Chapter

Emerging Technologies to Increase Extraction, Control Microorganisms, and Reduce SO_2

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Abstract

This chapter reviews the main non-thermal technologies with application in enology and their impact in: the extraction of phenolic compounds from grapes, the elimination of indigenous microorganisms, and the subsequent effect in SO_2 reduction. The technologies are physical processes with null or low repercussion in temperature and therefore gentle with sensory quality of grapes. High hydrostatic pressure (HHP), ultra high pressure homogenization (UHPH), pulsed electric fields (PEFs), electron-beam irradiation (eBeam), ultrasound (US), and pulsed light (PL) have interesting advantages and some drawbacks that are extensively reviewed highlighting the potential applications in current technology.

Keywords: non-thermal technologies, wine, grape, HHP, UHPH, PEF, eBeam, ultrasound, PL

1. Emerging non-thermal technologies for grape and must processing

Traditionally, must extraction for winemaking is different in whites and reds. White grapes are pressed and optionally destemmed, especially if they are going to be cold soaked. Later, the grape juice is cleaned by settling before fermentation (**Figure 1**). Red wines need maceration to extract polyphenolic compounds (tannins and pigments). Consequently, the grapes are crushed and later simultaneously macerated and fermented in a tank for several days until a suitable polyphenolic content is reached (**Figure 1**). Emerging non-thermal technologies can help to speed up the extraction of phenolic compounds and aromatic molecules from skins, to eliminate or reduce wild microorganisms from grapes thus facilitating new fermentation biotechnologies, and some of them can also destroy or reduce the activity of oxidative enzymes [1–3]. Since the use of non-thermal technologies does not increase significantly the temperature, usually, their application does not affect negatively the sensory quality of the wine. Additionally, the elimination of microorganisms and control of enzymes helps to reduce the SO_2 content in wines [1].

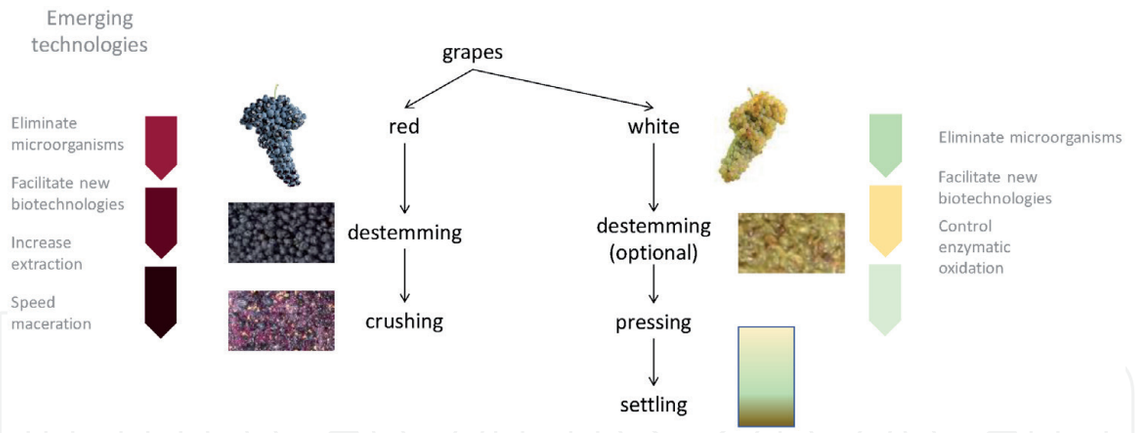

Figure 1.
Red and white winemaking and main steps where to apply emerging technologies and potential advantages.

The emerging technologies that showed the greatest potential for increasing the extraction of skin compounds and controlling indigenous microorganisms are as follows: high hydrostatic pressure (HHP) [4–6], ultra high pressure homogenization (UHPH) [7–9], pulsed electric fields (PEFs) [10–15], electron-beam irradiation (eBeam) [16–18], ultrasound (US) [15, 19–20], and pulsed light (PL) [21–23]. Most of these technologies are either approved or under evaluation as new enological practices in the International Organization of Vine and Wine (OIV) regulations [24–26]. The treatment conditions and the most significant effects on microbial loads, grape extraction, and enzyme inactivation are described in **Table 1**.

Wine freshness is a complex sensory perception in which acidity is strongly involved, but it is also represented by a certain aromatic profile in which floral and fruity esters predominate, as well as a bluish red color in young wines with no oxidation or a pale yellow color in white wines without browning [29–30]. The acidity makes the wines to be perceived more refreshing and this is currently well appreciated by consumers. Moreover, fresh aromas such as citric, fruity or floral add complexity and elegance to the wine. New emerging non-thermal technologies can eliminate or strongly reduce the wild microorganisms in grapes, thus facilitating the use of new biotechnologies such as fermentation with non-*Saccharomyces* yeasts which help modulate acidity and aroma [29–30]. Currently, acidity can be increased in several ways; one efficient way is by producing lactic acid during alcoholic fermentation with the non-*Saccharomyces* yeast *Lachancea thermotolerans* (Lt) [31–35]. The use of Lt during fermentation allows reducing pH values by 0.2–0.5 units, producing lactic acid from sugars and without significant side effects [34–35]. Moreover, some strains can also have positive sensory impacts by producing fruity or floral esters [34]. Concerning fruity or floral aroma, several non-*Saccharomyces* as *Torulaspora delbrueckii* (Td), *Wickerhamomyces anomalus* (Wa), *Metschnikowia pulcherrima* (Mp), *Hanseniaspora vineae* (Hv), and *Hanseniaspora/Kloeckera* spp., *Lachancea thermotolerans* or *Candida stellata* (Cs) have demonstrated their ability to modulate or influence wine aroma during fermentation by producing fermentation esters or by developing enzymatic activities that release varietal precursors of aromatic molecules [30]. Some of these species are also able to produce stable pyranoanthocyanins or promote the formation of polymeric pigments, thus improving and stabilizing wine color [36–39]. Among them, *Schizosaccharomyces pombe* (Sp) has interesting properties such as the formation of vitisin A-type pyranoanthocyanins because of its high production of pyruvate.

The use of non-*Saccharomyces* yeasts has the main drawback of being normally little competitive against *Saccharomyces*, so their successful implantation in the must is not easy and they cannot express their metabolic properties or enzymatic

Technology	Microbial inactivation	Extraction of anthocyanins	Extraction of polyphenols	Inactivation of oxidative enzymes	Processing flow and temperature	Reference
HHP	Yeast elimination: 400 MPa/10 min Lactic acid bacteria resistance even at 550 MPa	Increased	Improved	unclear	Until 3 t/h Room temperature or even refrigeration	[4–6, 27]
UHPH (EU Patent)	300 MPa/continuously yeast and bacteria elimination. Sporulated bacteria depending on in-valve temperature	Increased	Not described	>90%	Available 10,000 L/h pumps, higher rates in multi-modular systems. 20–25°C. In valve 98°C less than 0.2 s	[8, 28]
PEF	Depending on dose until 5 log cycles of bacteria not sporulated	4–10 kV/cm Increased 10–20%	4–10 kV/cm Increased 10–20%	Not described	Extraction: Flow 20 t/h Specific energy <10 kJ/kg ΔT^a < 3°C Inactivation: until 2000 L/h Specific energy >50 kJ/kg 20–25°C for less than 1 s	[11–15]
eBeam	1–2-log reductions for yeast and bacteria with 1 kGray, and elimination with 10 kGray	70% higher when 10 KGray dose is used	10–20% with 10 kGray	Not described	Increase 3–5°C at 10 kGray	[16]
US	Low effectivity, at high intensity thermal effects	Treatment and 3 days of maceration achieved similar contents to 8 days of conventional maceration	Treatment and 3 days of maceration yielded double content compared to 8 days of conventional maceration	Not described	ΔT^a: 2–3°C	[19–20]
PL	Effective against yeast and bacteria. 1–3-log reductions	Slightly higher, but some final degradation due to oxidizing effects	Not described	Not described	ΔT^a: 2–3°C	[21]

Table 1.
Main features and effects of the emerging non-thermal technologies used in grape processing.

activities. Most non-*Saccharomyces* usually have lower fermentative power than *S. cerevisiae* or slower fermentation kinetics (e.g., Sp), which reduces the possibilities of being well implanted [40]. New emerging non-thermal technologies facilitate the use of non-*Saccharomyces* yeasts by eliminating or strongly reducing wild yeasts from the grapes [2, 6, 8, 14, 16, 21].

2. High hydrostatic pressure (HHP)

HHP was developed at an industrial scale in the last century by Gec Alston Company in Europe, even when their basis and initial prototypes where defined by Hite in 1899 [41]. Technological difficulties delayed the production of industrial devices able to work at industrial scale until the 80s of XX century. HHP technique uses the hydrostatic pressure transmitted by a liquid, usually water, to pressurize a liquid or solid food in order to eliminate microorganisms or modify some food properties (**Figure 2**). In HHP systems, the pressures are applied by a liquid and therefore homogeneous pressurization is produced on the whole surface of the food. The low-pressure pump is used to quickly fill the vessel with water. It is important to reach a high filling ratio to reduce the volume of water necessary and therefore decrease the dead-times. When air is removed and the vessel is completely filled with water, the secondary high pressure pump is used to continue introducing water to increase the pressure. It is normally necessary to introduce an additional 4% volume of water to reach the working pressures of 400–600 MPa needed to eliminate the microorganisms.

Typical pressure ranges to eliminate microorganisms by HHP are 300–400 MPa for yeasts and molds, 400 MPa for Gram-negative bacteria, and 500–600 MPa for Gram-positive during processing times of 3–10 min [42]. Sporulated bacteria cannot be controlled by HHP because pressures around 1000 MPa are needed, which is not feasible for industrial equipment. The application of 200 MPa for 10 min on the grapes can decrease the indigenous yeast populations, with 400 MPa often being enough to eliminate the yeasts from the grapes [5–6]. However, even the treatment of 550 MPa for 10 min may not be able to completely eliminate bacterial populations [5]. The elimination of indigenous yeasts facilitates the implantation of inoculated non-*Saccharomyces* yeasts and the better expression of their metabolic activities [6]. Furthermore, the elimination of both wild yeasts and bacteria helps to reduce the levels of sulfites needed for their control.

HHP can be considered a non-thermal technology because the adiabatic compression heat is around 2–3°C/100 MPa, so pressurization at 500 MPa increases

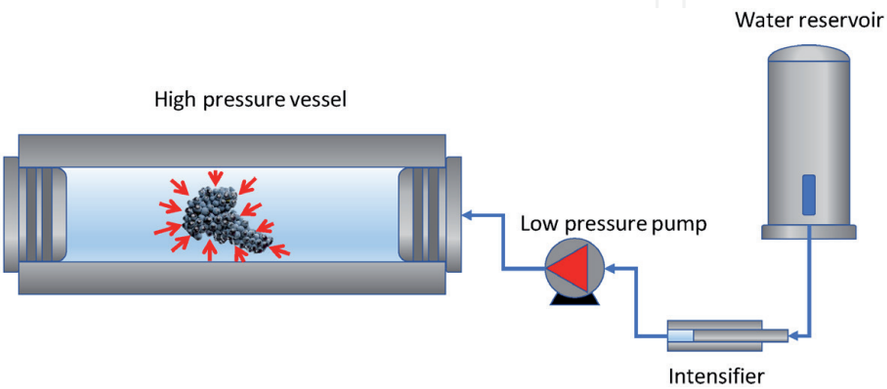

Figure 2.
High hydrostatic pressure system. The pressure vessel is filled with water by means of a low-pressure, high flow rate pump and then increased to working pressure by an intensifier.

the temperature by only 10–15°C. In addition, heat exchangers are incorporated in the pressurization vessel to either refrigerate or to apply thermo-pressurization. Moreover, pressure forces are unable to modify covalent bonds, so most of the molecules responsible for sensory properties (pigments, aromas and flavor substances) remain unaffected during HHP processing. Therefore, HHP can be considered a gentle technique in terms of preserving sensory quality. Moreover, even when grapes are pressurized with intense treatments (500–600 MPa), no heat treatment markers such as Maillard compounds are observed in the HHP-processed musts.

The antimicrobial effect of pressure mainly affects the microbial envelopes: cell wall, cell membrane, and nuclear membrane in yeasts. When microorganisms are pressurized and subsequently returned to atmospheric pressure, all these microbial structures are affected by the poration or completely broken, making the cells non-viable. At the same time, foods are unaffected or slightly affected by their texture and appearance. The pressure only produces a homogeneous volume reduction that can be quantified as 4% and the external appearance of the grapes remains unaltered even at pressures of 550 MPa (**Figure 3**).

The external aspect does not show the effect of the HHP processing but on a molecular scale both membranes and cell wall are altered, causing the migration of pigments and phenols from the grape skin to the pulp (**Figure 4**). Even anthocyanin staining of seeds is observed after HHP treatment [5]. This migration of phenolic compounds produces a faster extraction of these molecules and probably of the aromatic precursors from the skins to the pulp, facilitating grape maceration. Anthocyanin extraction may increase by 20–80% in HHP-processed grapes compared to controls [4, 5], and the wines produced have a significantly higher color intensity and tannin content [5].

HHP processing can be used as a gentle technique to control microorganisms in grapes, thus facilitating the implantation of inoculated starters, especially when they have weaker performance than *S. cerevisiae*. In addition, the intense effect on cell walls helps to increase extraction by reducing conventional maceration times in red winemaking [3].

Currently, industrial devices equipped with horizontal vessels of 55–525 L are available for HHP, able to work up to 600 MPa, and to process up to 3000 kg/h [27]. To reduce dead-times, HHP machines are equipped with a high number of intensifiers: 4–16 depending on the vessel capacity [27, 43]. The most important companies in HHP technology are Hiperbaric [27] and Avure [43].

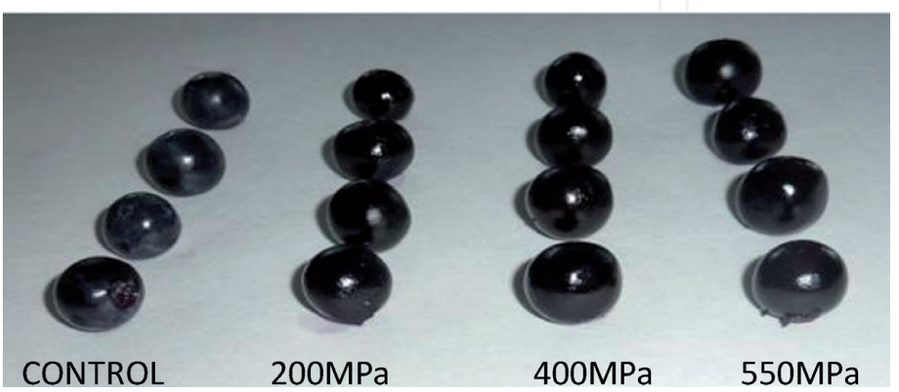

Figure 3.
External appearance of Vitis vinifera *L. cv. Tempranillo grapes processed by HHP at 200, 400, and 550 MPa for 10 min.*

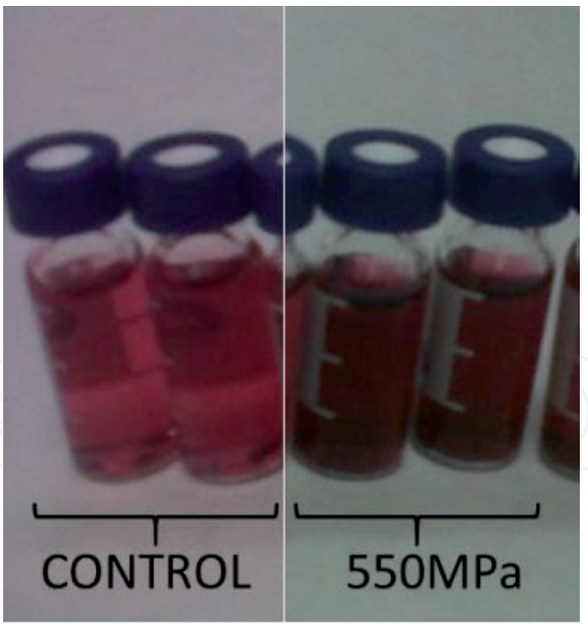

Figure 4.
Color of the grape juice extracted without maceration: unprocessed (control) or pressurized at 550 MPa for 10 min.

3. Ultra high pressure homogenization (UHPH)

High pressure homogenization (HPH) is a continuous treatment in which a food liquid is pumped at high pressure and later depressurized when the fluid passes through a special valve. It is normally called HPH when pressurization occurs at 100–200 MPa (**Figure 5A**) and ultra high pressure homogenization (UHPH) when the pressure range is higher than 200 MPa (**Figure 5B**) [7, 9].

The "UHPH sterilization system" is a novel process, patented in Europe (EP2409583B1) by UAB (Autonomous University of Barcelona), extended to a lot of countries and exclusively exploited and manufactured by Ypsicon Advanced

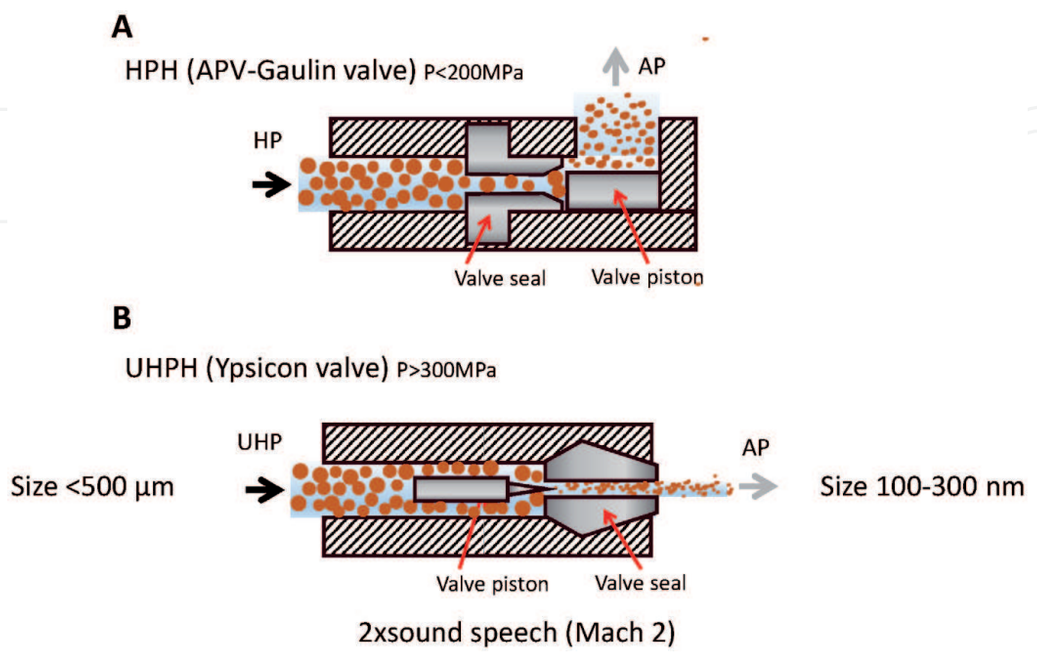

Figure 5.
Valve components in HPH (A) and UHPH (B) systems (Ypsicon [28]). HP: high pressure, UHP: ultra high pressure, AP: atmospheric pressure.

Technologies (Barcelona, Spain) (www.ypsicon.com). It consists of a continuous device capable of working from 200 to 400 MPa and applying shear, impact, cavitation, and turbulence forces in a special valve at high speed (Mach 2). As a consequence, the particles size is reduced from 100 to 300 nm, microorganisms are destroyed, enzymes inactivated and stable emulsions are produced without additives, and consuming less energy than thermal treatments.

UHPH is highly efficient in controlling microorganisms. The antimicrobial effect is produced by the strong impact forces together with the shear stresses and the complementary effect of local cavitation and friction [7–9]. This process produces intense heating in the in-valve time but during a really short period of time of 0.02 and 0.2 s for the global residence time. The temperature in the valve can reach 100°C when the inlet temperature is 20°C, being reduced to 25°C after the valve [8] by adiabatic-expansion chilling. Even when high temperatures are reached instantaneously in the valve, as a whole it can be considered a gentle technology with no thermal effect on sensory degradation. After the UHPH process, no formation of thermal markers such as furfural or 5-hydroxymethylfurfural is observed, probably because of the very low residence time. UHPH processing using suitable in-valve temperatures can produce sterilization capable of destroying even sporulated bacteria. Because of this feature, it can be considered a gentle alternative to UHT, since in this thermal technique a temperature of 140°C for 3–4 s is necessary. In contrast, UHPH only requires a total processing time of less than 0.2 s.

UHPH can be applied to liquids containing particles, but the particle size must be lower than 500 μm. The average particle size range at the valve outlet is 100–300 nm. The valve design is a critical point, as well as the performance of the UHPH process, but especially the antimicrobial effect depends on the geometry and materials of the valve piston and seal. Especially, efficient designs are manufactured by Ypsicon [44] (**Figure 6**). UHPH systems are currently available with flow rates of up to 10,000 L/h [28]. The processing rate can be increased in a modular way by using several systems working in parallel. Some UHPH pumps can work up to 400 MPa continuously with a pressure oscillation of 1 MPa.

Concerning the elimination of microorganisms, UHPH has proven to be highly effective with 6-log reductions for *Saccharomyces* and non-*Saccharomyces* yeasts

Figure 6.
UHPH industrial machine (Ypsicon [28]).

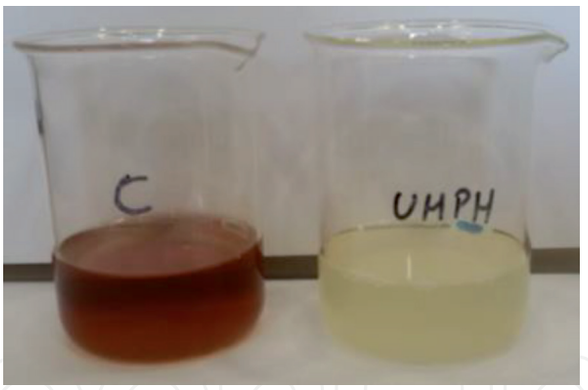

Figure 7.
Color evolution in the control and UHPH musts after 2 days at room temperature in the absence of SO_2. White must of Vitis vinifera *L. cv. Muscat.*

and 4-log for aerobic and lactic acid bacteria in the must [8]. All these wild microorganisms remained undetected in the must after UHPH processing at 300 MPa (inlet temperature 20°C, in-valve 98°C, outlet 26°C, and in-valve time 0.02 s) [8]. Therefore, UHPH is a powerful technology for eliminating indigenous microorganisms and facilitating the use of modern biotechnologies, such as the use of non-*Saccharomyces* or yeast-bacteria co-inoculations [2]. Simultaneously, microbial control facilitates the reduction of SO_2 content.

UHPH technology has also shown high efficiency in enzyme destruction. The intense impact and shear forces that the fluid undergoes when pumped through the valve produce a molecular depolymerization that reduces colloidal particles, microorganisms, and enzymes to small fragments. In the case of cells and spores, it causes microbial death, and in the case of enzymes, it causes denaturalization and inactivation. In musts processed by UHPH, a reduction in oxidase activity higher than 90% has been observed for polyphenol oxidase (PPO) enzymes [8]. In addition, the fragmentation effect of colloidal particles can increase the nutrient availability for alcoholic fermentation, which can have positive impact on the production of fermentative aroma [8].

When measuring the color intensity in white grape musts, the value was lower in UHPH than in the unprocessed controls. This is an indication of a paler color that can be correlated with low oxidation by PPO enzymes [8]. The same results have been observed when white musts were kept at room temperature under oxidation conditions and without SO_2: the UHPH musts remained pale and the controls quickly browned (**Figure 7**). UHPH is a key technology for reducing sulfites in must by controlling oxidative enzymatic activities which lead to browning and aroma degradation.

4. Pulsed electric fields (PEFs)

Pulsed electric fields (PEFs) are a non-thermal technology with a high potential in the extraction of phenolic and aromatic compounds from grapes [11–13] and also in the elimination of microorganisms [10, 14]. This technology uses high intensity voltages (10–40 kV) producing strong electric fields of 1–30 kV/cm. However, it can be considered a non-thermal technology because these high intensity fields are applied in ultra-short periods of a few microseconds which usually produce a temperature increase of only a few degrees Celsius.

Typical pulses are produced as bipolar square waves because of their higher efficiency compared to other types of waves. Exponential decay waves can also be used, but the energy transferred and the effectiveness of electroporation is lower for the same field intensity.

PEFs are applied to food products in a treatment chamber using two electrodes built with inert metals. The location of the electrodes is usually in two consecutives sections of the pipeline separated by an isolating section. In this design, the electrodes from the treatment chamber, keeping the same diameter of the pipeline through which the crushed grapes are pumped. The force lines of the electric field are tangential to the flow direction.

The effect of PEFs on the cell is the electroporation which increases the permeability of the cell membrane in microorganisms and plant cells. This increase in permeability is due to the formation of pores. The electric field strength required to produce the electroporation depends on the size of the cells, with intensities below 10 kV/cm and specific energies below 10 kJ/kg being sufficient to produce this effect in grape skin cells. However, higher electric fields (>10 kV/cm) and specific energies (>50 kJ/kg) are required for the electroporation of microbial cells [12].

In wine technology, electroporation is a powerful tool to increase the extraction of pigments and tannins, thus reducing maceration times (**Figure 8**). The skin contact time when crushed grapes are processed by PEFs can be reduced by 2–3 times in comparison with the unprocessed grapes, also allowing to finish the fermentation in the absence of solids which allows a cleaner and more controlled fermentation. The low temperature increase protects the aroma compounds and facilitates the preservation of the varietal aroma.

Moreover, PEF can be applied continuously to the crushed grapes during the pumping from the crusher to the tank (**Figure 9**). Industrial devices can process more than 10 t/h. PEF industrial treatments decrease maceration times, thus increasing the availability of the fermentation facilities. In addition, the equipment is moderate in size, it requires little space in the winery, and the energy inputs required are low compared to other traditional techniques.

The use of PEFs to control microorganisms in grapes needs higher field intensities due to the smaller size of the microbial cells [45–46]. These field intensities produce an inactivation ranging from 0.6 to 4.94 log cycles for several wine spoilage yeasts and lactic acid bacteria [46]. PEFs can be used as a powerful non-thermal tool to control indigenous microorganisms, thus helping to decrease SO_2 levels.

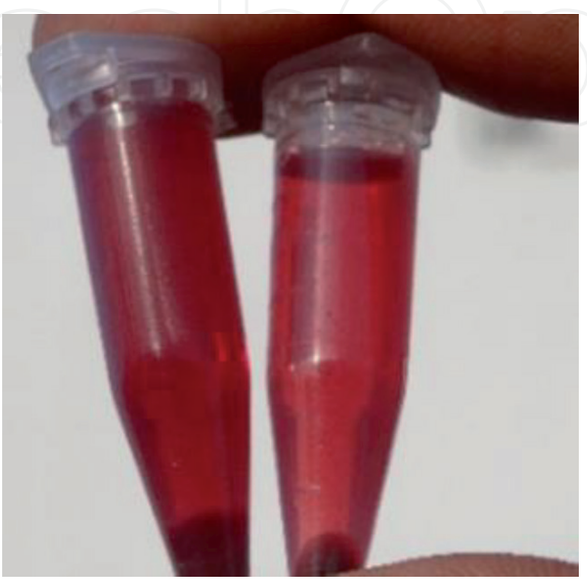

Figure 8.
*Color intensity and degree of pigment extraction in musts obtained from untreated (right) and PEF-treated (left) grapes (*V. vinifera *L. cv. Garnacha) after 1 h of maceration.*

Figure 9.
PEF experimental unit to process the crushed grapes at a flow of 3 t/h to improve the polyphenols extraction in the maceration-fermentation stage of red winemaking.

5. eBeam or β-irradiation

Electron-beam irradiation or beta-irradiation is a technology that uses low-dose ionizing radiation to eliminate microorganisms in food and also to delay ripening by extending shelf-life. eBeam irradiation and X-rays use ionizing radiation but they are not produced by radioactive materials. The radiation is generated by an electron accelerator that is a switch-on/off electronic technology that can be disconnected when is not in use. The electrons are generated in a heavy metal-doped cathode and then increase their speed by being accelerated by radio frequency fields in a high vacuum cavity (**Figure 10**). The maximum energy allowed is 10 MeV to avoid

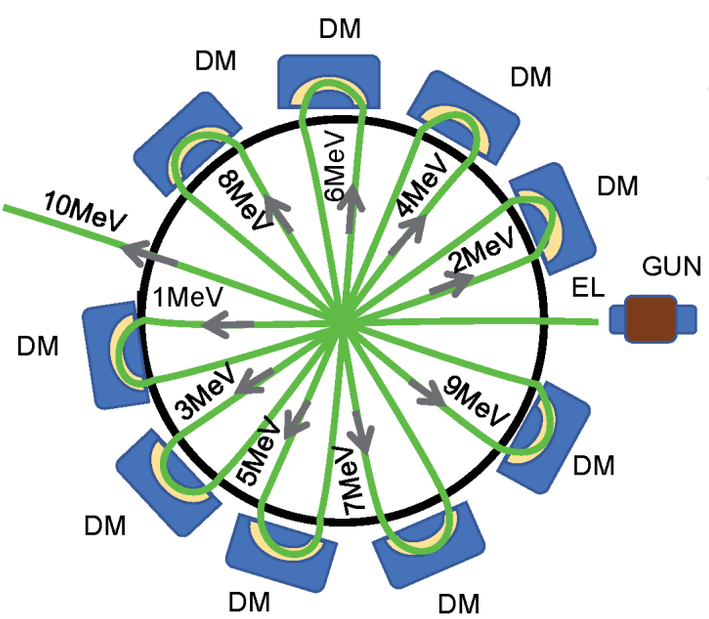

Figure 10.
Radiofrequency cavity in a rodothron™-type ebeam accelerator. GUN: low energy electron producing cathode, EL: electromagnetic lens, DM: deflector magnets.

the production of unstable nuclei in food. This maximum value is less than the 14 MeV necessary to generate radioactive isotopes. The energy of the radiation is related to its penetration power and it can be estimated as 3 mm depth in water per MeV. Therefore, 3 cm of water-like density products can be treated at 10 MeV.

The energy of 10 MeV makes the electrons capable of breaking the atomic and molecular bonds, thus generating ions and free radicals that can react with other molecules and produce secondary ions and radicals [47–48]. During food irradiation, water is mainly affected by the formation of hydroxyl ($^{\bullet}OH$), hydrogen ($H^{\bullet}$), superoxide ($HO_2^{\bullet}$) radicals or peroxide (H_2O_2) [48]. These free radicals may react with several molecules or biopolymers in the cells, including DNA, making them unviable. The irradiation dose is measured in kiloGray (1 kGy = 1000 J/kg). Food irradiation applications can be classified according to dose: low dose (<1 kGy) with disinfection applications, medium dose (1–10 kGy) with antimicrobial effect to extend shelf-life, and high dose (10–60 kGy) for sterilization purposes [48].

The dose must be checked after irradiation by locating radio-chromic dosimeters (**Figure 11**) on the treated foods in order to verify if the scheduled and applied doses correspond to actual values in the food. It is essential to check at all depths to ensure that the eBeam radiation is suitable for the entire material. Radiochromic dosimeters are formed by a transparent film, inside an aluminum envelope, containing a radiochromic pigment that is colored when is irradiated, and color intensity depends on received dose. After treatment, the irradiation dose can be measured spectrophotometrically. Additionally, a radiochromic sticker is added (**Figure 11**) to each food package to verify that it has been properly treated. It is often difficult or impossible to visually detect if foods have been treated, and this sticker is necessary to distinguish treated from untreated samples.

Irradiation can be considered a non-thermal technology with slight temperature increments of a few degrees in conventional treatments. Moreover, it can be applied in refrigerated foods. The effect on sensory quality is gentle, thus many molecules with sensory repercussion as pigments or flavor compounds remain unaffected. The oxidative effect of free radicals generated during the irradiation can produce some oxidative processes in some molecules.

When the electron beam is produced, it must scan from left to right to generate a treatment surface. Electron beam must move at high frequency, usually 100 Hz, to

Figure 11.
Grape sample with both, radiochromic dosimeter and sticker.

treat each irradiated section several times in a second. The electron beam is moved by using intense electromagnetic fields. When the food is moved below the treatment plane, all food volume is irradiated. The received dose depends on the speed at which the food moves below the irradiation section that is controlled by using a belt conveyor (**Figure 12**).

The external appearance of foods after irradiation frequently remains unaffected; sometimes, a brighter outer aspect can be observed (**Figure 13A**) [16]. Irradiation doses of 0.5–1 kGy produce 1 log reductions in yeast and lactic acid bacteria in grapes [16] without modifications in the external appearance and firmness [16]; similar effects have been observed in other fruits [49–50]. Irradiation dose of 10 kGy produces 6-log reductions in yeasts and 3-log in bacteria [16]; this dose decreases firmness and softens the texture of grapes, thus enhancing the extraction of pigments in juice (**Figure 13B**) [16].

Irradiation can also be considered non-thermal technology with high efficiency to eliminate indigenous microorganisms from grapes. This reduction in the population of microorganisms allows a better implantation of selected yeasts and the reduction in SO_2 doses. Depending on the irradiation dose (10 kGy), better

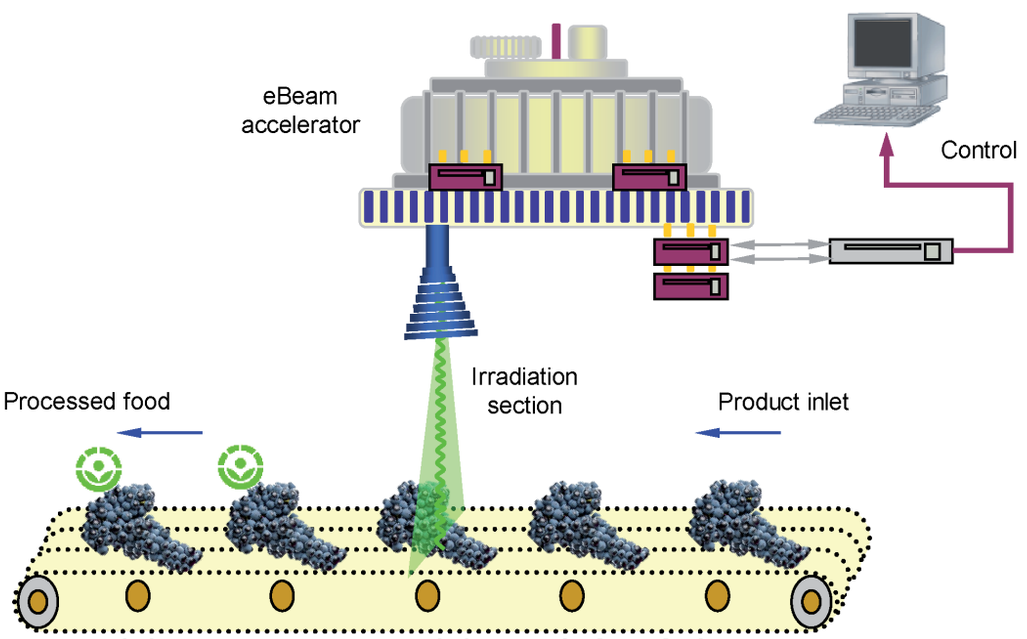

Figure 12.
Electron beam accelerator and detail of the irradiation process.

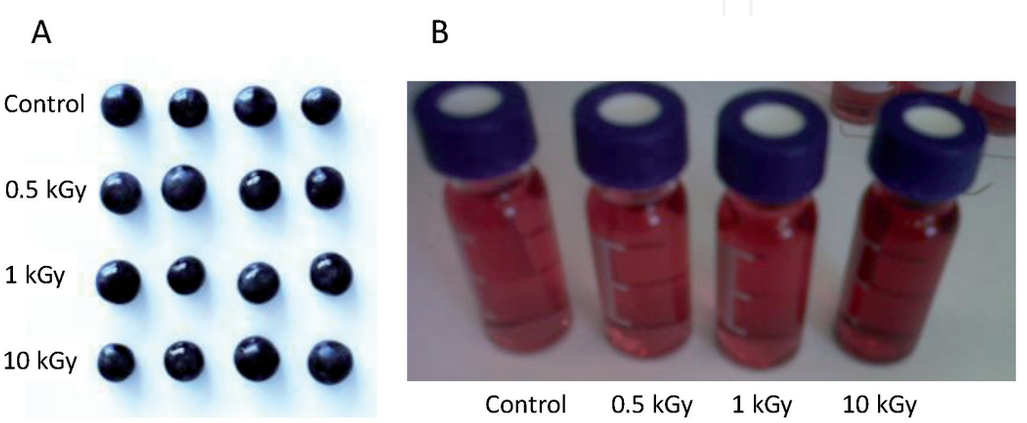

Figure 13.
(A) External appearance of grapes irradiated at 0.5, 1, and 10 kGy. (B) Color of juice from the irradiated grapes.

extraction of pigments and polyphenols can be observed with subsequent improvements on the maceration processes.

6. Ultrasound

Ultrasound (US) is sonic waves with a frequency range of 20–100 kHz producing cavitation phenomena with locally extreme temperature and pressure. Cavitation is generated by compression-decompression cycles producing the formation and implosion of gas bubbles [51] (**Figure 14**). This phenomenon produces intense agitation and dispersive effects that help to disrupt vegetal tissues, depolymerizing cell walls and favoring a better extraction. US efficiency in extraction processes improves when the frequency is closed to 30 kHz [52].

US technology can be used in winemaking for continuous processing of crushed grapes. As a consequence, there is a weakening of the cell wall and an increase in the extraction of tannins, pigments, and aromatic compounds [19–20, 52, 15]. This effect can also be enhanced with the use of pectolytic enzymes [53]. When enzymes are used as the sole extraction technique, tannin concentration is 13% higher while, after US treatment, there is an increase of 16%. The initial use of enzymes followed by the subsequent application of US is especially synergistic increasing color intensity (18%) and tannin content (30%) [53].

Antimicrobial effect of US is quite reduced, and the intensity and time needed normally produce significant increments of temperature. Therefore, it is difficult to consider US as a non-thermal technology. However, US produces synergistic antimicrobial effects when applied together with conventional thermal technologies or emerging non-thermal processes.

Industrial devices are currently available to process crushed grapes increasing extraction and reducing maceration times. The sonication device has a tubular structure to increase sonication surface, normally with a polygonal section to better dispose of the sonoplates (**Figure 15**). Currently, this technology is developed by several companies; among them, Agrovin inside a H2020 European project, has developed the Perseo™ system [54] with 50 kW of power and 8 cavitation cells to process up to 10 t/h [52, 54]. With this technology, it is possible to reduce maceration times from 7 to 2–3 days with similar contents of anthocyanins and tannins; moreover, the aromatic fraction is in the same time enhanced. Prof. Emilio Celotti

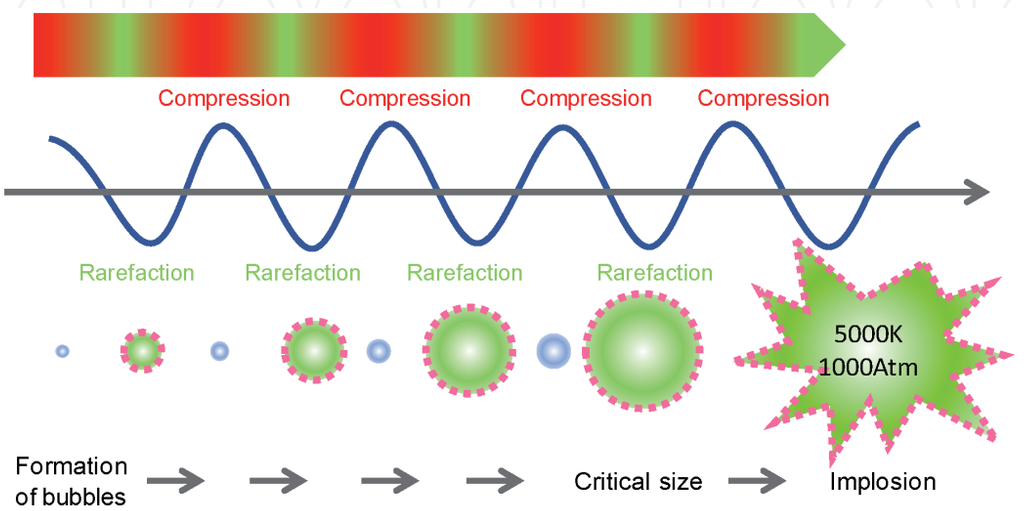

Figure 14.
Implosion of bubbles and cavitation produced by alternative compression-rarefaction effects generated by US waves.

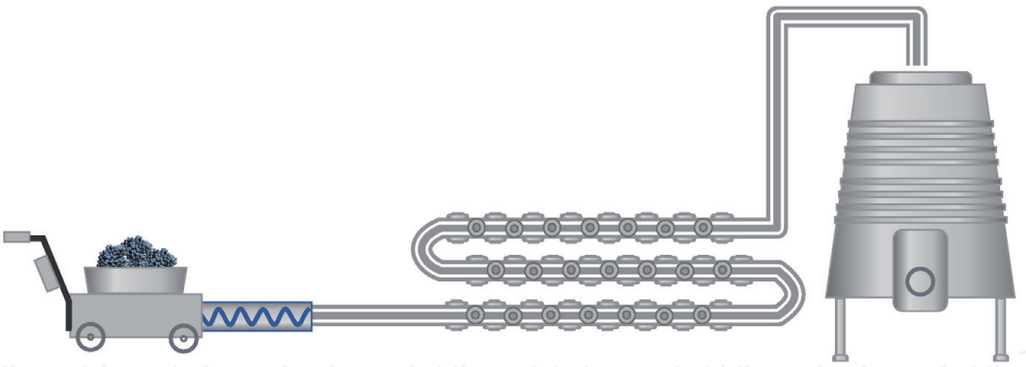

Figure 15.
Cavitation cells disposed in a tubular structure with the sonoplates to apply the US waves.

(Udine University, Italy) is also working in the use of US to improve the maceration process and has developed a prototype to favor a faster extraction of polyphenols, aroma, and precursors [55–56].

7. Pulsed light

Pulsed light (PL) is the use of wide spectrum (170–2600 nm) high intensity light [57] applied in short flashes during a few microseconds. The spectrum includes ultraviolet, visible, and near infrared radiation. PL spectrum is quite similar to the solar radiation, but having lower wavelengths from the UV (<320 nm), which, in the case of sun light, these are filtered by the ozone layer in the atmosphere. The intensity of light can reach values 10^5 folds higher than the sun radiation at sea level [58]. PL technology uses very short energization times, for example, a flash lamp applying energy of 300 J during 300 μs produces a peak intensity of 1 MW which produces ca. 1 kW/cm^2 on the irradiation surface. PL was initially used in Japan in the 1970s; later, in 1988, it was developed by a Californian company PurePulse Technologies Inc., but the applications in the food industry increased after it was approved by FDA for food processing [59].

The antimicrobial effect of PL is due to dimerization of DNA pyrimidines promoted by the 254 nm UV radiation but also due to the localized instantaneous heating producing membrane and cell wall breakage [57]. The effect on membrane

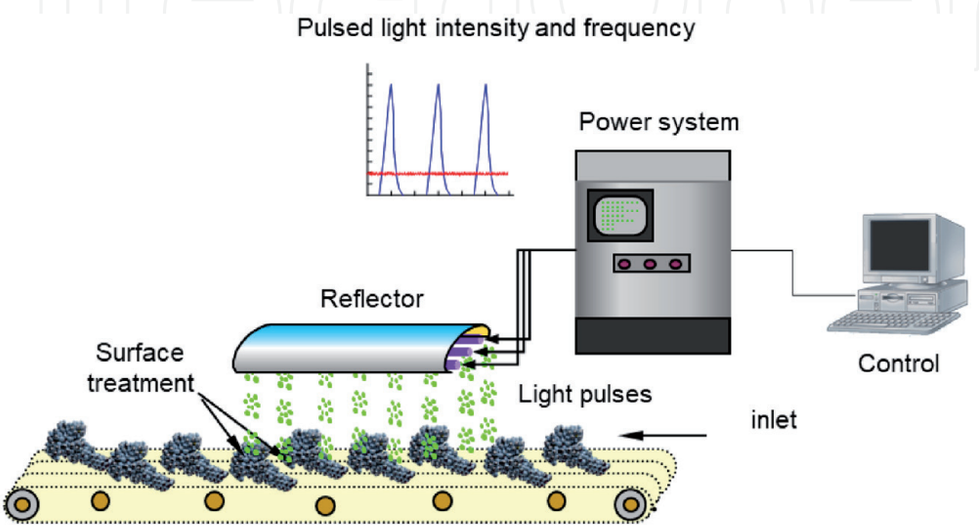

Figure 16.
Pulsed light system to treat grape surface for the elimination of microorganisms in a continuous flow.

and cell walls can be observed by electronic microscopy, and it is associated to a higher concentration of eluted protein [60]. These effects affect vegetative and spore forms of microorganisms. *Saccharomyces* and non-*Saccharomyces* yeasts and lactic acid bacteria are controlled in grapes at low temperature and without effect in external appearance and sensory quality of food [60] (**Figure 16**).

Currently, PL devices not only for continuous food treatment, but also for the sterilization of food packaging are available [61]. The PL equipment is formed by an electronic module containing both control and monitoring systems, high voltage components for the flashing lamps, cooling system, and, finally, the optical unit with the flash lamps. PL is another non-thermal technology with powerful applications to sterilize the grape surface, thus enabling the control of microbial loads and, therefore, facilitating the use of selected starters and the reduction of SO_2 levels in wines.

8. Conclusions

Emerging non-thermal technologies open new possibilities in winemaking technology, generally facilitating at the same time the control of indigenous microorganisms and the use of new biotechnologies such as the fermentation with non-*Saccharomyces* yeasts or the use of yeast-bacteria co-inoculations. Most of them (HHP, UHPH, PEF, eBeam, and US) also facilitates a faster extraction of phenolic compounds from the grape skins, including not only pigments and tannins, but also aromatic and flavor compounds, thus reducing the maceration times. Several of them (UHPH, PEF, US, and PL) can be applied in a continuous mode when the crushed grape or must is pumped to the fermentation tank, increasing the processing yield and reducing the dead times. Some of these technologies, such as UHPH, produce an intense inactivation of oxidative enzymes, preserving better the sensory quality and strongly reducing the SO_2 needs. Finally, most of these technologies have low energetic requirements, so the running costs are moderated.

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