UDC 579.864.1:543.92:664.8/.9 *review*
ISSN 1330-9862

(FTB-1145)

Effects of Lactic Acid Bacteria Autolysis on Sensorial Characteristics of Fermented Foods

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Received: April 16, 2002
Accepted: November 7, 2002

Summary

Lactic acid bacteria autolysis is due to intracellular hydrolytic enzymes that act on specific parts of the cell wall causing the release of biologically active compounds, in particular enzymes, to the medium. Consequently, the role of lactic acid bacteria in the transformation of foodstuffs does not involve merely the fermentation stage but also the later maturation and aging stage thanks to the compounds released to the medium that combine in order to change the sensorial profile of the product. This phenomenon is particularly important for cheese types subjected to very long aging, for salami ripening and for red aged wines.

Key words: lactic acid bacteria autolysis, sensorial characteristics, cheese, salami, wine

Sensorial Qualities and their Source

Fermented food sensorial qualities can be divided into three types: pre-fermentative, fermentative and post--fermentative.

1) Pre-fermentative qualities are those typical of and proper to the raw material. They can be quite homogenous or vary depending on the source.

2) Fermentative qualities are those added by the fermenting agent and can be due to primary or secondary fermentation products or the transformation of compounds in the raw material.

3) Post-fermentative qualities are those that appear during aging or ripening, when microorganism growth has ended, and they are due to physical, chemical or biological reactions by enzymes that have conserved their activity.

In many cases post-fermentative qualities do not have enough time to appear because the product is consumed shortly after the end of fermentation (for example, many types of cheese and wine). But in other instances, it is necessary to subject the products to long aging periods in order to achieve the quality and typical characteristics essential for the quality of these products. Many post-fermentative composition variations can be attributed to enzymes already in the product before the beginning of the fermentation, such as the enzymes brought by the rennet for cheese, grape enzymes and meat cathepsins. But unquestionably a predominant role is played by the enzymes released by bacteria, especially lactic acid bacteria, when their growth has ended.

It is well known that lactic acid bacteria and yeasts are microorganisms responsible for producing fermented foods. In more detail, lactic acid bacteria cause the primary fermentation of all food products, from milk (fermented milk and cheese) and meat (salami and similar products). They can also be active in the wine-making sector as agents of malolactic fermentation following the alcoholic fermentation. At the end of the fermentation process, their cells are not removed

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(though sometimes this is not possible) but remain in the product at length, often until it is consumed and when they finally lose their vitality. In the case of lactic acid bacteria cell death is caused by autolysis that occurs under entirely specific conditions.

Lactic Acid Bacteria Autolysis

The biochemical mechanism that causes cell autolysis in lactic acid bacteria (and also other bacteria) has been the subject of numerous research projects (*1-7*). These have shed light on the way in which it happens. The hydrolytic enzymes in bacteria cells, N-acetyl-muramidases, act on cell wall polysaccharides and thus permit the insertion of more basic molecules and, hence, the formation of new cells. N-acetyl-muramidases are especially active during the multiplication stage but remain so even when it stops. These enzymes then act in an uncontrolled manner and cell wall components rather than increasing are hydrolysed and destroyed. Cell wall rupture does not affect the entire structure but is localised at a number of places where the hydrolytic enzymes are present (*8,9*). As a result, cell walls with autolysis at the beginning stage, display small scattered holes whose diameter gradually increases to become vast fractures (*10*). At this point the cells lose their typical appearance. Cell wall appearance at different autolysis stages can be clearly seen under a scanning electron microscope (Figs. 1–3).

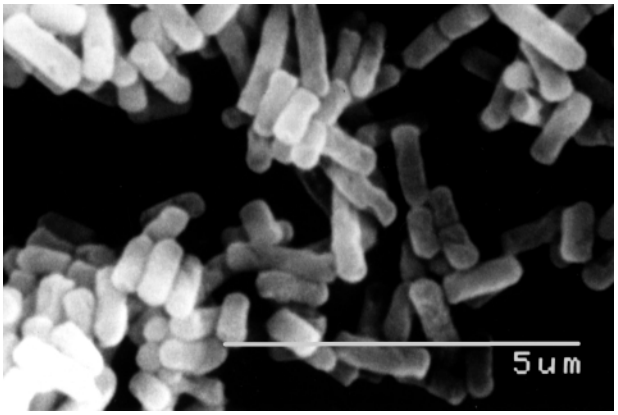

Fig. 1. *Lactobacillus brevis* cells at 3 days in MRS. The cellular wall is intact. Magnification x 10 000

Lactic acid bacteria autolysis involves cell walls but not necessarily other components such as intracellular enzymes, which are unaltered. Thus, when cell walls break down, these still biologically active compounds are released to the medium where they can continue their action. Enzyme release, particularly of hydrolytic enzymes, and not just of these, has important consequences in various fermented food products, when the multiplication stage draws to an end.

Cheese Aging

The transformation of all types of cheese relies on the activity of lactic acid bacteria added, as general rule, to pasteurised milk as selected starter. They multiply at

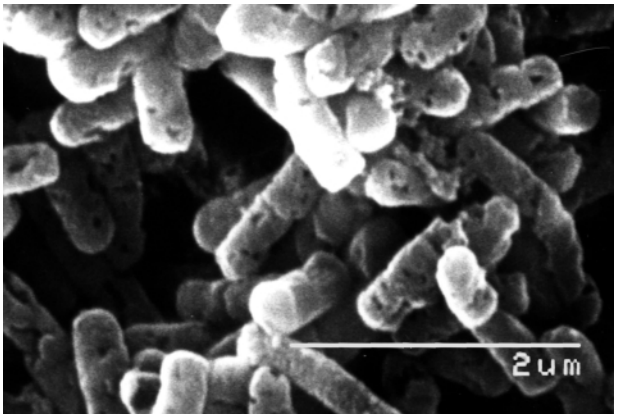

Fig. 2. *Lactobacillus brevis* cells at 30 days in MRS. The cellular walls show lesions and holes. Magnification x 20 000

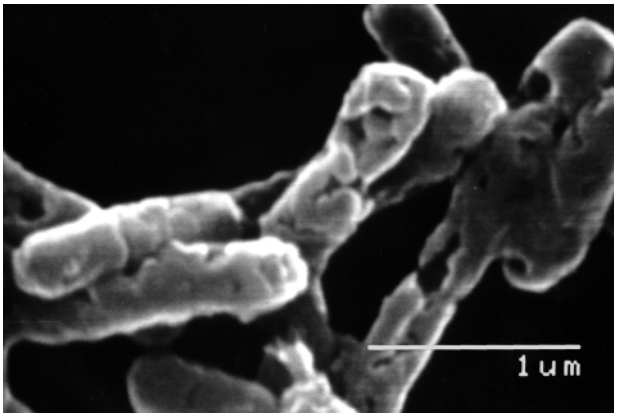

Fig. 3. *Lactobacillus brevis* cells at 60 days in MRS. The cellular walls are destroyed. Magnification x 30 000

the expense of lactose and lower pH, mainly through the formation of lactic acid. They produce small amounts of other compounds that contribute to fermentative sensorial traits. The species that act in the fermentative process are: *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Lb. acidophilus*. These are all homofermentative, but there are also others belonging to facultatively homofermentative heterofermentative species. The choice of one or the other strain will depend largely on the technology used but also on the temperature at which the curd, once formed, is held (36 °C = raw cheese) or is treated (up to 56 °C = partly cooked or cooked cheese). Many types of cheese such as Squacquerone, Mozzarella, Robiola, typical Italian cheeses are intended for immediate consumption, or after a brief aging time and their sensorial traits are solely pre-fermentative and fermentative. Others are subjected to an aging period of many months or some years as in the case of grating cheese such as Parmigiano-Reggiano and Grana padano. In these latter, the sensorial traits are mainly post-fermentative and give the products their typicalness (*11–13*).

The major change that happens during cheese aging is protein hydrolysis (*14*) with the release of low molecular weight peptides and amino acids; the percentage of soluble nitrogen compounds compared to the total is the parametre used to determine the aging index. It is the

combination of soluble nitrogen compounds that gives the fragrance and taste which are chacteristic of aged cheeses (*15*). Casein hydrolysis attributes to the action of the protease in the rennet and which continues at length in soft body cheese, while in cooked or stretched body cheeses it can no longer be found in the curd after just a few hours (*16*).

In addition to this proteolytic action, the lipolytic action is important during cheese ripening. This acts on the fat content releasing fatty acids and glycerol, thus playing an important role in taste formation. Fat hydrolysis is very limited in hard body cheeses (*17*) and more dynamic in soft body and blue cheeses. The lipase enzymes, responsible for this hydrolysis, are partly natural components of milk, which are inert at low pH and easily destroyed by heat, and partly thermoresistant microbial enzymes (*18*,*19*).

During the fermentation stage, lactic acid bacteria multiply very intensely (*20*) but slow down during the different aging stages (*21*). In addition to soft cheese products, cheeses made from raw milk (those to be aged longer) sometimes develop other bacteria (*22*). The bacteria cells are evenly distributed throughout the entire volume of the cheese and are an integral part of it. A scanning electron microscope has shown that lactic acid bacteria cells and propionic bacteria in the cheese are subjected to autolysis as described in the preceding section (*23–30*). Autolysis is immediately followed by release of all the intracellular compounds including proteolytic enzymes and still intact lipolytic enzymes.

It is commonly admitted that hydrolytic enzymes released by lactic acid bacteria are mainly responsible for the post-fermentative traits of long aging cheese. In support of this hypothesis, it should be recalled that, for Grana type cheese, the concluding stage of the dairy process is cooking at 56 °C for 30 min, a time sufficient to cause a sharp decrease in the action of the enzymes in the milk or added with the starter.

Salami Ripening

Salami are fermented food products whose transformation is due to a number of different microorganisms; a large number of micrococci are always in the raw material, while some, such as lactic acid bacteria, develop during the fermentation stage (*31*,*32*). They work jointly towards product transformation, the former by their lipolytic action and the latter by their fermentative action and formation of lactic acid. During the ripening stage, the role of the mould that forms on the natural casing is that its mycelium penetrates deep into the meat mixture (*33*).

The proteolytic enzymes brought by meat, especially the cathepsins, have a predominant role in salami ripening. The cathepsins hydrolyse the muscle fibres to release nitrogen compounds and take away from the product the sensorial traits proper to fresh meat. The proteolytic action added to that of the cathepsins is the one performed by the mould that grows on the natural casing. Caboni *et al*. (*34*) determined that there is a major release of fatty acids in the stage following the end of fermentation. This means that in salami as in cheese during ripening there is a major lipolytic action that can be attributed to both micrococci and enzymes and perhaps also to mould (*35–37*).

It is hard to say whether, in addition to direct microorganism action and indirect intrinsic enzyme activity, we must add the action of the enzymes released by autolysis of the micrococci and lactic acid bacteria responsible for the fermentative stage. Research by Chiavari *et al*. (*38*) and Grazia *et al*. (*39*) has shown that some lactic acid bacteria strains possess the trait of slowing down the growth of mould on the natural casing and that this inhibition can be attributed to compounds released after autolysis. It is highly likely that, in addition to these, intracellular enzymes are also released, particularly protease and lipase that contribute to the formation of post-fermentative characteristics (*40*).

Wine Aging

The microorganisms that act to change grape must into wine are yeasts, especially those belonging to the genus *Saccharomyces*. At the end of must fermentation the yeast cells are removed in good time to prevent them, through autolysis, from enriching the wine with compounds that facilitate the growth of lactic acid bacteria, which could be agents of wine diseases. Only in the case of sparkling wine refermented in the bottle, the wine remains in contact with the yeast cells for a long time because it is presumed that due to autolysis of the yeast, compounds are released (especially amino acids) that refine product quality (*41*,*42*). It should be pointed out, however, that yeast autolysis takes place through biochemical processes different from those for bacteria. The former is due to enzyme activity with protease and nuclease that perform their action inside the cells without any involvement of the cell walls, which remain practically unaltered (*43*). The compounds released are of low molecular weight and are biochemically inert.

The action of lactic acid bacteria in wine making can occur, if the conditions allow it, at the end of alcoholic fermentation. It is due to the growth of *Oenococcus oeni*, agent of the so-called malolactic fermentation, whose main consequence is the conversion of malic acid to lactic acid and carbon dioxide. This conversion, accompanied by the formation of other secondary compounds, typical of lactic fermentation, modifies the composition of the wine by adding new sensorial characteristics still belonging to the second group (*44–47*).

Malolactic fermentation does not have univocal importance (*48*). For immediate consumption wine (within a year from production) it is considered a negative factor as it takes the necessary characteristic of freshness from the wine and gives it an »old« taste profile. In this case, every wine making skill is used to prevent it from taking place. However, it is regarded as the essential preliminary stage for the production of high quality wine (especially red) to be aged (*49*). In this latter case the bacteria cells are not removed at the end of fermentation but remain in the wine for a long time.

The post-fermentative sensorial traits that appear during long aging of wine are due to chemical, physical and biochemical mechanisms completely different from

those in other fermented foodstuffs. Protein levels in wine are so low as not to affect the composition even after hydrolysis and, in addition, lipids are not present. The changes involve minor components such as anthocyanins, glucosides and others whose modification leads to the formation and release of components that give the product the fragrance and taste profile typical of aged wine. It seems highly likely that cell autolysis of *Oe. oeni* has a primary role in the development of post-fermentative sensorial quality.

The *Oenococcus oeni* cells, once their growth stage has ended, do not survive for long in wine because this can be considered a hostile environment due to the low pH and the large amount of ethanol present. These negative factors cause rapid cell death and it is probable that they offer no obstacle to autolysis. Viewed under an electron scanning microscope, the bacteria cells a month after fermentation has ended have an entirely special exterior appearance. As highlighted in Figs. 4 and 5, the cell walls are porous as observed in other conditions and display a sort of overall shapelessness that results in an increased diameter. This entirely peculiar appearance of the cells proves without a shadow of doubt that the cell wall has collapsed but this could have happened due to an autolysing mechanism other than that which causes the holes.

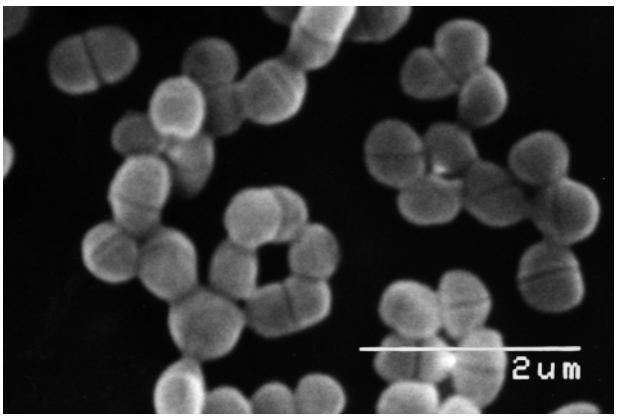

Fig. 4. *Oenococcus oeni* cells at 2 days in MRS. The cellular wall is intact. Magnification x 15 000

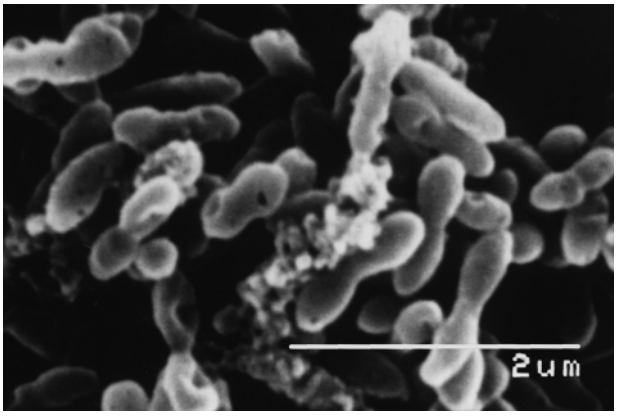

Fig. 5. *Oeonococcus oeni* cells at 30 days in MRS. The cells are collapsed with lesions and holes in the cellular wall. Magnification x 20 000

A research project conducted by Zambonelli *et al.* (unpublished data) compared the chemical and sensorial characteristics of different types of red wine at the end of alcoholic fermentation, at the end of malolactic fermentation and three months after the end of malolactic fermentation. At the end of malolactic fermentation, the changes in the chemical profile of the wine compared with the starting wine were entirely evident and as expected, because they only involved some of the basic components. This was not the case after the three-month holdover of bacteria cells in the wine, that is, after cell autolysis. In this latter case, the clearly perceived differences were in the sensorial profile. It seemed quite likely that biochemically active compounds released by the cell walls acted on the minor components responsible for the formation of aromatic products.

Conclusions

The role of lactic acid bacteria in the transformation of foodstuffs does not involve only the primary fermentation process with modifications to the composition that guarantee product stability but also during the ripening or aging stage when the post-fermentative sensorial profile appears. Lactic acid bacteria action at this stage is the result of their autolysis and also of biochemical mechanisms by which autolysis takes place: cell wall collapses with the release of compounds, especially of intact and still active hydrolytic enzymes. Bacterial enzymes are an addition to those already in the raw material and combine to make product ripening more intense and complete. Research projects on this subject are still at a very early stage and our knowledge about reactions due to the compounds released by bacteria is at present partial and incomplete.

One fact that emerged was that the ability to undergo fast autolysis at the end of the multiplication stage and, hence, to release intact compounds is diverse in various lactic bacteria species and within each species, in the different strains. In other words, it is a species and strain specific trait. As a result, the trait must be given careful consideration when selecting the strains to be used as starters to guide fermentation.

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Utjecaj autolize bakterija mliječno-kiselog vrenja na senzorske značajke fermentiranih namirnica

Sažetak

Autoliza bakterija mliječno-kiselog vrenja nastaje djelovanjem intracelularnih hidrolitičkih enzima koji u reakciji sa specifičnim dijelovima staničnog zida uzrokuju izlazak biološki aktivnih spojeva, osobito enzima, u podlogu. Stoga utjecaj tih bakterija na promjene u namirnicama nije ograničen samo na fermentaciju već i na vrijeme zrenja i starenja zbog spojeva koji su iz stanica ušli u medij, što dovodi do promjena senzorskih značajki proizvoda. Taj je proces posebno važan za vrste sira koje dugo sazrijevaju, za zrenje salame i za proizvodnju odležanih crvenih vina.