of Agro-Industrial Waste. *Molecules*, 25, 2811. https://doi.org/10.3390/molecules25122811

Kurtzman, R.H. Jr. (2010). Pasteurization of mushroom substrate and other solids. *African Journal of Environmental Science and Technology*, 4, 936-941.

Li, X., & Farid, M. (2016). A review on recent development in non-conventional food sterilization technologies. *Journal of Food Engineering*, 182, 33-45. https://doi.org/10.1016/j.jfoodeng.2016.02.026

Ma, G., Du, H., Hu, Q., Yang, W., Pei, F., & Xiao, H. (2021). Health benefits of edible mushroom polysaccharides and associated gut microbiota regulation. *Critical Reviews in Food Science and Nutrition*, 1,1-18. https://doi.org/10.1080/10408398.2021.1903385

Madigan, M.T., Bender, K.S., Buckley, D.H., Sattley, W.M., & Stahl, D.A. (2018). *Brock Biology of Microorganisms* (15th ed.), Pearson, London UK, Pearson.

Mejía, S.J., & Albertó, E. (2013). Heat treatment of wheat straw by immersion in hot water decreases mushroom yield in *Pleurotus ostreatus*. *Revista Iberoamericana de Micología*, 30, 125-129. https://doi.org/10.1016/j.riam.2012.11.004

Mignuccci, J.S., Hernández-Bacó, C., Rivera-Vargas, L., Betancourt, C., & Alameda, M. (2000). Diseases and Pests Research on Oyster Mushroom (*Pleurotus* spp.) in Puerto Rico. *The International Journal of Mushroom Sciences*, 3, 21-26.

Moda, E.M., Horii, J., & Spoto, M.H.F. (2005). Edible Mushroom *Pleurotus sajor-caju* Production on washed and supplemented sugarcane bagasse. *Scientia Agraria*, 62, 127-132. https://doi.org/10.1590/S0103-90162005000200006

Moreno Galindo, J.N. (2013). *Modelamiento y control de planta pasteurizadora*. Trabajo de grado, Informe final, Pontificia Universidad Javeriana, Bogotá, Colombia.

Mújica-Paz, H., Valdez-Fragoso, A., Samson, C.T., Welti-Chanes, J., & Torres, J.A. (2011). High-Pressure Processing Technologies for the Pasteurization and Sterilization of Foods. *Food and Bioprocess Technology*, 4, 969. https://doi.org/10.1007/s11947-011-0543-5

Orge, F.R., & Leal, L.V. (2018). Utilizing heat from rice hull biochar production for steam pasteurization of mushroom fruiting bags. *Cogent Engineering*, 5, 1453972. https://doi.org/10.1080/23311916.2018.1453972

Oseni, T.O., Dlamini, S.O., Earnshaw, D.M., & Masarirambi, M.T. (2012). Effect of Substrate Pre-treatment Methods on Oyster Mushroom (*Pleurotus ostreatus*) Production. *International Journal of Agricultural Biology*, 14, 251-255.

Palou, E., López-Malo, A., Barbosa-Cánovas, G.V., & Swanson, B.G. (2007). High-pressure treatment in food preservation. In Rahman MS (Ed.). *Handbook of Food Preservation* (2nd ed.). Boca Raton, CRC Press, 815-854. https://doi.org/10.1201/9780429091483-54

Picart-Palmade, L., Cunault, C., Chevlier-Lucia, D., Belleville, M-P., & Marchesseau, S. (2019). Potentialities and limits of some non-thermal technologies to improve sustainibility of food processing. *Frontiers in Nutrition*, 5, 130. https://doi.org/10.3389/fnut.2018.00130

Peng, J., Tang, J., Barrett, D.M., Sablani, S.S., Anderson, N., & Powers, J.R. (2017). Thermal Pasteurization of ready-to-eat foods and vegetables. Critical Factors for Process Design and Effects on Quality. *Critical Reviews in Food Science and Nutrition*, 57, 2970-2995. https://doi.org/10.1080/10408398.2015.1082126

Reineke, K., & Mathys, A. (2020). Endospore Inactivation by Emerging Technologies, A Review of Target Structures and Inactivation Mechanisms. *Annual Review of Food Science and Technology*, 11, 255-274. https://doi.org/10.1146/annurev-food-032519-051632

Richards, J.W. (1968). *Introduction to Industrial Sterilisation*. London, Academic Press.

Roncero-Ramos, I., & Delgado-Andrade, C. (2017). The beneficial role of edible mushrooms in human health. *Current Opinion in Food Science*, 14, 122-128. https://doi.org/10.1016/j.cofs.2017.04.002

Roupas, P., Keogh, J., Noakes, M., Margetts, C., & Taylor, P. (2012). The role of edible mushrooms in health, Evaluation of the evidence. *Journal of Functional Foods*, 4, 687-709. https://doi.org/10.1016/j.jff.2012.05.003

Royse, D.J., Baars, J., & Tan, Q. (2017). Current Overview of Mushroom Production in the World. In: Diego CZ, Pardo-Giménez A (Eds.). *Edible and Medicinal Mushrooms, Technology and Applications*. Hoboken NJ, USA, John Wiley & Sons Ltd, pp 5-13.

Royse D., & Beelman, R. (2014). *Six steps to mushroom farming*. College of Agricultural Sciences, The Pennsylvania State University, 2nd Edition.

Rózsa, S., Danuț-Nicolae, M., Sima, R., Gocan, T.M., & Andreica, I. (2016). Research on the influence of hybrid, culture substrate and method of disinfection on oyster mushrooms - *Pleurotus* spp. *Lucrări Ştiinţifice*, 59, 35-38.

Saldaña, G., Puértolas, E., Monfort, S., Raso, J., & Álvarez, I. (2011). Defining treatment conditions for pulsed electric field pasteurization of apple juice. *International Journal of Food Microbiology*, 151, 29-35. https://doi.org/10.1016/j.ijfoodmicro.2011.07.033

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*, 85, 1321-1337. https://doi.org/10.1007/s00253-009-2343-7

Shrestha, S., Bhattarai, S., Shrestha, R.K., & Shrestha, J. (2021). Effect of different substrate sterilization methods on performance of oyster mushroom (*Pleurotus ostreatus*). *Journal of Agricultural Science*, 32, 127-132. https://doi.org/10.15159/jas.21.03

Siddhant, O., Yadav, S., Mishra, R., & Singh, R. (2014). Effect of Substrate Disinfection on the Biological Efficiency of *Pleurotus sajor-caju* (Fr) Singer. *Plant Archives*, 14, 205-209.

Silva, F.V.M., & Evelyn, E. (2020). Resistant moulds as pasteurization target for cold distributed high pressure and heat assisted high pressure processed fruit products. *Journal of Food Engineering*, 282, 109998. https://doi.org/10.1016/j.jfoodeng.2020.109998

Siqueira, F.G., Maciel, W.P., Martos, E.T., Duarte, G.C., Miller, R.N., Silva, R.D., *et al.* (2012). Cultivation of *Pleurotus* mushrooms in substrates obtained by short composting and steam pasteurization. *African Journal of Biotechnology*, 11, 11630-11635. https://doi.org/10.5897/AJB12.451

Stanbury, P.F., Whitaker, A., Hall, S.J. (1995). *Principles of fermentation technology*. Oxford, UK, Pergamon Press Ltd.

Tang, J., Hong, Y.K., Inanoglu, S., & Liu, F. (2018). Microwave pasteurization for ready-to-eat meals. *Current Opinion in Food Science*, 23, 133-141. https://doi.org/10.1016/j.cofs.2018.10.004

Taurachand, D. (2004). Sugarcane bagase. In: Choi KW (Ed.). *Mushroom Growers' Handbook 1*. *Oyster mushroom cultivation*. Seoul, MushWorld, pp 118-121.

Tewari, G., & Juneja, V.K. (2007). *Advances in thermal and non-thermal food preservation*. Ames, Iowa USA, Blackwell Publishing, pp 3, 96, 116.

Wilbey, R.A. (2014). Heat treatment of foods, principles of pasteurization. *Encyclopedia of Food Microbiology* (2nd ed.), San Diego CA USA, Academic Press, pp 169-174.

Yang, P., Rao, L., Zhao, L., Wu, X., Wang, Y., & Liao, X. (2021). High pressure processing combined with selected hurdles: Enhancement in the inactivation of vegetative microorganisms. *Comprehensive Reviews in Food Science and Food Safety*, 20, 2, 1800-1828. https://doi.org/10.1111/1541-4337.12724

Yu, T., Niu, L., & Iwahashi, H. (2020). High-pressure carbon dioxide used for pasteurization in food industry. *Food Engineering Reviews*, 12, 364-380. https://doi.org/10.1007/s12393-020-09240-1

Zhang, C., Huang, T., Shen, C., Wang, X., Qi, Y., Shen, J., *et al*. (2016). Downregulation of ethylene production increases mycelial growth and primordia formation in the button culinary-medicinal mushroom, *Agaricus bisporus* (*Agaricomycetes*). *International Journal of Medicine Mushrooms*, 18, 1131–1140. https://doi.org/10.1615/intjmedmushrooms.v18.i12.80