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Pasteurisation at the heart of the food revolution

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Abstract

Louis Pasteur devoted much of his early work to wine, its fermentation and diseases. Surrounded by his colleagues, he set up a field laboratory in Arbois and analysed each diseased wine, creating a microbial landscape by observing it under the microscope. Pasteur deduced that unusual ferments were responsible for the wine's deviations from alcoholic fermentation. He realised that successful fermentations had to be carried out by human intervention, combining the principles of restraint and hygiene to stop the development of disease-causing microbes. Pasteur then sought a method to kill or inhibit the bad ferments without hindering the development and activities of the good ferments. The history of pasteurisation is thus that of a compromise between destroying undesirable microbes and preserving the treated foodstuff, its colour, taste and smell. His work paved the way for the rationalisation of preservation processes, and his method was to have a universal impact, inspiring scientific and technological developments that continue to benefit the quality of food and drink to this day.

Wine and its diseases as a source of scientific research

Louis Pasteur was born in Dole in 1822, and in 1830 his family moved to Arbois, a small town in the heart of the Jura vineyards below the Revermont massif. Wine is everywhere in Arbois, with hillside vineyards on the horizon, wineries, cooperatives and wine merchants. The "Biou", a processional ceremony "to offer the first fruits of the harvest to the patron saint of the parish", has been held here for hundreds of years (first documented in 1665). The wine-growing society, founded in 1858, became the organiser, and in 1885 Louis Pasteur himself led the traditional procession. At the time, the Arbois vineyards covered 20,000 hectares, compared with 2,000 hectares today. Years of poor harvests, as well as deviant wine production that made it unmarketable, quickly had repercussions on the local economy. Is this the source of Louis Pasteur's curiosity about fermentation and the diseases associated with fermented products? In any case, it is certain that Louis Pasteur devoted some of his work to wine, its fermentation and its diseases. In 1863, he was asked by Napoleon III to seek urgent solutions to improve the quality of wine, the economy of which, far beyond Arbois, was a national issue. This attention to the economic issues involved in processing agricultural products was a constant in the first part of his scientific work, since in Lille, where he was appointed Dean of the Faculty in 1854 at the age of 32, his work focused on beer, vinegar and beet alcohol.

Microbes cause deseases in wine, beer and many other foods

What did Louis Pasteur know, or what could he have known, when he began his work on the principles of transforming grape juice into wine and the causes of its deterioration? Not much about the main players in these processes, which are infinitely small living organisms in the form of yeasts, moulds and bacteria. The word bacterium, inspired by the Greek "bacterion", was first used in 1831 when Louis Pasteur was 9 years old [1]. However, Antoine Laurent de Lavoisier had already worked out the equation for alcoholic fermentation, in which sugars are converted into ethyl alcohol [2]. In 1837, the physicist Charles Cagniard de Latour had also indicated that the transformation of sugar into alcohol was the result of yeast activity [3]. In

1837, Theodor Schwann had shown that yeasts are living organisms and that if they develop in an aqueous sugar solution in the absence of oxygen, the sugar is transformed into ethanol [4]. Pasteur's thinking was rooted in these early days of microbiology, and he was also able to take advantage of the development of microscopes, which he did not hesitate to incorporate into original experimental set-ups, enabling him, for example, to observe changes in the micrometric particle content of fermenting liquids, including anaerobic liquids (Figure 1).



Figure 1: Experimental apparatus used by Louis Pasteur to observe fermenting liquids on a microscopic scale. In Studies on Beer by Louis Pasteur (1876). Credits : Institut Pasteur

Pasteur, who was not a winemaking specialist, was commissioned by Napoleon III to develop a wide-ranging investigation strategy and methods. Surrounded by his collaborators, he set up the field laboratory in Arbois, where he conducted surveys among the winegrowers, visiting the region's wineries. He took countless notes and analysed each diseased wine, creating a microbial landscape by direct observation under the microscope, transcribed into faithful drawings, graded and annotated. In this way, each wine disease is associated with ferments that are generally found alongside the correct yeast for alcoholic fermentation. Each disease ferment is identified by its shape and size. Pasteur deduced that unusual ferments were responsible for the diseases observed and that they caused deviations in the wine through fermentations that competed with alcoholic fermentation. He realised that successful fermentations had to be carried out by human intervention, combining the principles of hygiene, to avoid contamination, and constraints, to promote the development of good ferments and stop the development of disease-causing ferments. These principles, applied using empirical methods derived from thousands of years of processing and preserving agricultural materials, were until then based on invisible and unexplained phenomena. Louis Pasteur, by scrutinising the progress of fermentations (Figure 1), revealed them to the world and paved the way for the rationalisation of processes for producing and preserving wine, beer, vinegar, beetroot alcohol and so many other products that he did not study himself but which benefited from his work as a scientific trailblazer. His work on wine and beer led him to meticulously identify the microbes responsible for deviations in taste, smell, texture and colour (Figure 2). Pasteur was looking for a method to inactivate these bad ferments without hindering the development and activities of the good ferment, alcoholic yeast. Pasteur, a chemist by training, initially thought of using chemical agents in the form of natural organic antiseptics or sulphur, the use of which was described in ancient wine production methods. But the results were not convincing.



Figure 2 : Main disease-causing ferments of wort and beer commented on by Louis Pasteur " n° 1 the ferments of turned beer, n° 2 are given the lactic ferments of wort and beer, n° 3 are given the ferments of putrid wort and beer... almost always the result of very faulty workmanship, No. 4 lists the ferments of viscous wort and those of spun beer, which the French call filante, No. 5 represents the ferments of pungent and sour beer, which has an acetic odour, No. 6 represents one of the deposits belonging to wort. The ferments given at n° 7 characterise beer with a particular acidity, more or less reminiscent of unripe acid fruit, with an odour sui generis. When n° 7 is present, alone or with n° 1, the beer acquires a sour taste and smell that make it detestable". In Studies on Beer by Louis Pasteur (1876). Credits : Institut Pasteur

Heat as a simple treatment

Louis Pasteur was also acquainted with Alfred de Vergnette de Lamotte, whom he had met to learn about wine. In 1850, de Vergnette de Lamotte published a memoir on heating wine and observed that the method resulted in much better preservation [5]. Alfred de Vergnette de Lamotte wrote that "heat makes ferments less organised". However, the word "ferment" first appeared in the 16th century (Dictionnaire de l'Académie Française), derived from the Roman

term *fermentum*, which described the result of fermentation and was not associated with the microbes themselves. The word fermentation comes from *fervere*, which described the bubbling observed in the must. In the long history of food technology, made up of experimental empiricism preceding the rational approach, Louis Pasteur was to explain the effectiveness of the method developed by Nicolas Appert and published in 1810 under the title "The Art of preserving all animal and vegetable substances for several years" [6]. A certain Balthazard Grimod de la Reynière had praised Appert's method, which he claimed made it possible to "naturalise spring and summer in the middle of the harshest winter". Appert, a confectioner by trade, had perfected the method step by step through numerous trials and experiments designed to find heating and packaging conditions that would preserve fruit, vegetables, meat and fish for several months or years without any apparent damage. Appertisation changed society and even led to the disappearance of scurvy among sailors, but remained without an irrefutable explanation for several decades. Joseph Louis Gay-Lussac, a physicist and chemist, studied the process and postulated a reduction in oxygen content and gas pressure in the container to explain the improved preservation, whereas Appert attributed its effectiveness to the action of heat on mould. Appert's method was largely based on the destructive effect of heat on microbes, and Louis Pasteur was probably the first to understand this. Similarly, machines for heating wine to preserve it or ferment it properly were already being made, and Louis Pasteur was also aware of them. His knowledge of the microbes that needed to be destroyed by heat in order to produce disease-free wine enabled him to find the ideal conditions for stabilising wine while altering it as little as possible. The microbiological techniques developed by Louis Pasteur gave him a great advantage in perfecting and optimising his process. He showed that by heating wine to a temperature of between 60 and 100°C, away from the air, the wine did not ferment irregularly and retained an acceptable taste. The history of pasteurisation is the story of a compromise between destroying undesirable microbes and preserving the treated foodstuff, its colour, taste and smell. The partial sterilisation process to which the name "pasteurisation" was later attributed was born, and Pasteur presented it to the Académie des Sciences on 1 May 1865 in a note entitled "A practical process for preserving and improving wines" [7]. He had previously applied for a patent to cover the process for fifteen years. However, Louis Pasteur had to fight hard to claim the originality of his invention in line with earlier work. His patent for the invention of a process for preserving wine dates from 11 April 1865 and he described its originality and scope in the following terms: "I have recognised that diseases or spontaneous alterations in wines are produced by microscopic beings, the germs of which exist in the wine before it becomes diseased. Wine does not deteriorate if these germs are killed. A simple and practical way of killing these germs is to heat the wine to between 60 and 100°C. I declare that I have taken out a patent for the application of this process. It prevents all irregular fermentations in wines, whatever their nature, without altering the quality of the wine". In Lille, he became interested in beer, and helped develop methods to guarantee its quality and that of the must. As with wine, Pasteur showed that microbes were responsible for beer diseases and that heat could stop their harmful effects. Pasteur's method became universal in scope, inspiring almost two centuries of scientific and technological development to improve the quality of foodstuffs and many other products. His process was even better suited to other foods, and was only used for a short time on wine, where other processes altered the taste even less. In fact, the process also killed off good microbes, which inhibited another natural fermentation that occurs in wine as it ages. This fermentation transforms malic acid into lactic acid and softens the acidic perception of the wine. Heating the wine was therefore gradually replaced by a combination of rigorous hygiene practices and measures to control microbial activity. Controlling temperature, acidity and oxidation-reduction potential during winemaking inhibits the proliferation of the main disease-causing microbes, while encouraging the persistence and activity of the 'good' microbes, which are not limited to the yeast observed by Pasteur. After Louis Pasteur's death, other scientific advances showed that certain microbes can adopt two main states, the vegetative state and the spore-forming state. By studying wine, beer and vinegar, Louis Pasteur avoided the development of active bacterial spores, which would have complicated the development of his preservation method. Bacterial spores are highly resistant to heat and their inactivation requires higher temperatures and longer application times than for vegetative forms. So, for unfermented foods without a high alcohol or organic acid content, which may be naturally contaminated by bacterial spores, the heat treatment invented by Louis Pasteur would not have been sufficient. Bacterial spores were studied by Cohn (1876), who was trying to understand why 100°C was not enough to kill bacteria in hay infusions, whereas much lower temperatures were enough to kill other living organisms [8]. He observed bacteria in this infusion and gradually saw refractive bodies appear. He thus described for the first time the process of sporulation of certain bacteria. In other experiments, he observed that the refractive bodies could take the form of bacteria when they germinated, provided they were transferred to a fresh infusion of hay. His work led him to identify two key phases in the behaviour of spore-forming bacteria: spore formation and germination. By observing the sporulation of anthrax and other spores in cultures under a microscope, Koch in 1876 was able to describe for the first time their complete life cycle : sporulation - germination - multiplication - new sporulation [9]. These scientific advances led us to consider the possibility of spores forming or germinating within foods. A major development in the pasteurisation process was therefore required to eliminate all microbial forms, because one of the essential characteristics of spores is that they are highly resistant to heat and adverse conditions. In 1875, John Tyndall sterilised beef, lamb and hay broths by boiling them, and compared what happened when he placed the different broths in purified air and in ordinary air. The broths placed in pure air remained without apparent change for several months, while those placed in ordinary air became putrid after a few days [10]. This result extended Louis Pasteur's earlier demonstrations that the presence of micro-organisms was a prerequisite for fermentation and decomposition. However, within a year, some heated broths rotted on contact with purified air [10]. Tyndall deduced from this the presence of bacterial spores in broths supposedly sterilised by heat, since a spore can survive boiling. His work led him to develop a method of eradicating spore-forming bacteria based on the life cycle of sporeforming bacteria [11]. This method, known as Tyndallisation, consisted of a cyclic treatment spread over several days that allowed the spores to germinate and adopt their heat-sensitive form.

From hot water baths to continuous heat exchangers

Following Louis Pasteur's invention, thermal processes underwent a major boom, with numerous technical developments arising from engineering and process research. Louis Pasteur himself saw himself as a player in this technological progress, writing: "Several people have kindly communicated to me their ideas on various processes. [...] I believe that everyone will do well to put to the test the device he has imagined, so that experience may judge in the last resort". [12]. Technological developments consist of intensifying the heat transfer potential of machines to achieve heat treatments compatible with large production volumes. For wine treatment alone, a number of devices have been developed to create the temperature conditions needed to destroy spoilage microbes, and these will undergo rapid technical development (Figure 3). In early technologies, wine bottles or even casks were placed directly in a bath of hot water or steam. These processes for treating wine in closed containers are slow and not very productive. Continuous processes have been developed that allow the wine to be heated as it is transferred from barrel to barrel, or from barrel to bottle, as it circulates through a pipe.



Figure 3. Different devices used for heating wine. A : Pasteurisation method, developed by Louis Pasteur around 1870, for heating by steam or hot air and for preserving wines in bottles stacked in an oven (fig.42, p. 271, in Œuvres de Pasteur réunis par Pasteur Valléry-Radot, tome 3, études sur le vin, ed. 1924, Paris). B : Heating wine in barrels to a temperature of about 60° for about ten minutes, in order to destroy microbes without damaging the taste of the wine (fig. 42, 167, in Etudes sur le vin, ed. Victor masson et fils, 1866). C : Apparatus by M. Sourdeval (fig.50, p. 293, in Œuvres de Pasteur réunies par Pasteur Valléry-Radot, tome 3, études sur le vin, ed. 1924, Paris). D : Apparatus of M. de Lapparent. Heating 1 hectolitre per half hour (fig. 51, p. 295 in Œuvres de Pasteur réunies par Pasteur Valléry-Radot, tome 3, in Etudes sur le vin, ed. 1924, Paris). E : Apparatus installed in Toulon by Monsieur de Lapparent. Heating 40 hectolitres per hour (fig.49, p. 291, in Œuvres de Pasteur réunies par Pasteur Valléry-Radot, tome 3, études sur le vin, ed. 1924, Paris). Credits : Institut Pasteur

Heat is transferred through the walls of the pipes or ducts. The principle of circulating through immersed pipes increases the heat transfer surface area in relation to the volume contained. The circulation of the wine in the pipe also agitates it, facilitating convective heat transfer. These machines enable more intense heat transfer and facilitate rapid temperature rises. Louis Pasteur himself worked closely with engineers to improve the efficiency of these processes. He commented on a large-scale trial : "We heated 650 hectolitres in two days. The speed of the operation lends itself to the largest and fastest supplies. These 650 hectolitres will be sent to the west coast of Africa (where our sailors hardly drink anything but vinegar) along with 50

hectolitres of the same unheated wine. If the trial succeeds, that is, if the 650 hectolitres (65000 liters) arrive and can stay without deterioration, and the 50 hectolitres deteriorate, which I do not doubt on the basis of my laboratory experiments, the question will be resolved and, in the future, all the navy's wine will be insured against disease by prior heating." [12]. So, in the last decades of the 19th century, process engineering set to work to develop efficient, rapid and cost-effective solutions. The different heating processes developed, whether discontinuous or continuous, had different effects on the quality of the wine, just as the method of heat treatment of the milk or fruit juices we consume today affects the colour and taste quality. Technology will gradually make it possible for heating machines to produce temperatures in excess of 100°C, and to destroy all forms of microbes in food, including spores.

The death of microbes depends on both temperature and time

In the wake of Louis Pasteur's pioneering work, microbiologists had highlighted the link between temperature and the time it is applied to destroy microbes. At virtually the same time, chemists demonstrated that heat had effects on molecules and that these effects were also linked to the time of application and the temperature level. The denaturation of certain constituent molecules of foodstuffs during pasteurisation - vitamins, pigmentary or aromatic compounds degrades their nutritional and sensory quality.



Figure 4 : Main changes observed after heat treatment of water-soluble soy extract : inactivation of 90% (pink) and 99.999% (red) of *Clostridium sporogenes*, limits of acceptable changes in colour (orange) and flavour (green). Adapted from [13]

Comparison of the kinetics of the degradation reactions of microbes and molecules shows that these entities do not react at the same rate to treatments (Figure 4). This difference makes it possible to define heat treatment conditions that are lethal for microorganisms and nondestructive for food molecules. These conditions correspond to temperatures in excess of 120°C and sometimes approaching 150°C. At these temperatures, treatment times can be reduced to hundred (temperature between 120 and 125°C) or dozen (temperature over 140°C) seconds. It should be noted that treatments carried out by boiling water at atmospheric pressure, i.e. at a maximum of 100°C, degrade the sensory quality without necessarily destroying the microorganisms. However, for a long time to come, the main difficulty will lie in designing machines and processes that can heat food almost instantaneously to temperatures well above the boiling point of water at atmospheric pressure, and then cool it just as quickly.

Processes to kill only microbes and all microbes

The work of Joseph Fourier (1768-1830) and Jean-Baptiste Biot (1774-1862) was decisive at the time, as they respectively established the physical law that describes heat flow between two

systems of different temperatures and solved the resulting multifactorial equations. Applied to pasteurisation or sterilisation machines, these laws give rise to equations containing the variables that influence the rate of temperature change in a stirred heated or cooled system (Figure 5).



Figure 5 : Equation describing the evolution over time (t) of the temperature (T) in a container as a function of the heating temperature (T_{ext}) and the initial temperature of the product (T_0). U is the overall heat transfer coefficient and depends on convection on the outside of the container (h_{ext}), convection on the inside (hint), the thickness of the container wall (e) and the thermal conductivity of the wall material (k). The surface area of the wall (A) and the volume (V) of the container, as well as the density (ρ) and heat capacity (Cp) of the contents of the container, influence the change in temperature over time.

Machines developed on this theoretical basis are characterised by very large heat transfer surfaces in relation to their volume. They frequently use water vapour as a heat transfer fluid, as its condensation releases colossal amounts of energy, which is then transferred by convection. To ensure that the heat progresses very quickly through the thickness of the product being treated, the flows are organised in very thin layers, circulating, for example, between two plates or in thin pipes. Finally, when they operate continuously (circulation of the product to be heated or cooled between an inlet and an outlet), these machines are highly energy-efficient, since the heat contained in the heated product is recovered to preheat the product entering the appliance. To transfer heat even more quickly, other heat sources have also been implemented (Figure 6). Pasteurisation and, by extension, sterilisation by heating with a heat transfer fluid are still the most widely used techniques today for guaranteeing the preservation and safety of food and drink.



Figure 6 : Chronology of the development of food preservation heat treatment processes in the 19th and 20th centuries.

Pasteurisation in the 21st century

The end of the 20th century and the beginning of the 21st saw the emergence of new concepts with the discovery of the extraordinary biodiversity, ubiquity and complexity of the microbial world. Since Louis Pasteur, we have become aware of the presence of microorganisms that are harmless and even beneficial in protecting the living organisms they colonise. Science has also revealed the impact of agri-food activities on health, the environment and the climate. Progress in food production and processing processes and technologies is therefore expected. The main challenges are to reduce the use of fossil energy sources, and to reduce the use of pollutants that are toxic for the environment and living beings. The idea of using natural, bio-inspired processes to process and preserve food has developed. This approach involves, for example, using certain micro-organisms as preservatives [14]. In this context, the future disappearance of the microbial heat treatment invented by Louis Pasteur could be imagined... and yet it has perhaps never been so popular. Thus, "flash pasteurization" at 74°C is a saving process, authorized since 2021, to help stabilize organic wines with low sulfur content. Another area of

application for pasteurization is also emerging and concerns the inactivation of good microbes such as probiotics. Indeed, certain positive health effects of these "good microbes" could be explained by the molecular entities that constitute them. These compounds are the foundation of the emerging post-biotic era [15] for which it will probably be necessary to develop extraction processes by disrupting microbial membranes while preserving the molecular structures of these active constituents. In line with thermal processes for inactivating viruses to make vaccines, a pasteurization technology for "good microbes" would thus see the light of day.

To conclude this text dedicated to the essential contribution of Louis Pasteur and his scientific approach to the evolution of food quality, it is interesting to reread an article that he published in 1868 and in which he gives his own idea of science : "No, a thousand times no, there does not exist a category of sciences to which we can give the name of applied sciences. There is science and the applications of science, linked together like the fruit to the tree that bore it. » [16]. Louis Pasteur's approach remains irreplaceable and is the driving force behind technological advances in food preservation for his many scientific descendants. It is therefore very probably at the fundamental roots of processes and more particularly the microbial Achilles heels that new means of food preservation will be born that respond to modern health and environmental challenges.

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