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review

Malolactic Fermentation of Wines

Jabučno-mliječna fermentacija vina

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Summary

Malolactic fermentation is a bacterial fermentation which occurs in wines and results in the conversion of malic acid to lactic acid and CO_2. Lactic acid bacteria (LAB) which are responsible for this process belong to the genera ***Leuconostoc, Pediococcus*** *and* ***Lactobacillus****. The growth of LAB in wine is often limited by nutritional factors and physico-chemical factors such as storage temperature, low pH, and alcohol as well as sulphur dioxide concentrations. Recently, the investigations in this area have focussed on physiological and biochemical properties which determine the ability of malolactic bacteria to grow in wines, and on the biochemical mechanisms by which these bacteria affect the wine quality. Such fundamental knowledge is now becoming increasingly important because the wine industry is moving in the direction of controlled malolactic fermentation with specific strains of malolactic bacteria. These basic informations will also indicate the conditions under which lactic acid bacteria could be most efficiently used in the winery.*

Sažetak

Jabučno-mliječna fermentacija jest bakterijska fermentacija koja se odvija u vinima konverzijom jabučne kiseline u mliječnu kiselinu i ugljik(IV)-oksid. Bakterije mliječne kiseline (BMK) koje provode ovaj proces pripadaju rodovima ***Leuconostoc, Pediococcus*** *i* ***Lactobacillus****. Rast BMK u vinu često je ograničen hranidbenim čimbenicima te fizikalno-kemijskim, kao što su temperatura, niski pH, koncentracija etanola i sumpor(IV)- oksida. U posljednje su vrijeme istraživanja na ovom području usmjerena na fiziološka i biokemijska svojstva koja određuju sposobnost rasta ovih bakterija u vinima te na biokemijske mehanizme kojima utječu na kakvoću vina. Ovakve temeljne spoznaje postaju vrlo značajne zbog usmjeravanja vinske industrije prema kontroliranju jabučno-mliječne fermentacije sa specifičnim vrstama/sojevima bakterija mliječne kiseline koje provode jabučno-mliječnu fermentaciju. Te osnovne informacije također upućuju i na uvjete pod kojim se bakterije mliječne kiseline mogu najdjelotvornije primijeniti u vinariji.*

Introduction

The conversion of L-malic acid to L-lactic acid, i.e. malolactic fermentation, as well as the utilisation of the main sugars of grape juice (glucose and fructose) by lactic acid bacteria (LAB) is well known and has been extensively studied *(1-5)*. It is most generally thought of in connection with wine production, but it can also be an important aspect of other food fermentations *(6,7)*.

For the malolactic fermentation of wines the heterofermentative as well as homofermentative LAB are responsible. These bacteria which are capable to decarboxylate malic acid to lactic acid are usually referred as malolactic bacteria. Heterofermentative LAB convert glucose to lactic acid, ethanol or acetic acid and carbon dioxide, while fructose is converted to mannitol, lactic acid and acetic acid. Homofermentative LAB convert glucose almost exlusively to lactic acid and fructose to mannitol and lactic acid *(8)*.

The malolactic fermentation in wines is desirable for two main reasons:

1. The bacterial transformation of malic acid to lactic acid deacidifies wines conveniently and confers a mellowness to it. This is essential in order to decrease the excess of acidity resulting from the high content of malic acid in grapes grown in cold wine-regions.

2. The disappearance of malic acid provides a biological stability of wines with regard to the action of LAB, which is very important for the wines from warm regions *(1,9)*. However, a practical application of malolactic fermentation in winemaking is a subject of great controversies in the past two decades. It is very difficult to describe this fermentation as clearly desirable or undesirable in sense of final wine quality. This is so because the advantage or disadvantage of malolactic fermentation depends on a number of factors such as wine-growing hills, grape species, composition of wine, winemaking technique and, of course, manufacturers style. Furthermore, malolactic fermentation is often difficult to promote and/or control even when inducing the fermentation by inoculation with commercial strains of malolactic bacteria.

Lactic acid bacteria in musts and wines

Numerous studies have been conducted on the lactic acid bacteria that occur on grape, musts and wines *(10-17)*. It is well known that only a restricted number of species develops in musts and wines since this medium is quite selective due to its acidic pH, its alcohol concentration and SO_2 addition. Ribérau-Gayon et al. *(18)* using the morphology and metabolic route for the assimilation of sugars as a criterion for identification, classified the LAB of musts and wines principally in three genera: *Pediococcus, Leuconostoc* and *Lactobacillus* (Table 1, *19*).

There were many difficulties in classification of the wine leuconostocs and pediococci *(20-23)*. Namely, the members of the genera *Leuconostoc* and *Pediococcus* are phenotypically very similar and it is nearly impossible to separate them by using only phenotypic characteristics. Besides the similar carbohydrate utilisation patterns, their percentage of the sum of guanine and cytosine residues (G+C) is also similar *(19)*. So, in their numerical taxonomic study of *Leuconostoc oenos* strains from wine Tracey and Britz *(23)* recommended cell morphology, the type of lactic acid produced and cell wall composition as primary characteristics, at the genus level, to differentiate the wine leuconostocs from other related genera. Indeed, when phenotypic properties are considered even the most important species in malolactic fermentation, *Leuconostoc oenos*, is not an homogeneous group, and it is difficult to select a type of strain. It seems that all confusions in wine leuconostocs classification arose from great variability in the physiological properties of *L.oenos*, depending on habitat and growth conditions *(3,20,23,24)*. At present, all leuconostoc strains isolated from wine are generally included in the species *L.oenos (22)*, although earlier investigators suggested that this species should be divided into two, or even three species based on carbohydrate utilisation.

However, although the results of their study confirm the observations that *L.oenos* is not an homogeneous group, Tracey and Britz *(23)* stated that more research (including DNA homology, metabolites formed and cellular fatty acids) is required before any subdivision of this species.

Very little information exists on the development of LAB during vinification process. But, the evolution of rapid methods for the identification of LAB (API galleries) has provided a quantitative examination of the species that evolve during different stages of vinification. Lactic acid bacteria can be found on grapes, but usually to a lesser extent than the acetic acid bacteria or the yeasts. During the vinification process there is a succesion of LAB species that actually occur, depending on the particular vineyard.

In Swiss vineyards, for example, there is a predominance of homofermentative lactobacilli *Lb.plantarum* and *Lb.casei*, which rapidly disappear during the alcoholic fermentation to be replaced by heterofermentative *Lb.hilgardii* and *Lb.brevis (25)*, usually found in spoiled wines.

In Bordeaux wines (Merlot and Semillon) Fleet et al. *(26)* found *Pediococcus cerevisiae, Leuconostoc mesenteroides* and some unidentified *Lactobacillus* species in musts at very low level ($\approx 10^2$–10^3 cells/mL), but they did not grow and died off by the end of alcoholic fermentation. The authors noticed the presence of *Leuconostoc oenos* just after alcoholic fermentation had finished, and that was the only species of LAB isolated during malolactic fermentation in these Bordeaux wines.

After the isolation and enological characterisation of malolactic bacteria from the vineyards of northwestern Spain, Sieiro et al. *(16)* concluded that there are some differences even between two regions on that location. They isolated *Lb.curvatus* and *Lb.buchneri* from sulfited musts, while during the active alcoholic fermentation the malolactic bacteria were not isolated. *Leuconostoc oenos* was isolated after alcoholic fermentation had finished, i.e.during the malolactic fermentation. Predominant species in malolactic fermentation were *Lb.plantarum* and *L.oenos*, although *Lb.curvatus* and *Lb.brevis* were also isolated at this stage of vinification *(16)*.

Parameters affecting the development of lactic acid bacteria in wines

It is well known that the growth of lactic acid bacteria and malolactic fermentation of wines are often limited by nutritional and physico-chemical factors.

Table 1. Classification of lactic acid bacteria of musts and wines *(18)*
Tablica 1. Klasifikacija bakterija mliječne kiseline izoliranih iz moštova i vina *(18)*

Bacterial species Vrsta bakterije	Morphological form Morfološki oblik	Differences / Razlike		
		Pentose fermentation Fermentacija pentoza		Metabolic pathway of glucose ferment. Metabolički put ferment. glukoze
		Arabinose Arabinoza	Xylose Ksiloza	
*Pediococcus cerevisiae**	spherical / sferni	–	–	homoferm.
*Leuconostoc gracile**	spherical / sferni	–	–	heteroferm.
Leuconostoc oenos	spherical / sferni	±	±	heteroferm.
Lactobacillus plantarum	rods / štapićasti	±	±	homoferm.
Lactobacillus casei	rods / štapićasti	–	–	homoferm.
Lactobacillus fructivorans	rods / štapićasti	–	–	heteroferm.
Lactobacillus hilgardii	rods / štapićasti	–	+	heteroferm.
Lactobacillus brevis	rods / štapićasti	+	+	heteroferm.

* In the latest Bergey's Manual of Systematic Bacteriology *(19)* these two species are not listed
* U posljednjem Priručniku za sistematiku bakterija *(19)* ove dvije vrste nisu navedene

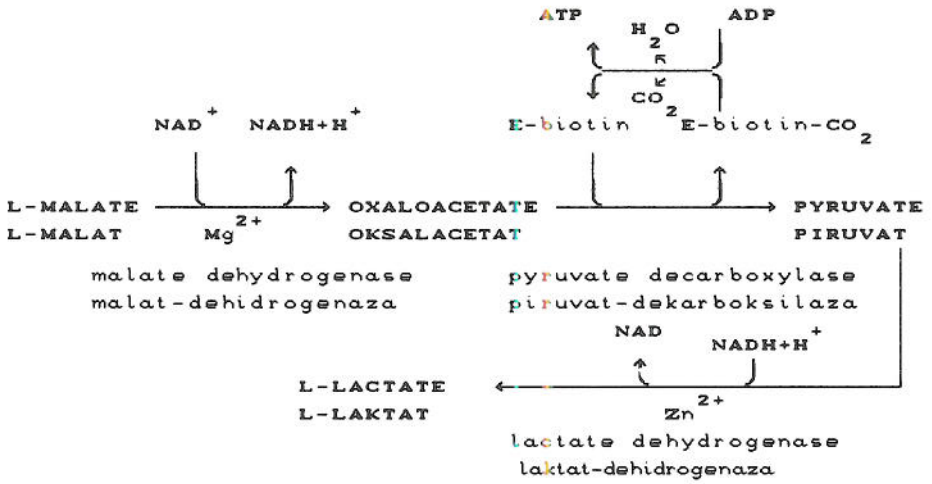

Fig. 1. Proposed mechanism for the utilisation of L-malic acid and the generation of ATP for growth *(28)*
Slika 1. Pretpostavljeni mehanizam korištenja L-jabučne kiseline i proizvodnja ATP za rast *(28)*

Nutritional factors

Lactic acid bacteria have complex growth factors and amino acid requirements *(19)*. The energy requirements are relatively small. Radler *(27)* has calculated that 100 mg/L of carbohydrates are sufficient to supply energy for the growth of 100 mg/L of bacterial biomass (dry weight) which is capable of fermenting 1 g/L of malic acid. This requirement can be fulfilled by residual sugars of the wine (namely fructose, glucose, arabinose, xylose and trehalose). Malolactic bacteria can use malate as carbon and/or energy source *(28)* (Fig. 1), but some of them only in the presence of fermentable sugars (usually glucose) *(28-30)*.

Various studies which have been carried out on the nutritional requirements of malolactic bacteria reported that amino acids are important for the growth, both as nitrogen sources *(31)* and as carbon sources *(32)*. The amino acids requirements of these bacteria are in most cases not fully satisfied by amino acid compositions of wines *(2)*. The beneficial effect of yeast extract on growth of LAB is well known *(33)* and has been studied extensively also on malolactic bacteria *(25,31-36)*, because of the presence of yeast cell components in wine, and because of the legally allowable addition of limited amounts of yeast extract to wine *(37)*. Yeast extract, in comparison with the combination of amino acids and growth factors included in basal medium *(36)*, gave the best growth of several *L.oenos* species and *Pediococcus damnosus* DSM 20331 *(28)*, but it caused only a small increase in the rate of malolactic fermentation *(36)*.

Physico-chemical factors

In spite of complex nutrient requirements of malolactic bacteria mentioned above it seems that the physico-chemical factors are decisive for the development of these bacteria in wine. Among them, the temperature of storage, low pH, alcohol concentration and SO_2 content have great influence.

Influence of wine storage temperature

In accordance with the optimal growth temperature the malolactic bacteria belong to mesophylic group of microorganisms, so their growth is often limited by the temperature of wine storage (i.e. from 18 to 22 °C). However, the growth response to low temperature depends on the species or even the strain of bacteria *(3,14)*. Lowering the growth temperature from 30 to 10 °C Silver and Leighton *(14)* obtained different results with three strains of *L.oenos* (ML-34, PSU-1 and B44.40) at pH = 4.5 and in the optimal growth medium. Only one of them (strain B44.40) was able to grow at 10 °C, while the other two strains showed already at 20 °C the retardation in growth rate as well as in cell density. Lafon-Lafourcade *(2)* stated that a lowering of the temperature by 5 °C (from 19 to 14 °C) is sufficient (together with other unfavourable factors) to kill a great part of the initial population of LAB in must, or decrease the formed biomass thus prolonging the malolactic fermentation by 3 weeks. She mentioned also that the temperature optimum for growth of malolactic bacteria in wine lies between 20 and 25 °C.

Influence of pH value

The acidity of musts and wines is much below the optimal ranges for the growth of malolactic bacteria (i.e. pH = 4.2 to 6.5). In their comprehensive study which included a large, representative number of isolates (81 isolates of *L.oenos*, 23 of *Pediococcus parvulus*, and 22 of *Lactobacillus* spp.) Davis et al. *(3)* investigated the influence of initial pH values (from pH = 3.0 to 7.5) on bacterial growth. They found that all but one strain of *L.oenos* grew in the investigated pH range. It was necessary to decrease the pH to 3.0 before a substantial reduction in the proportion of strains able to grow occured. Compared with *L.oenos* the pediococci and lactobacilli were less tolerant to pH levels below 3.4. None of the isolates of pediococci grew at pH = 3.0, while 31 % of *L.oenos* strains grew at this pH.

Because of the influence on bacterial growth low pH has a great effect on malolactic fermentation. In wines ino-

culated with *L.oenos* ML-34, lowering of the initial pH values from 3.83 to 3.15 prolonged the time for the completion of malolactic fermentation for about 20 weeks, and the fermentation rate decreased from 0.50 to 0.04 $weeks^{-1}$, respectively *(20)*.

Influence of alcohol concentration

Among chemical factors ethanol is the principal inhibitor of the bacterial growth. The toxicity of ethanol is in correlation with its lipid-buffer partition coefficient, suggesting its interference with hydrophobic regions of cell membranes *(38)*. The plasma membrane is the possible target for the ethanol inhibition of nutrient transport, but the inner mitochondrial membrane was determined to be the target region of ethanol-enhanced thermal death *(39)*. Ethanol interacts with membranes possibly by insertion into the hydrophobic interior, increasing the polarity of this region, weakening the hydrophobic barrier to the free exchange of polar molecules, and weakening the hydrophobic interactions affecting thus the positioning of proteins within the membranes *(38)*. The loss of membrane integrity decreases the ability of the cell to maintaine a concentration gradient across the plasma membrane.

In winemaking there is a multiplicity of variables which affect the alcohol tolerance of microorganisms involved. Temperature, pH, sugar concentration are only the easily recognized ones *(40)*. Most malolactic bacteria can grow in the presence of volume fraction of ethanol φ = 10 % *(3)*, but the proportion of strains capable of growing decreased as the ethanol concentration was increased. However, some bacterial growth has been observed in wines with more than φ(ethanol) = 20 % (34). Generally, *Lactobacillus* species are more tolerant to higher ethanol concentrations than either *Pediococcus* spp. or *L.oenos (3,15)*.

Influence of SO_2

The use of sulphur dioxide in winemaking is well established. Its efficiency as an antimicrobial agent depends on several factors. One of them is pH since SO_2 exists in various forms at different pH values, and has two dissociation constants. Between pH = 5.0 and 9.0 this substance exists as a mixture of hydrogensulphite (HSO_3^-) and sulphite (SO_3^{2-}) ions, while below pH = 5.0 the mixture changes to one of hydrogensulphite ions and molecular SO_2 in solution. As the pH decreases the proportion of molecular SO_2 – the form which has the most potent antimicrobial effect – increases *(7)*.

Testing the effect of wide range of total sulphur dioxide mass concentrations (from 32 to 256 mg/L) on *Leuconostoc, Pediococcus* and *Lactobacillus* species, Davis et al. *(3)* found that all species were able to grow in the presence of 64 mg/L of total SO_2 under investigated conditions. *L.oenos* strains were more sensitive at higher concentrations of SO_2. Only 4.9 % of tested strains were able to grow in presence of 160 mg/L total SO_2, while 39.1 % of *P.parvulus* strains, and 27.3 % of *Lactobacillus* spp. grew at this concentration. These growth studies *(3)* have confirmed the conclusions of the authors' earlier investigations *(41,42)* namely, that wines of high total SO_2 concentration are more likely to undergo natural malolactic fermentation with species of *Pediococcus* or *Lactobacillus*.

Biochemistry of malolactic fermentation

The kinetics of malolactic fermentation is directly connected with the bacterial biomass formed, and the biochemical reactions are carried out by LAB during their growth.

Different hypotheses regarding malate utilisation have been proposed: **first,** malate can be used as carbon and/or energy source by many of LAB *(43)* by malic enzyme-catalyzed decarboxylation of malate to pyruvate and CO_2 and concomitant production of NADH, or by the action of malate-dehydrogenase which lead to malate assimilation producing oxalacetate and generating NADH (Fig. 1) *(2,43,44)*; **second**, LAB also have a second decarboxylating enzyme called malolactic enzyme *(45,46)* which transforms L-malate to L-lactate directly without production of NADH or other energetic compounds.

In the last pathway mentioned above malate enters into cells and is decarboxylated to yield lactate and CO_2, after which lactate and carbon dioxide leave the cells. Although the decarboxylation of L-malate is a non-energy-yielding reaction catalyzed by a single enzyme, malolactic fermentation supplies cells with additional metabolic energy (Fig. 2) *(47)*. Since substrate-level phosphorylation or direct ion extrusion by a membrane-bound decarboxylase *(48)* does not occur during malolactic fermentation, Poolman et al. *(47)* stated that the metabolic energy must originate from the movement of L-malate, L-lactate and/or carbon dioxide across the membrane. Assuming that CO_2 diffuses out of the cell without affecting the pH gradient, they proposed three distinct mechanisms of metabolic energy generation during malolactic fermentation (Fig.2). A membrane potential is generated either by antiport reaction, malate uptake, or lactate efflux, and a pH gradient is generated as a result of proton consumption in the cytoplasm.

Strasser et al. *(49)* purified the malolactic enzyme from *Lactobacillus murinus* and found that enzymatic activity was optimal at pH = 5.5 and at 37 °C. They also assumed that the malolactic enzyme consists of two probably identical subunits as well as the malolactic enzyme purified from *L.plantarum (50)*. This work does not support the hypothesis that the malolactic enzyme is an aggregation of several different enzymes *(2)* which was established earlier.

Use of LAB starter cultures

Numerous factors mentioned earlier in this work which effect the malolactic fermentation cause great difficulties in initiating the bacterial growth and the deacidification process in wines. Therefore, attempts have been made to stimulate the malolactic fermentation through bacterial inoculation *(1,6,51-54)*. The most widely used malolactic bacteria are strains of *Leuconostoc oenos* and *Lactobacillus plantarum (37)*.

The question which is still open is the time of inoculation with lactic acid bacteria: before, during or after alcoholic fermentation. The results of Gallander *(53)* indicate that the time of bacterial inoculation had an influence on induction of malolactic fermentation. Although natural fermentation occured in controled wines, it was slower than that in inoculated ones. Addition of *L.oenos* (about 10^8 cells/mL) during and after the alcoholic fermentation

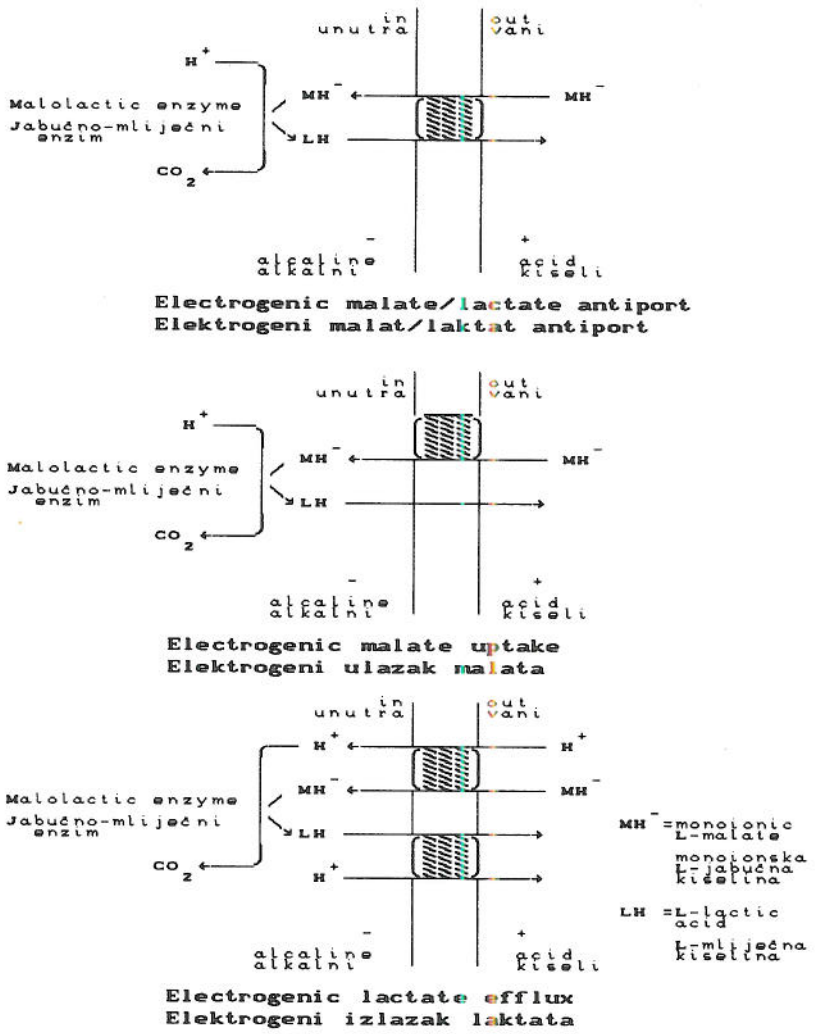

Fig. 2. Possible meshanisms for the generation of metabolic energy by malolactic fermentation *(47)*
Slika 2. Pretpostavljeni mehanizmi proizvodnje energije jabučno-mliječnom fermentacijom *(47)*

resulted in higher rates of malolactic fermentation, which was completed about 80 days earlier than in controlled wines and those inoculated before the alcoholic fermentation. These results do not support the findings of Kunkee et al. *(51)* that the rate of malolactic fermentation showed no dependence upon the time of bacterial inoculation. It seems that, besides the type of wine, the size of an inoculum has a great influence on initiating the malolactic fermentation, and the starter culture should be added to the wine in the minimum concentration of 10^8 cells/mL.

Recently, there has been great interest in the use of immobilized cells of LAB in malolactic fermentation of wines in separate bioreactors *(55-57)* in order to achieve its better control. Fluidized-bed reactor is the one mentioned in literature, and cells were immobilized in calcium alginate *(56,57)* or in K-carrageenan *(55)*. The immobilized cells showed good efficiency in decreasing L-malate concentration with the conversion ratio of 53.9 % *(55)*.

Because of the increased use of malolactic bacteria inoculations in deacidification of wines, there has been a corresponding increase in the commercial availability of starter cultures. The production of dry active bacterial preparations is extensively studied *(58)* particularly on lactic acid bacteria, because of their wide application in food industry. Kunkee stated *(37)* that there are at least five preparations of malolactic bacteria currently on the USA market, which are usually purchased as lyophilized (i.e. freeze-dried) cultures rather than frozen.

The efficiency of lactic acid bacteria starter culture application in malolactic fermentation of wines would be better if conducted as the pure-culture fermentation. So, the prevention of growth of naturally occuring LAB in musts and wines is necessary. In winemaking, sulphure dioxide is the most important agent to suppress unwanted lactic acid bacteria. However, the use of SO_2 is strictly regulated, and for many years the wine industry has strived to use as little of this aggressive acid as possible.

Recent studies *(59,60)* have shown that indigenous LAB in wines can be inhibited by nisin, which does not affect the sensory characteristics of wines, and as polypeptide bacteriocin is destroyed in the gastrointestinal tract of the wine consumers and has no known toxicity *(61)*. But,

if nisin is used to inhibit the growth of naturally occurring lactic acid bacteria, the nisin-resistant malolactic culture is necessary to conduct the desired malolactic fermentation. The results of Deaschel et al. *(62)* showed that in the presence of nisin the nisin-resistant strain *L.oenos* Ey2d-NR1 was able to grow and use more than 95 % of malic acid, whereas a sensitive parent was inhibited. Nisin-resistant strain was obtained by the stepwise exposure of *L.oenos* Ey2d to increasing concentration of nisin (from 5 to 100 U/mL).

The observations of these authors demonstrated that nisin and nisin-resistant strains of desirable malolactic bacteria can be used to promote a pure-culture malolactic fermentation in the presence of other lactic acid bacteria.

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Utjecaj dodataka na procese zrenja fermentiranih kobasica

The Influence of Additives on the Ripening of Fermented Sausages

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Sažetak

Brz razvoj biotehnoloških znanosti te procesne i mjerne tehnike doprinio je objašnjavanju procesa zrenja fermentiranih kobasica u njihovu uvođenju u industrijsku proizvodnju. U proizvodnji fermentiranih kobasica mišićno i masno tkivo su supstrati na kojima se zbivaju brojni procesi uzrokovani ili ubrzani međudjelovanjem prirodnih aktivnih sastojaka nadjeva i dodataka.

U radu su prikazane novije spoznaje o svojstvima i međuovisnosti pojedinih dodataka: starter-kultura mikroorganizama, šećera, kuhinjske soli, nitrata, nitrita i začina s osnovnim sastojcima nadjeva. Prijeko je potrebno znati promjene koje nastaju u nadjevu tijekom proizvodnje, to više što se na njihov intenzitet može utjecati brojnim čimbenicima. Samo poznavanjem mehanizama i dinamike promjena, koje se zbivaju u kompleksnoj biološkoj sirovini, mogu se odabrati približno optimalni uvjeti proizvodnje fermentiranih kobasica. Nadalje, prikazane su važnije reakcije u nadjevu ove vrste proizvoda, kao: fermentacija šećera, tvorba proteinskoga gela, nastanak nitrozilmioglobina, razgradnja masti i proteina, koje sve zajednički čine složeni mehanizam pretvorbe sirovog nadjeva u gotov proizvod velike biološke vrijednosti.

Summary

The rapid development of the biotechnological sciences and of processing and measuring techniques has led to a better understanding of the ripening process in fermented sausages and to the commercial processing of this group of products. In the production of fermented sausages, the muscles and the fatty tissue are the substrates on which occur numerous processes caused or speeded up by the interaction of the natural active ingredients of the filling and of added ingredients.

Some recent insights into the properties and interdependence of the individual ingredients, i.e. the starter culture of microorganisms, sugar, common salt, nitrate, nitrite and spices, and the basic components of the filling during processing are presented in this paper. A knowledge of the changes that occur in the filling during processing is essential, especially since the intensity of the changes can be influenced by numerous factors. Only a knowledge of the mechanisms and dynamics of the changes that occur in a complex biological raw material makes it possible to select nearly optimal conditions for processing fermented sausages. Furthermore, the more important reactions occurring in the filling of this group of products, such as sugar fermentation, the formation of protein gel, the formation of nitrosylmyoglobin, and the decomposition of fats and proteins, which together make up the complex mechanism of conversion of the raw filling into a finished product of great biological value, are presented.

Uvod

U još nedefiniranoj sistematizaciji sirovih proizvoda od mesa fermentirane su kobasice proizvod koji nije konzerviran toplinom već je donekle fermentiran i osušen kako bi mu se povećala trajnost, a okus i miris tako promijenio da je proizvod primamljiv potrošaču. Proučavanje problematike proizvodnje te vrste proizvoda aktualno je tek posljednjih tridesetaka godina pa je danas najsloženije, a ujedno i najviše proučavano područje suvremene znanosti o mesu. U proizvodnji fermentiranih kobasica najviše su ispitivane biokemijske, fizikalne, kemijske, mikrobiološke, strukturne, organoleptičke i druge promjene sustava koji sadržava meso, masna tkiva i dodatke što zrenjem prelaze u proizvod, namirnicu velike biološke i hranjive vrijednosti. Novije spoznaje s područja biokemije, posebno kemije makromolekula, fizikalne kemije i mikrobiologije omogućile su industrijsku proizvodnju fermentiranih kobasica primjenom starter-kultura mikroorganizama i drugih dodataka. Cilj je ovoga rada sistematizirati svojstva i djelovanje dodataka nadjeva, koji se primjenjuju u industrijskoj proizvodnji na temelju do sada objavljenih podataka u literaturi, te prikazati međudjelovanje dodataka i osnovnih sastojaka, potrebnih za dobivanje proizvoda određene i stalne kakvoće.

Izbor sirovina i sastav nadjeva

Uporaba odgovarajućih sirovina i dodataka jedan je od osnovnih uvjeta za izradu fermentiranih kobasica dobre kakvoće. Brojni znanstveni radovi obrađuju tu problematiku *(1-8)*. Vrsta mesa i masnog tkiva, stanje u kojem se nalaze prilikom izrade nadjeva, te njihov međuodnos u smjesi također utječu na kakvoću proizvoda. Dodatak starter-kultura mikroorganizama, šećera, kuhinjske soli, nitrata, nitrita i drugo znatno utječe na procese u nadjevu *(5,7,9-14)*. Također začini, koji se dodaju da bi se poboljšala senzorna svojstva, mogu pospješiti promjene u nadjevu tijekom zrenja i sušenja *(15,16)*.

Meso i masno tkivo

U tradicionalnoj (uglavnom zanatskoj) proizvodnoj praksi mislilo se da je izbor mesa i masnog tkiva starijih i dobro uhranjenih životinja, najčešće primitivnih pasmina, važan preduvjet za proizvodnju fermentirane kobasice dobre kakvoće *(1,17-19)*. Zbog intenzivnije stočarske proizvodnje, uz istodobno povećanu proizvodnju fermentiranih kobasica, pomanjkalo je tradicionalne sirovine, ali se pokazalo da se proizvod dobre kakvoće može proizvesti i od mesa intenzivno tovljenih životinja plemenitih pasmina *(20,21)*. Bitno je da se za izradu nadjeva upotrebljava dobro ohlađeno meso zdravih životinja s normalno izraženim postmortalnim promjenama *(22-26)*. Masno tkivo treba biti svježe, čvrsto, zrnate konzistencije, dobro ohlađeno bez degradacijskih promjena *(1,21,27)*.

Da bi se postigao što bolji izgled presjeka proizvoda, meso i masno tkivo se namrzavaju prije usitnjavanja i homogenizacije *(6,17)* ili se upotrijebi smrznuta sirovina. Ne smije se koristiti dulje skladišteno smrznuto masno tkivo *(28)* na kojem je došlo do oksidacijskih promjena, posebno kada se u proizvodnji fermentiranih kobasica primjenjuje glukono-delta lakton (GdL) *(29,30)*.

U Italiji, Mađarskoj i Francuskoj najkvalitetnije se fermentirane sušene kobasice proizvode samo od svinjetine i masnog tkiva, a u Njemačkoj uz dodatak 30 % govedine *(28,31)*. U Turskoj se proizvodi suđuk od mesa i masnog tkiva goveda i bivola, a dodaje se i do 10 % ovčjeg masnog tkiva *(32)*.

Odnos mišićnog i masnog tkiva u pripremanju nadjeva ovisi o vrsti kobasice, ali se kobasice dobre kakvoće najčešće proizvode sa 70 % mišićnog i 30 % masnog tkiva *(33)*.

Dodaci

Meso i masno tkivo osnovni su sastojci nadjeva u kojem se zbivaju promjene što uzrokuju nastanak senzornih i nutritivnih svojstava fermentirane kobasice. Promjene ne ovise samo o osnovnom sastavu nadjeva već i o tehnološkim postupcima, a posebno o dodanim tvarima koje utječu na proces prijelaza nadjeva u fermentirani proizvod.

Starter-kulture mikroorganizama

Dok se tradicionalna proizvodnja oslanjala na fermentaciju nadjeva djelovanjem enzima mišićnog tkiva i prirodno prisutnih mikroorganizama ili na inokulaciju nadjeva s kobasicom dobre kakvoće iz prethodne proizvodnje, u posljednje se vrijeme u industrijskoj proizvodnji primjenjuju starter-kulture mikroorganizama. Primjena starter-kultura ima niz prednosti. U prvom redu ubrzava se proces proizvodnje zbog skraćene »lag«-faze rasta mikroorganizama te zbog sigurnije fermentacije kobasica pri višim temperaturama *(34,35)*. Drugi je važan činitelj, koji govori tome u prilog, da poželjni mikroorganizmi starter-kulture brzo prerastu prirodno prisutne mikroorganizme koji mogu uzrokovati nepoželjne promjene u nadjevu ili što više proizvesti toksine *(31)*. Ujedno fermentacija nadjeva dobro proučenim bakterijama, čije je enzimsko djelovanje istraženo, osigurava stalnu i standardnu kakvoću proizvoda.

Bakterije mliječno-kiselinskog vrenja: *Lactobacillus plantarum, L. sake, L. curvatus, Pediococcus acidilactici, P. pentosaceus te Micrococcaceae: Staphylococcus carnosus, S. xylosus, Micrococcus varians (35)* danas su najčešće primjenjivani mikroorganizmi u obliku starter-kultura za pripremu nadjeva.

Mikroorganizmi dodani ili spontano prisutni važan su sastojak nadjeva fermentiranih kobasica. Osim što izravno utječu na intenzitet i kakvoću procesa zrenja i sušenja, produktima svog metabolizma (kombinirano sa spojevima nastalim razgradnjom mišićnog i masnog tkiva) sudjeluju u nastanku karakterističnih svojstava proizvoda. Laktobacili i bakterije porodice *Micrococcaceae* najčešće su i najdjelotvornije starter-kulture koje se dodaju nadjevu fermentiranih kobasica *(36)*. Laktobacili i druge bakterije mliječno-kiselinskog vrenja u prvom redu razgrađuju šećere i snizuju vrijednost pH. Snizivanjem vrijednosti pH utječe se na promjene proteina mesa i inhibiranje rasta nepoželjnih mikroorganizama *(5)*. Brzo snizivanje pH nadjeva na 5,3 inhibira rast bakterija vrsta *Salmonella typhimurium* i *Staphylococcus aureus (9)*. Bakterijske vrste *Lactobacillus sake* i *Lactobacillus carnosus* u nadjevu pri 20 °C dobro inhibiraju rast bakterija roda *Salmonella (10)*. Neki sojevi vrste *L. sake* mogu se primijeniti i kao zaštitne kulture jer sprečavaju rast bakterija vrste *Listeria monocytogenes (9)*. Bakterije porodice *Micrococcaceae* pak potrošnjom kisika i razgradnjom peroksida pomažu stabilizaciji boje te štite masno tkivo od oksidacijskih promjena. Nadalje katalaza-pozitivni koki utječu na lipolitičke promjene u mastima, a time i na poboljšanje okusa i arome kobasica *(2,36,37)*.

Šećeri

Razgradnja šećera u mliječnu kiselinu, djelovanjem bakterija mliječno-kiselinskog vrenja, bit je fermentacije kojom se osigurava zrenje nadjeva. U mesu obično nema dovoljno šećera za brzo postizanje poželjne vrijednosti pH pa se šećeri trebaju dodavati u nadjev. *Lücke* i *Hechelmann (35)* utvrdili su da oko 1 % šećera snizuje vrijednost pH za jedinicu pa se, ovisno o udjelu šećera u mesu te željenoj konačnoj vrijednosti pH, preporučuje dodatak od 0,4 do 0,8 % šećera u nadjev *(6,23,38)*. Da bi se postigao željeni tijek fermentacije, bitno je odabrati vrstu i količinu šećera te odgovarajuću starter-kulturu. To je važno zbog toga jer polagana razgradnja šećera, uz postupno snizivanje vrijednosti pH produljuje vrijeme djelovanja nepoželjnih mikroorganizama.

Naprotiv, prevelika brzina razgradnje šećera i brzo smanjenje pH ispod izoelektrične točke proteina mesa mogu biti uzroci loše konzistencije i arome kobasica *(12)*. Danas se najčešće koriste monosaharidi: (glukoza i galaktoza), disaharidi (saharoza i laktoza) te polisaharid (škrob).

Usporednim ispitivanjem glukoze, saharoze, laktoze, škroba i škrobnog sirupa u istim masenim udjelima *(0,5 %)* pokazalo se da glukoza, saharoza i škrobni sirup brzo snizuju pH uz značajnu proizvodnju mliječne kiseline i stvaranje čvrste konzistencije kobasice *(39)*. Brzina difuzije šećera u mišićnom tkivu nadjeva te njegova razgradnja ovise o veličini molekule šećera i njezinoj topljivosti *(40)*.

Kuhinjska sol

Kuhinjska sol, koje se u nadjev dodaje 2-3 % *(28,31)*, utječe na tvorbu poželjnih organoleptičkih svojstava proizvoda, ali isto tako djeluje na mikrobiološke i fizikalno-kemijske procese u nadjevu tijekom zrenja i sušenja. Kuhinjska sol uglavnom neizravno djeluje na mikroorganizme jer se njezinim dodavanjem u nadjev smanjuje aktivnost vode. Kuhinjska sol masenog udjela 2,5-4,0 % selektivno ihibira rast gram-negativnih bakterija, a ne utječe na razvoj mikrokoka, laktobacila i kvasaca *(41)*. Dokazan je utjecaj kuhinjske soli na konzistenciju i povezanost nadjeva, a kao posljedica reakcija kuhinjske soli i proteina miofilamenata *(42,43)*.

Nitriti i nitrati

Iako se u nadjev dodaje mala količina nitrita i nitrata, oni imaju značajnu ulogu u proizvodnji fermentiranih kobasica. U reakcijskom nizu s mioglobinom, uz kemijske i mikrobne agense, tvore nitrozilmioglobin i tako izravno sudjeluju u nastanku boje proizvoda *(44)*. Osim toga glavnog djelovanja, nije zanemariva ni inhibicija rasta nepoželjnih mikroorganizama uzrokovana nitritom. Utvrđeno je da maseni udjel nitrita, veći od 0,0125 %, inhibira rast salmonela i drugih patogenih bakterija *(31)*. Isto je tako utvrđeno da nitrit doprinosi tvorbi poželjnog okusa i mirisa fermentiranih kobasica *(45)*. Optimalni je maseni udjel nitrita u nadjevu 0,010-0,015 % *(46)*, iako postoje podaci da bi zadovoljili i manji udjeli *(47)*. Preveliki su udjeli nitrita nepoželjni jer preostali nitrit može reagirati s proteinima mesa uz tvorbu nitrozilamina, za koje se znade da su kancerogeni.

Začini

Začini se dodaju da bi se poboljšao okus i miris proizvoda *(48)*. Ovisno o vrsti kobasice dodaje se 1-3 % začina *(31,34)*, uglavnog bijelog i crnog papra, slatke i ljute papire, češnjaka i manji udjeli drugih specifičnih začina *(49)*. Utvrđeno je da najčešće primjenjivani prirodni začini, za razliku od oleoresina, stimuliraju rast bakterija vrste *L. plantarum* i tako ubrzavaju fermentaciju *(50)*. Neki začini ograničavaju rast pojedinih mikroorganizama. Češnjak djeluje inhibitorno na rast patogenih mikroorganizama, kapsicidin iz paprike sprečava razmnožavanje kvasaca, a piperin iz papra rast *E. coli (15)*. Začini djeluju i antioksidacijski i tako sprečavaju ranu užeženost proizvoda *(16)*.

Ostali dodaci

U nadjev se mogu dodavati i neki drugi sastojci kako bi se poboljšala kakvoća proizvoda i brzina njihove proizvodnje.

Tako se dodatkom od 0,3 do 0,8 % GdL-a, već za nekoliko sati, snizuje vrijednost pH nadjeva.

Sve većom primjenom starter-kultura manje se koristi dodatak GdL-a jer fermentirane kobasice proizvedene sa starter-kulturama imaju bolja organoleptička svojstva od proizvoda pripravljenih s dodatkom GdL-a.

Udjel od 0,01 do 0,05 % askorbinske kiseline ili njezine soli može se dodavati u nadjev da bi se brže postigla željena boja salamurenog mesa te bolje održala boja proizvoda *(51)*.

Dodatak natrij-glutaminata od 0,1 do 0,3 % omogućava bolji okus proizvoda *(51)*. U posljednje vrijeme ispituje se utjecaj dodataka lipaze pankreasa *(52)*, zatim lipaze i proteinaze bakterija mliječno-kiselinskog vrenja na tijek fermentacije te kakvoću proizvoda *(53)*.

Nadjev pripremljen od osnovnih sirovina i ostalih dodataka, kao heterogena masa, puni se u ovitke različitih promjera u kojima fermentira sirovi nadjev u proizvod pod određenim i strogo kontroliranim mikroklimatskim uvjetima, kao što su temperatura, relativna vlažnost, cirkulacija i izmjena zraka.

Promjene tijekom proizvodnje

Promjene su u nadjevu dvostupanjske. U prvom stupnju koji je kraći, ali kudikamo intenzivniji, osnovni je proces (popraćen značajnim učincima) fermentacija šećera u mliječnu kiselinu i dehidratacija. U drugom stupnju, duljem po trajanju, uz daljnji gubitak vode iz nadjeva postupno nastaju organoleptičke promjene značajne za kakvoću proizvoda.

Primjenom starter-kultura u nadjev se unose bakterije mliječno-kiselinskog vrenja i veliki broj mikrokoka. Aktivnost unesenih bakterija mliječno-kiselinskog vrenja ovisi u prvom redu o temperaturi, vrijednosti pH, udjelu šećera i kuhinjske soli *(54)*. Pripremljen od ohlađene ili čak smrznute sirovine, nadjev se izlaže temperaturi od 15 do 24 °C, povoljnoj za djelovanje i razmnožavanje mikroorganizama. Bakterije su neravnomjerno razdijeljene u nadjevu, a nalaze se u nakupinama međusobno udaljenim 0,1- 5,0 mm. Prelaskom miofibrilarnih proteina iz otopljenog stanja u gel-stanje, imobiliziraju se bakterije u šupljinama u kojima se zbiva fermentacija šećera i drugi procesi što utječu na zrenje i razvoj organoleptičkih svojstava proizvoda. Brzina fermentacije u takvu modelu ovisi o difuziji šećera, uvjetovanoj vrstom šećera te brzinom izlaska mliječne kiseline iz nakupina *(55)*. Fermentacijom nastala mliječna kiselina snizuje pH od početne vrijednosti 5,8-6,0 na vrijednost 5,3-5,4 za što su pri 22 °C potrebna dva dana *(56,57)*. Dakako, ovisno o početnom udjelu šećera, fermentacija se može nastaviti, tako da konačna vrijednost pH nadjeva može biti niža od 5,0 *(58)*.

Sniženje pH nadjeva na vrijednost 5,3-5,4 značajno je radi nekoliko popratnih pojava. Pri nižoj vrijednosti pH (5,4) inhibira se većina nepoželjnih mikroorganizama, čime se ostvaruje sigurnost proizvodnje *(9)*. Posebno je to značajno za inhibiciju vrsta stafilokoka čiji se rast može inhibirati niskim vrijednostima pH, a ne može smanjivanjem aktivnosti vode u nadjevu *(59)*.

U području izoelektrične točke proteini mesa (pH= =5,3-5,4) prelaze u gel-stanje u kojem pokazuju najmanju sposobnost vezanja vode, čime je stvorena predispozicija za odvajanje vode iz nadjeva sušenjem *(60)*. Isto su tako ljepljivi proteini u gel-stanju pa utječu na povezivanje mi-

šićnog i masnog tkiva i na nastanak poželjne konzistencije proizvoda. Pri povećanom početnom udjelu šećera i sniženoj vrijednosti pH ispod 5,0 povećava se sposobnost hidratacije proteina. Istodobno se gel djelomično otapa, što uzrokuje manji gubitak mase tijekom proizvodnje i mekšu konzistenciju proizvoda.

U nadjevu se oksimioglobin oksidira preko mioglobina u metmioglobin. Reakcija je ubrzana i djelovanjem nitrita koji prelazi u nitrat. Djelovanjem enzima mikrokoka nitrat ponovno prelazi u nitrit, a sniženjem vrijednosti pH ispod 5,5 nitriti se enzimski dalje razgrađuju do dušik-monoksida. Nastali se dušik-monoksid veže na metmioglobin uz stvaranje nitrozilmetmioglobina koji se u mesu pretvara u nitrozilmioglobin, poželjni pigment fermentiranih kobasica *(61,62)*.

Istodobno reakcijama uzrokovanim fermentacijom šećera i sniženjem pH nastaju promjene na mišićnom i masnom tkivu, bitne za nastanak organoleptičkih i strukturnih svojstava proizvoda. Započete reakcije u nadjevu na mišićnom i masnom tkivu, usmjerene i ubrzane u prvom stupnju zrenja, nastavljaju se u drugom stupnju, kada dolazi do postupne difuzije vode iz nadjeva i sušenja proizvoda.

Pezacki *(42)* upućuje na mogući put promjena proteina mišićnog tkiva tijekom proizvodnje fermentiranih kobasica. Po tom se modelu proteini (topljivi pri pH=5,5) razgrađuju, a razgradni produkti mogu utjecati na aromu proizvoda. Pretpostavlja se da netopljivi proteini utječu na konzistenciju proizvoda. Interfibrilarni netopljivi proteini (kolagen i elastin) ne razgrađuju se tijekom zrenja pa se povećava njihov relativni udio. Intrafibrilarni netopljivi proteini sarkoplazme i miofibrila intenzivnije se razgrađuju dok se ne počne stvarati gel, a zatim je razgradnja usporena *(42)*.

Razgradnja proteina mišićnog tkiva tijekom zrenja ove vrste proizvoda dokazana je stalnim povećanjem slobodnih aminokiselina i neproteinskih dušikovih spojeva *(63)*.

Najčešće se pojavljuju ove aminokiseline: alanin, leucin, izoleucin i glutaminska kiselina *(64)*. Dio slobodnih aminokiselina razgrađuje se osobito pri kraju zrenja uz nastanak amonijaka, a dio se može dekarboksilirati u nepoželjne biogene amine, u prvom redu histamin. Utvrđeno je da udjel histamina u sušenim fermentiranim kobasicama ovisi o slobodnom histidinu kao supstratu i mikroorganizmima koji omogućuju proizvodnju histamina te o starosti mesa upotrijebljenog u proizvodnji *(65)*.

Tijekom zrenja zbivaju se značajne promjene i u masnom tkivu *(8,29)*. Posebno su važne hidrolitičke promjene zbog djelovanja tkivnih lipaza i lipaza mikrokoka *(66)*. Djelovanjem enzima postupno se razgrađuju neutralne masti, esteri kolesterola i fosfolipidi pa nastaju nezasićene, a djelomično i zasićene masne kiseline. Masne kiseline pomoću enzima mogu prijeći u hlapljive karbonile te doprinijeti nastanku karakteristične arome fermentiranih kobasica *(48,67,68)*.

Reakcije oksidacije nezasićenih masnih kiselina, čiji produkti daju pretežito nepoželjan okus, slabije su izražene u fermentiranim kobasicama tijekom zrenja i skladištenja, što se može povezati s antioksidacijskim djelovanjem dima i dodataka *(8,36)*.

Usporedo s navedenim promjenama nadjev se postupno dehidrira tijekom procesa proizvodnje. Dehidratacija nadjeva ovisi u prvom redu o vanjskim činiteljima: temperaturi, relativnoj vlažnosti i cirkulaciji zraka, a zatim o promjeru crijeva, ali i o sastavu nadjeva, najčešće o omjeru mišićnog i masnog tkiva. Stupanj dehidratacije je veći što nadjev sadržava manje masnog tkiva *(69)*. Rezultat dehidratacije je postupno smanjivanje aktivnosti vode (a_v) u nadjevu, koji se tijekom proizvodnje smanjuje od početne oko 0,97 do vrijednosti manje od 0,88 na kraju proizvodnje *(58,70)*.

Vrijednosti aktivnosti vode u nadjevu i pH zacijelo su najznačajniji čimbenici koji osiguravaju uspješnu proizvodnju i stabilnost fermentiranih kobasica. Manja aktivnost vode i niže vrijednosti pH inhibiraju rast nepoželjnih mikroorganizama. Vrijednost pH ima i dodatno značenje jer su sve reakcije u nadjevu na neki način vezane uz promjene pH, što se može vidjeti iz navedenog pregleda znanstvenih radova.

Od reakcija koje izravno utječu na promjene pH, u prvom redu to je fermentacija šećera u mliječnu kiselinu *(56-58)*. Zatim, hidrolizom masti nastaju slobodne masne kiseline, što također utječe na promjenu pH *(66)*. Neznatno povećanje vrijednosti pH pri kraju proizvodnje fermentiranih sušenih kobasica rezultat je stvaranja amonijaka razgradnjom aminokiselina *(7,70)*.

S druge strane, postoje reakcije koje se ne bi provodile u drugim pH-područjima. To su najčešće one što omogućuju da proteini iz topljivog prijeđu u gel-stanje te reakcije nastanka nitrozilmioglobina *(60,62)*. Stoga je tijekom proizvodnje važno pratiti promjene vrijednosti pH nadjeva jer ono upućuje na tijek i stupanj kemijskih, biokemijskih i mikrobioloških procesa.

Navedene promjene na mišićnom i masnom tkivu, fermentacija šećera i stvaranje poželjne boje nadjeva fermentiranih kobasica samo su dio kompleksnog mehanizma reakcija u proizvodnji te vrste proizvoda. Poznavanje tog osnovnog mehanizma značajno je zbog tehnološkog vođenja procesa i izbora odgovarajućih vanjskih mikroklimatskih i drugih čimbenika. Zadatak je tehnologa da njihovim pravilnim odabirom, primjenom suvremenih procesnih i mjernih postupaka te tehnologije, usmjerava tijek promjena u poželjnom smjeru.

Zaključak

U novije je vrijeme ostvaren značajan napredak u proizvodnji fermentiranih kobasica na temelju dobrog poznavanja procesa proizvodnje. Najbolje je objašnjena primjena određenih dodataka koji pospješuju proces zrenja i dobivanje proizvoda bolje kakvoće i veće stabilnosti.

Posebno je istaknuta važnost kontrolirane uporabe šećera i starter-kultura te kontrola pH, kao pokazatelja uspješno vođenog fermentacijskog procesa.

Novije spoznaje te odabir sirovina i dodataka temelji su uspješno provedenog tehnološkog procesa proizvodnje fermentiranih kobasica, što je danas osobito značajno zbog povećanih zahtjeva na tržištu hrane i sve veće potražnje takvih proizvoda.

Extended Abstract

Some recent insights in the field of fermented sausages production are presented in this paper, with special

reference to the use of additives and to the processes which make up the complex mechanism of conversion of the raw filling into the final product.

The basic prerequisite for a high-quality product is the use of suitable raw materials and additives. Meat and fatty tissue are the basic ingredients of the filling. These are used well cooled or almost frozen.

Changes in the filling depend, among other factors, on the presence of added substances which influence the process by which the filling becomes the fermented product. Foremost among these are starter cultures of microorganisms. The starter cultures most frequently added to the filling of fermented sausages, and the most efficient ones, are bacteria belonging to the genus *Lactobacillus* and to the family *Micrococcaceae.*

Lactobacilli and other lactic acid bacteria decompose sugars and cause the pH to drop rapidly to the isoelectric point of the meat protein, which results in desirable changes in the proteins and in the inhibition of the growth of undesirable microorganisms.

Micrococcaceae, by consuming oxygen and decomposing peroxides, help to stabilize the colour and protect the fatty tissue from oxidative changes.

Meat does not contain sufficient sugar for a normal course of fermentation and for the development of a desirable pH. For this reason, sugar is added to the filling of fermented sausages in a mass fraction of 0.4 to 0.8 %.

Common salt is added to the filling in a mass fraction of 2 to 3 %. Apart from providing desirable organoleptic properties, it reduces water activity and contributes to the microbiological stability of the product. Furthermore, common salt triggers the extraction of myosin, thus helping to improve the cohesion of the filling.

Nitrates and nitrites, added to the filling in a mass fraction of 0.010 to 0.0125 %, are directly involved in the formation of the colour of fermented sausages, as well as inhibiting the growth of some pathogenic microorganisms.

Spices, used in a mass fraction of 1 to 3 %, improve the sensory properties of the product, but they may also, depending on the kind of spice, have an inhibitory influence on the growth of microorganisms.

Some other components are also added to the filling of fermented sausages to decrease the pH of the filling. The advantages and disadvantages of GdL are pointed out. Ascorbic acid may be added in a mass fraction of 0.1 to 0.5 % to speed up the pickling process and to preserve the colour, while 0.1 to 0.3 % of sodium glutaminate may be added to enhance the taste.

Research is also being carried out into the effects of the addition of the enzymes lipase and proteinase on the course of fermentation and on the product quality.

Changes in the filling of fermented sausages take place in two main phases. In the first phase, which is shorter but much more intensive, the basic process which has significant side effects is the fermentation of sugar into lactic acid. During the second phase, which lasts longer, the loss of water from the filling is accompanied by the formation of the organoleptic characteristics of the final product.

In the first phase, the starter cultures of microorganisms are activated by exposing the filling to increased temperatures. Sugar fermentation then starts and the pH is reduced to 5.3 or 5.4. At this pH level most undesirable microorganisms are inhibited and proteins pass into a gel state, losing their ability to bind water, which facilitates the next phase of drying the filling. The drop in pH, as well as the activity of the micrococcal enzymes, creates the conditions required for nitrite and nitrate to react with myoglobin and form nitrosyl myoglobin.

Parallel with reactions connected to sugar fermentation and pH reduction, reactions take place in the muscle and fatty tissue which are essential for the development of desirable organoleptic properties in the product. The reactions in the muscle and fatty tissue start before the filling is made and are speeded up and channeled into a desirable direction in the filling; they become prominent in the second phase of fermentation when a gradual reduction of the relative humidity in the environment leads to water separation and the drying of the filling.

The soluble proteins of muscle tissue are completely decomposed during fermentation, while the insoluble proteins make up the consistency of their product and their decomposition is less marked. The decomposition of muscle tissue proteins is evident from the constant increase of free amino acids and non-protein nitrogen compounds during the fermentation of dry sausages.

During fermentation, significant changes also take place in the fatty tissue. The hydrolytic changes which have a direct effect on the development of a characteristic and desirable aroma in fermented sausages are especially important.

During processing, the filling is gradually dehydrated, which leads to a reduction in water activity. The dehydration of the filling depends primarily on external factors, i.e. the relative humidity and circulation of the air, as well as on the sausage diameter and the composition of the filling, primarily the ratio of muscle tissue to fatty tissue.

The water activity and pH values are the most important factors in the processing of fermented sausages, since low values inhibit the growth of undesirable microorganisms. The pH has an additional significance because all the reactions taking place in the filling are connected in some way to changes in the pH.

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