Application of hop β-acids and rosin acids in the sugar industry

Anwendung von Hopfen-β-Säuren und Harzsäuren in der Zuckerindustrie

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In 1994, hop products were successfully used for the first time to combat bacteria in beet extraction. This was a totally new field for the use of hop products, compared with their traditional use in breweries. Today an alkaline solution of hop β-acids is used in sugar factories under the trademark "BetaStab". Hop β-acids have turned out to be very effective against formation of NO_2 and anaerobic infections in tower extractors, which are often operated intentionally with lactic acid fermentation. Hop β-acids have additionally proved effective in the field of thick juice storage. Sometimes, in the case of lactic acid control, a selection of less sensitive organisms is observed and a second disinfectant has to be used, alternating with hops. Thus an idea was born to use rosin acids as a further harmless natural biocide. Results from laboratory trials, full scale trials and some first studies on residues are presented. Rosin acids show a potential to be used in the sugar industry, either alternating with hop products or to create products which are more cost effective.

Keywords: Hop acids, rosin acids, abietic acid, biocides, Bacillus, *Leuconostoc*

Im Jahre 1994 wurden erstmals Hopfenprodukte zur Bekämpfung von Bakterien in der Rübenextraktion erfolgreich eingesetzt. Dies stellte im Vergleich zur traditionellen Anwendung von Hopfenprodukten in Brauereien ein völlig neues Einsatzgebiet dar. Heute wird eine alkalische Lösung von Hopfen-β-Säuren unter der Marke „BetaStab" in Zuckerfabriken eingesetzt. Hopfen-β-Säuren erwiesen sich als sehr effektiv gegen Nitritbildung und anaerobe Infektionen in Extraktionstürmen, welche oft mit absichtlich zugelassenen Milchsäuregärungen betrieben werden. Zusätzlich haben sich die Hopfen-β-Säuren auf dem Gebiet der Dicksaftlagerung als wirksam erwiesen. Manchmal wird bei der Bekämpfung von Milchsäure eine Selektion von weniger empfindlichen Organismen beobachtet und es muss, alternierend mit Hopfenprodukten, ein zweites Mittel eingesetzt werden. Daher wurde die Idee geboren, Harzsäuren als weiteres gefahrloses, natürliches Biozid einzusetzen. Es werden Ergebnisse von Laborversuchen, großtechnischen Versuchen und einige erste Rückstands-Studien präsentiert. Harzsäuren stellen ein weiteres Potenzial für die Zuckerindustrie dar und könnten entweder alternierend mit Hopfenprodukten oder zur Formulierung von kostengünstigeren Kombinationsprodukten eingesetzt werden.

Stichwörter: Hopfensäuren, Harzsäuren, Abietinsäure, Biozide, Bacillus, *Leuconostoc*

1 Introduction

In 1994 the first experiments with hop β-acids were carried out at the Agrana factory Tulln, to control bacteria in beet extraction [1, 2]. Agrana voluntarily abstained from using formalin and dithiocarbamates in sugar factories in 1991, when formalin hit the headlines due to emissions from chipboards. It was not always possible to eliminate bacterial problems simply by using higher extraction temperatures, so the idea to use hop β-acids in sugar factories occurred at the right moment. These natural water-insoluble hop-components are considered harmless for human beings and mammals and are regularly consumed by people favoring turbid white beer. By now, these β-acids have already been used in the sugar industry for as long as 8 years. Compared with an 800-year tradition of hopped beer, obviously hop

Fig. 1: New useful plants for the sugar industry (left: hop plant, right: pine tree)

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acids in the sugar industry do not have an excessively long history, but at least some international experience already exists.
On the other hand, the use of rosin acids for bacterial control in the sugar industry is quite a new idea without any international experience. Rosin acids from pine trees (Fig. 1) would fit well into a range of natural products for bacterial control and the original idea was derived from the Greek "Retsina" wine, produced with pine gum from the Aleppo-pine. The pine gum is added during fermentation and is effective against oxidation and acetic acid production under hot climate conditions [3]. After 2000 years of tradition, this Retsina wine with its characteristic taste even tops the long history of hopped beer. Fortunately, the non-volatile, water-insoluble fraction of pine gum, the so-called "rosin acids" (or "resin acids"), show a similar bacteriostatic effect to hop β-acids, although at higher concentrations. It was preferred to start work with these rosin acids, to be sure that white sugar will not show any characteristic odor of turpentine oil, the volatile fraction of pine gum.

2 Retrospective view on hop application in the sugar industry

Most of the scientific papers dealing with the mode of action of hop acids on bacteria were published within brewing science. *Shimwell* [4] found a parallelism between bacterial sensitivity against hop acids and staining properties and also the great importance of pH value on the effect. Gram-positive bacteria are affected by hop acids, but not spores and Gram-negative bacteria, although there are some exceptions [5]. Fortunately, most of the known bacterial species, growing in hot juices of beet sugar factories, are Gram-positive or at least sensitive against hop β-acids. In recent decades, scientific papers explained the effect of hop acids on bacteria as a damaging effect on the function of the bacterial membrane [6] and as lowering of the intracellular pH value [7]. A more detailed quotation of papers outside the sugar industry was given in the first paper about hop application in the sugar industry [2].
After the first paper on hop application, eight further lectures or posters have been presented at conferences or meetings, where at least summaries have been published [8–15]. Unpublished information from BetaStab customers is regarded as confidential. In the earlier papers a so-called "Baseextract" was used in emulsified form [2, 8–9], containing roughly 50% β-acids, the least soluble but most effective hop acids. Later on β-acids were isolated and offered as clear, well-defined and even more effective alkaline solution, which today is well-known as "BetaStab 10A" or simply "BetaStab".
Further practical experience can be drawn from publications of other authors, who worked under different conditions rather than from a lot of repetitions in one country. Lowering of lactic acid levels is priority in factories without severe microbial problems, in order to reduce sugar losses. As lactic acid production is possible for a lot of bacterial species, it is imaginable that less sensitive organisms occur, which cannot be controlled by minimal inhibitory concentrations, but only by shocks with high concentrations.

2.1 "Sterile" beet extraction with lactic acid suppression

A very good result for a DDS extractor was reached in 1998 in an Italian factory [12], operated with 250 mg/kg of SO_2 on beet. For this trial BetaStab emulsion was still used. The emulsion was poured into press water, port 2 and port 3, dependent on individual lactic acid and NO_2 content. This led to a ten days average of 68 mg/kg of lactic acid in raw juice, which was very close to the beet values. If a tower extraction is kept as "sterile" as possible, shock application to, at least, press water, mid-tower position and to cossette/juice pump are required (Fig. 2). Dosing at mid-tower requires an especially good distribution between the outside wall and central cylinder, while the distribution around the periphery will arise from rotation.

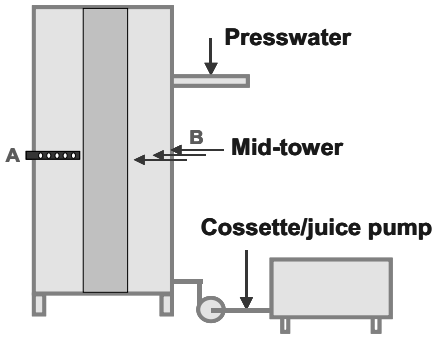

Fig. 2: "Sterile" beet extraction with lactic acid suppression – Addition points for β-acids (A + B see text)

Tubes with several holes, leading from the wall close to the central cylinder and shown as variant A, were often installed for dosing of formalin. If shocks with lower volumes of BetaStab are not heavy enough, the inner holes will not be served and biofilms on the inner part of the tower will not be affected. Continuous dilution of BetaStab with water during shocks may improve the distribution. In Austria there are three tubes, shown as variant B in Figure 2, with different lengths, which are served alternatively by valves in periods of 20 s.
During the first days of a trial, BetaStab is often very effective with a single dosing point at the mixer, because traces of β-acids are carried upwards with the cossettes and dissolved in the juice. The minimal inhibitory concentration for bacteria, which are not able to grow in presence of hop β-acids, will be exceeded in favorable cases. If the effect diminishes, the existing know-how about dosing will be important. Installations can be improved after the campaign, but usually not during trials. If the knowledge about effective dosing does not help, a rotating use of two different biocides may do. For example, a combination of BetaStab and ABS (ammonium-bi-sulfite or -hydrogen-sulfite) has been used in such a way, as reported by *M. Fowers* [14].

2.2 Beet extraction with intentional lactic acid fermentation

More and more European sugar factories use micro-organisms in extraction towers to improve pulp pressing. A so-called homo-fermentative lactic acid fermentation without gas formation would be welcome in the upper part of the tower (Fig. 3).
Therefore no biocides are added to this upper part. A certain amount of lactic acid, formed in this part, will appear in the raw juice and has to be accepted. Normally Austrian factories are satisfied with a maximum of 400 mg/L of lactic acid in the raw juice. According to Austrian findings, six shocks a day at mid-tower position and fewer shocks at the cossette/juice-pump are used to limit excessive lactic acid formation. As the upper part of the tower acts as inoculum for the lower part, it is not always possible to get a lowering of lactic acid contents with an economically acceptable dosage of hop β-acids.

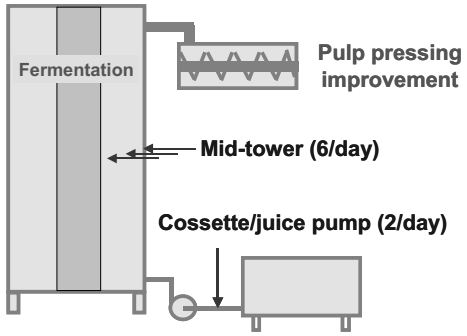

Fig. 3: Beet extraction with intentional lactic acid fermentation – Additions points of hop β-acids

2.3 Lactic acid fermentation and misfermentation

In contrast to the Italian DDS extraction already mentioned, lowering of lactic acid content nearly failed in a Czech DDS trough, operated with sulfuric acid addition to the fresh water [8]. Additionally, the press water temperature was low and a lot of D-lactic acid was produced, indicating a growth of Lactobacilli. But the authors learned a lot about misfermentation and specific effects of hop acids on the occasion of this failure.

Acetic acid and NO_2 contents dropped in parallel to very low levels, and in an earlier paper the authors were able to show that they are connected to each other [11]. Recently a pathway was found, reported for *Escherichia coli*, which shows a production of acetic acid and CO_2 as a consequence of NO_2 formation [16, 17] and which fits well to the observation.

In contrast to lactic acid, NO_2 is completely unwanted in the sugar industry and thus is a product of misfermentation, which can be avoided by hop addition. A further degradation of NO_2 leads to a heavy gas formation, as already shown by *Carruthers* et al. [18]. The intensity will be dependent on the properties of the actual factory flora, growing in lower parts of the tower and in the mixer. This gas formation will be inhibited by blocking the formation of NO_2 with BetaStab. German reports [13] about an economic drop in antifoam consumption on BetaStab addition could be explained by this mechanism. But this is not definite, because a second type of gas forming infection occurs in extraction towers, which does not use nitrate: It is the hydrogen producing Clostridia-type (Fig. 4), with the new taxonomic designation "Thermoanaerobacter(ium)" [19, 20].

As the good effect against NO_2 formation was connected to acetic acid formation, the question was if hop products could suppress other acetic acid forming mechanisms, as for example in Clostridia, in the same way. If an important pathway of Clostridia is blocked, their growth and all these unwanted acids and gases would disappear. Indeed it was possible to convert a fermentation,

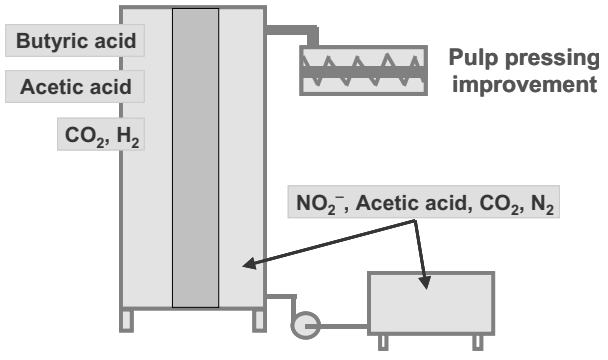

Fig. 4: Points of metabolite formation in tower extractors

caused by Clostridia, to a lactic acid fermentation [11]. Low dosing rates or shocks are necessary to maintain the lactic acid fermentation.

The well-known selection of alcoholic fermentation by yeasts over other fermentation was the original purpose of hops for beer and pine gum for wine. This is comparable to a selection of lactic acid fermentation over an unfavorable misfermentation in the sugar industry. Normally no drop in effect will appear because of a common effect of hop β-acids and lactic acid formation. It is impossible to get a similar effect with formalin, which is one of the least selective biocides [21].

2.4 Effects under slight alkaline conditions

Under slight alkaline conditions hop β-acids are more dissociated and thus should be less effective. But they are more soluble and have shown surprising effects on control of *Thermus* in thin juice, which passed an ion exchange column and lead to increase of NO_2 contents (Fig. 5) [2, 5].

This positive effect under slight alkaline conditions was encouraging to start trials in another field of slight alkaline conditions: Thick juice storage (Fig. 5). BetaStab was a little more effective against invert sugar formation than against a pH value drop [15]. As a detectable invert sugar formation is an extracellular reaction, the question arises if the mechanism of hop β-acids is particularly effective against excretion of enzymes by bacterial cells.

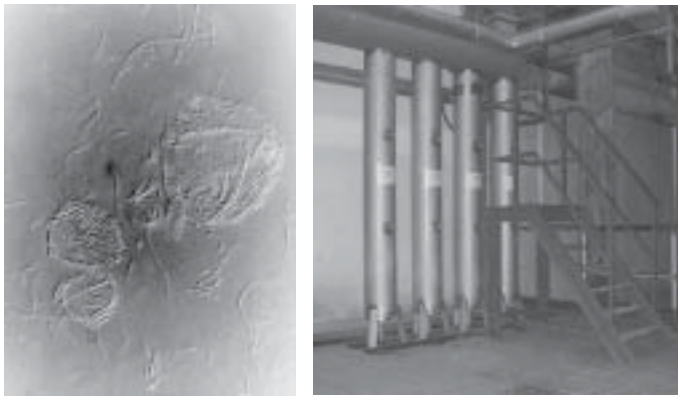

Fig. 5: Effects under slight alkaline conditions (left: control of *Thermus* sp.; right: thick juice strorage trial)

2.5 Problems with dextran in a Czech factory

There is a very important excretion of a bacterial enzyme for the sugar industry, which appears in raw juice as well as under slight alkaline conditions of preliming: It is the dextran-forming hexosyl-transferase from *Leuconostoc*. In a Czech factory problems with dextran, stemming from this organism, occurred in spite of routine disinfection with formalin and other biocides [8], but disappeared after application of BetaStab. As manual work was necessary to remove dextran pellets (Fig. 6), BetaStab was very welcome.

Till now two observations from two campaigns exist, but the problems did not come back after dosing was stopped. As a return of infection is important to get repeated blanks, analytical results from these full scale trials in the experimental part cannot be shown. But for completion of the review and with respect to the importance for cane sugar manufacture the observations about polysaccharide inhibition should be mentioned and a preliminary laboratory trial will be shown in the experimental part.

Fig. 6: Problems with dextran in a Czech factory

3 Information on rosin and rosin acids

3.1 Pine gum components

Of course, a lot of progress is still necessary to put this new idea into practice and possible handicaps should be found out as soon as possible. The authors hope that the "family of hop-users", who may have benefited from this new variant of natural biocide, will contribute in the same way as for hop application in the past.
In contrast to many recently developed chemical biocides, a mixture of rosin acids is already well-known in many applications, but not in the sugar industry. Gum products from the pine tree are distilled into about 20% volatile and 80% non-volatile components. The volatile fraction is called "turpentine oil". The mixture of rosin acids is called rosin or often "colophony" (Fig. 7, on the right), and this popular name should be at least mentioned here.

Fig. 7: Volatile and non-volatile pine gum fractions: turpentine oil and colophony

Turpentine oil, containing the volatile fraction of pine gum with the main components α-pinene and β-pinene (Fig. 8), is harmful to skin and if swallowed neat. It is included in the European Union Biocide List. As already mentioned, it was not used for sugar manufacture to avoid its strong characteristic odor. On the other hand, the particular effect of abietic acid (Fig. 8) and their isomers (Fig. 9) against thermophiles is not known in the food industry.
A patent shows minimum inhibitory concentrations of 20 mg/kg for abietic acid against acne bacterium [22]. In the field of food technology no information could be found and therefore patents have been filed [23, 24].
In literature, no information comparable to brewing science was found which explains the effect of rosin acids on bacteria. But scientific papers dealing with properties of rosin acids, present in waste water of paper mills, as they are toxic for fish are available.

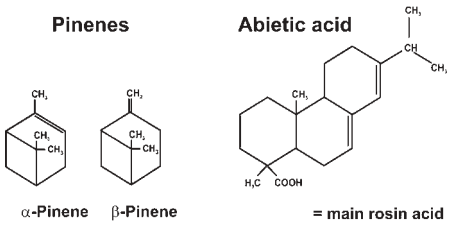

Fig. 8: Volatile and non-volatile pine gum components

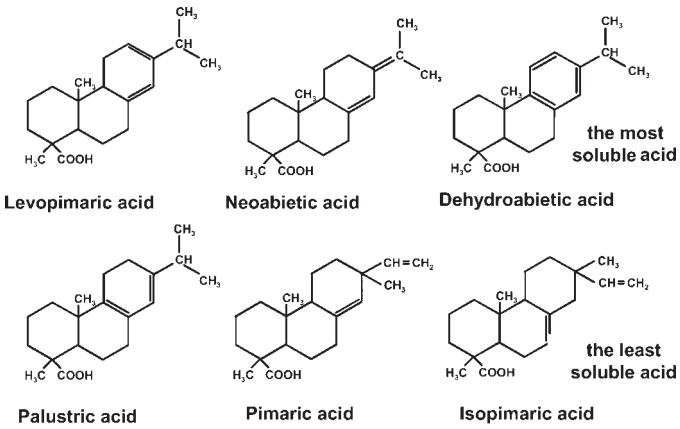

Fig. 9: Abietic acid isomers and dehydroabietic acid

It is stated that isopimaric acid is the most toxic and least soluble acid, whilst dehydroabietic acid is the least toxic and most soluble one (Fig. 9) [25]. This shows a parallel behavior to hop products and their effect against bacteria, although different biological contact surfaces (gills and bacterial membranes) are influenced. More papers have been published about degradation of rosin acids by bacteria, demonstrating their natural character [26–28].

3.2 Toxicology of rosin products

Indeed, the statement shown as Figure 10 about toxicology of rosin products [29] doesn't show much danger for rosin acids. "Harmless" and "nontoxic", even "beneficial" are used as attributes. Besides Retsina wine, chewing gum is mentioned as a foodstuff and people will extract rosin acid derivatives and traces of rosin acids on chewing. Additionally, although not mentioned here, barrels for beer were covered inside with brewer's pitch, consisting of paraffin and rosin acids [30]. Long term studies of more than 25 years did not show health risks. However, rosin dust may be an allergic risk for some people.

Ullmann, 1993, Volume A23, Page 87:

„Native rosin can be regarded as harmless and nontoxic. This also applies to many of its derivatives and modified rosins. Rosin esters are used, for example, in chewing gum, and rosin wine (retsina) is considered to be particularly beneficial.

About a hundred workers, who were in daily contact with a large number of modified rosins, have been checked over a period of more than 25 years. No damaging effects on health could be established.

Allergic reactions of certain people cannot, however, be ruled out. There is no evidence that allergy initiation can be attributed to any toxicological properties of rosin."

Fig. 10: Toxicology of rosin products

In the sugar industry rosin acids will be used as alkaline solution, similar to BetaStab. Thus allergy risks from dust will be kept away from sugar factory premises and considering all the other statements, a certification process for the sugar industry should not meet with big difficulties.

Till now only little is known on the effect on rosin acids on the feed quality of beet pulp. But as rosin acids are harmless for human beings, traces in pulp may be harmless for animals, compared to traces of chemical biocides which are even active at stomach temperature. Even a beneficial effect could be possible, as reported for Retsina wine and human beings (Fig. 10) [29] or for larch tree extracts and pigs [31].

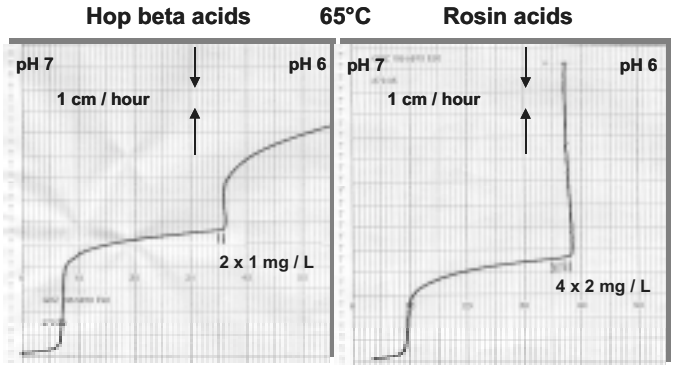

Fig. 12: pH curve after addition of hop β-acids and rosin acids

4 Experimental part

4.1 Materials and methods

Detailed information about materials and methods is given in the appendix.

The laboratory trials were carried out in sterile glass vessels with pH value recording and slow magnetic stirring, in order to avoid artificial aeration and to simulate anaerobic conditions in extraction towers. In addition to the most important information from pH value recording (Fig. 11), samples were drawn for manual measurement of optical density and for bacterial staining. The laboratory trials were carried out with clear liquid nutrients as usual in microbiology, inoculated with pure strains or simply with raw juice.

Parallel to these laboratory trials full scale trials have been carried out, with measurement of relevant parameters, such as lactic acid and NO_2. Considering the resources and the problems in Austrian factories, no polysaccharides have been determined till now.

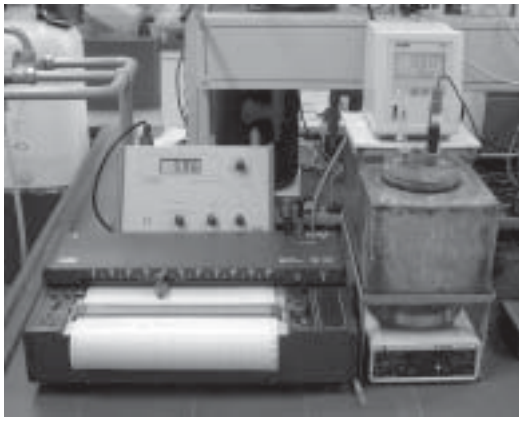

Fig. 11: Equipment for laboratory trials

4.2 Results

4.2.1 Results from laboratory trials

Typical results from trials with pH value-recording and raw juice as inoculum are shown in Figure 12. After two additions of 1 mg per kg of β-acids, the pH value drop stops immediately and the curve is stable for about two hours. *Teuber* and *Schmalreck* [6] have used minimum inhibitory concentrations, abbreviated MIC values, for studies on the inhibitory mechanism of hop acids, defined as inhibition during at least 1 h at 37 °C. The inhibition at 65 °C fulfills this time condition.

It is typical for hop products and this experimental model, that MIC values are only effective for some hours and a further drop in pH value will appear. Therefore other sugar scientists may be disappointed, if they want to see an effect from overnight laboratory trials without recording. But in sugar factories shock doses with high local concentration, differing significantly from MIC values, are applied every 4 h and effects are obtained, in spite of this further drop in pH value.

Normally the trials were shut off by a timer after some hours during the evening, when the pen is already outside the scale. It was surprising that in the case of rosin acids the trials were shut off by the timer during an interesting period. For rosin acids higher MIC values are necessary to get a first sharp stop in pH value drop, but no further drop appears, even in overnight trials. A parallel behavior was found in full scale trials.

Table 1 shows MIC values, obtained by the same method, for single rosin acids. The results are in good agreement with statements of an already mentioned paper, dealing with toxicity for fish [25]. Dehydroabietic acid, the most soluble acid, is least toxic. Isopimaric acid, the least soluble, is one of the most toxic acids. Of course the absolute values are dependent on temperature, strain and pH value, but the relative distribution is in agreement.

Table 1: Minimum inhibitory concentration (MIC) values for strain DSM 457, "Titration" method at 65 °C

Rosin acid	MIC-T65	Remarks
Dehydroabietic acid	12	most soluble acid
Abietic acid	8	main rosin acid
Neoabietic acid	8	
Pimaric acid	8	
Palustric acid	8	
Levopimaric acid	6	
Isopimaric acid	6	least soluble acid

Figure 13 shows an important difference between hop β-acids and rosin acids on the one hand and formalin on the other hand. For formalin it would be impossible to determine a minimal inhibitory concentration by titration. After addition of neutralized formalin at 35% of the recorder scale, comparable to the hop trial, the pH value drop was inhibited slowly after some time, showing a curve instead of an angle. As formalin denatures proteins [21], the mechanism is quite different to membrane leakage, as reported for hop β-acids [6]. But after the slow inhibition of the pH value drop no further drop appears during overnight. Glutaraldehyde shows a similar curve, while dithiocarbamates show an almost right angle, similar to hop β-acids and rosin acids.

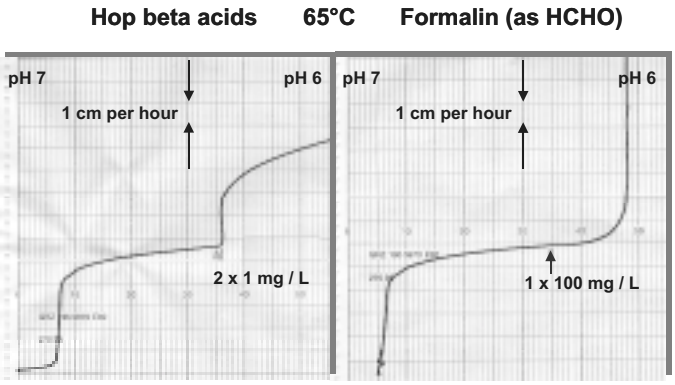

Fig. 13: pH curve after addition of hop β-acids and formalin

It is usual to follow up microbial growth in clear artificial nutrients by measuring optical density at 600 nm, abbreviated as OD_{600}. The aim was to show the influence of both hop β-acids and rosin acids, on optical density, measured against clear culture medium. 6 mg/kg of rosin acids were necessary to stop the drop in pH value and the increase of optical density (Fig. 14). It was surprising that the optical density dropped back heavily and immediately after the addition. The optical density was measured by manual sampling for eight hours and only the pH value was recorded for a longer time, showing a constant pH value until the trial was switched off by the timer.

With hop β-acids the same effect occurred in optical density measurements (Fig. 15). Ten small steps of addition led to a more rounded pH value curve in this trial, but the optical density

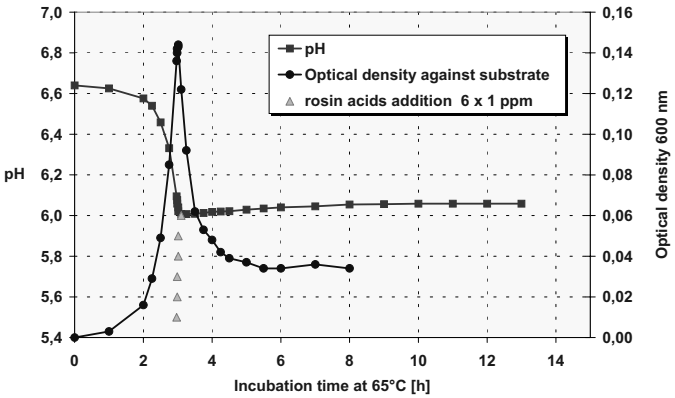

Fig. 14: Influence of rosin acids on pH value and OD_{600} (pure strain DSM 2027)

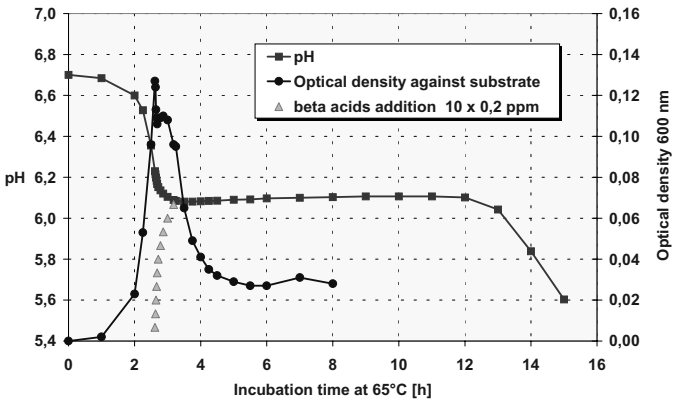

Fig. 15: Influence of hop β-acids on pH value and OD_{600} (pure strain DSM 2027)

dropped heavily during this time. After several hours a second pH value drop is visible in this culture with a pure strain. The same difference between hop β-acids and rosin acid was already shown with a raw juice culture and was obtained with a further strain DSM 22. The authors are not able to explain what happens in the bacterial cell with hop β-acids, but are only able to demonstrate the difference between hop β-acids and rosin acids. Thermophiles are reported to be extreme flexible organisms [32, 33] and therefore an adaptation of strains could appear in the case of hop β-acids. Furthermore, these acids are more insoluble than rosin acids and could precipitate to an equilibrium below the MIC value of the original or the adapted strain.

The same drop in optical density appears with different inocula, either pure strains or simply 20 mL of frozen raw juice from a sugar factory (Fig. 16). The MIC value is dependent on the type of inoculum. For raw juice from the Tulln factory, used as inoculum, the highest concentration of 8 mg/kg was necessary to stop an increase in optical density.

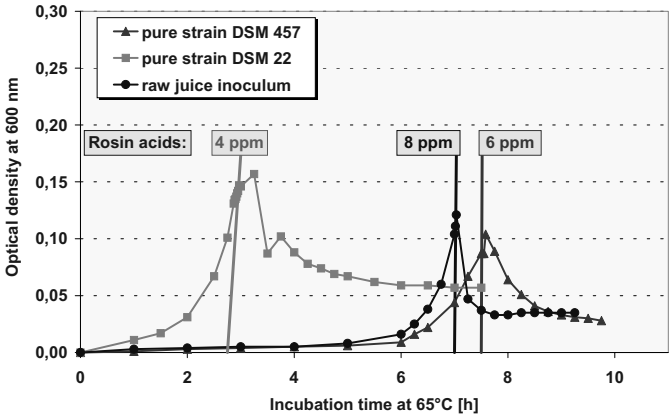

Fig. 16: Development of OD_{600} at 65 °C with different inocula

The congo red method is a vital staining method, which was introduced to the sugar industry by *Weidenhagen* et al. [34]. Living cells are able to resist to the color (which changes from red to blue during preparation) and look white on a blue background. Dead cells are blue on blue and it is difficult to make them out. Shortly after rosin acid addition the cells already show a lower contrast and after 22 min no cells are visible with the same method of preparation (Fig. 17).

In a trial with raw juice inoculation cells with good contrast disappeared after 2 min (Fig. 18). Considering the resources, it was impossible to analyze all possible combinations of hop β-acids, pure strains and methods. Therefore the new rosin acids have been put to the foreground and parallel conclusions may be drawn: The drop in optical density is caused by a dissolution of thermophiles at their growing temperatures, if the metabolism is blocked by hop β-acids or rosin acids. It is not simply a bacteriostatic effect with continuous growth after rinsing out of the "thermo"-biocides. Of course, spores are not effected and will be able to germinate within some hours after a biocide shock dose.

To demonstrate the particular effect of the natural acids at high temperatures, the optical density was measured for cultures of different temperatures (Fig. 19). All trials were inoculated with 20 mL of raw juice. In contrast to the high temperature variant, it was impossible to stop cultures at lower temperatures with useful amounts of rosin acids and the addition was stopped at a very high level. Additionally, it was impossible to stop a drop in pH value

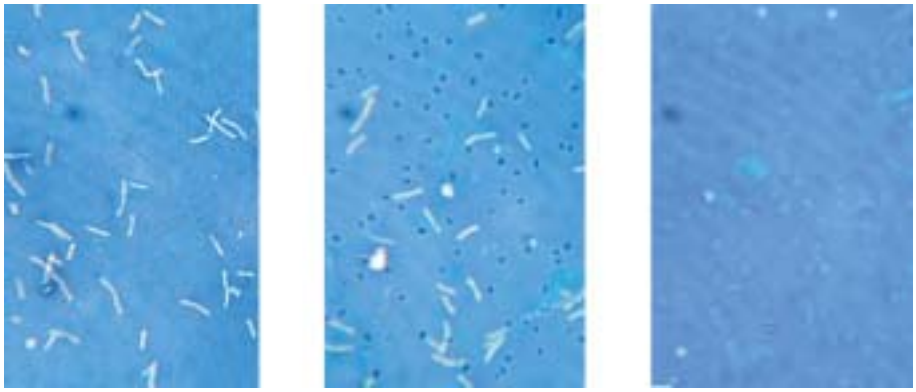

Fig. 17: Congo red/blue staining of pure strain DSM 457 with rosin acids addition (left: 8 min before addition; mid: shortly after addition; right: 22 min after addition)

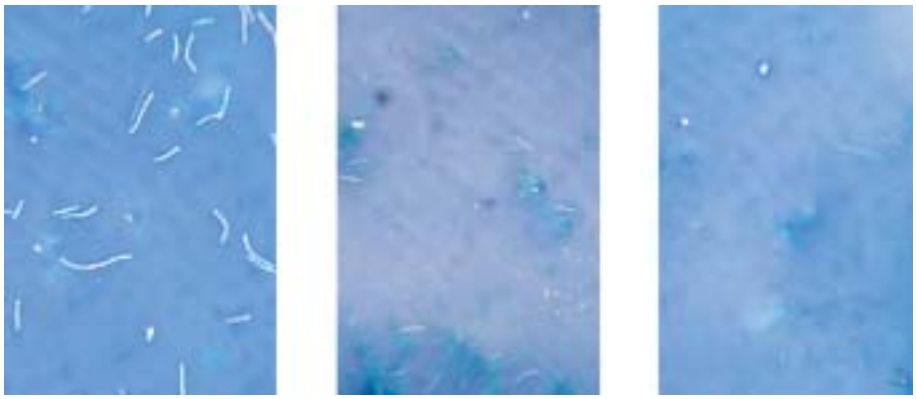

Fig. 18: Congo red/blue staining of raw juice inoculated culture with rosin acids addition (left: 4 min before addition; mid: 2 min after addition; right: 22 min after addition)

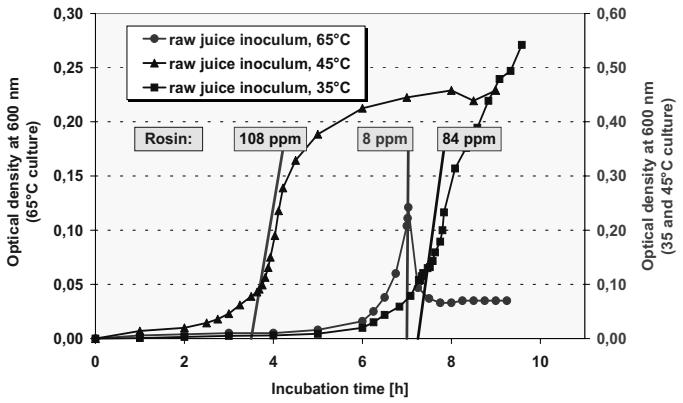

Fig. 19: Development of OD_{600} at different temperatures with rosin acids additions

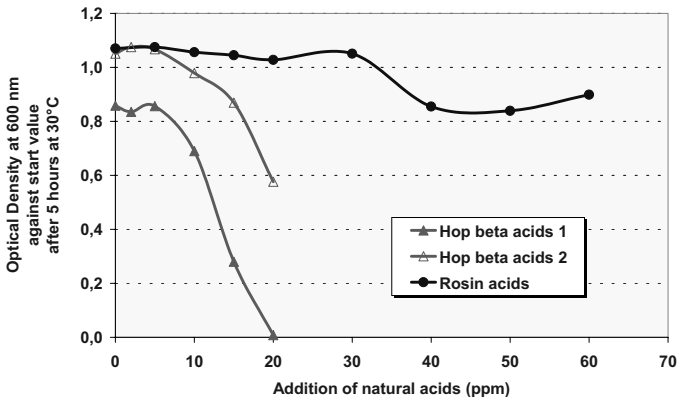

Fig. 20: Preliminary aerobic trials with Leuconostoc and addition of natural acids

with both, hop β-acids and rosin acids, at temperatures of 40 and 35 °C in further trials.

Leuconostoc is a mesophilic organism and it was not possible to stop a pure strain under these anaerobic conditions. But under aerobic conditions, in shaked cultures, an effect was obtained, rather with hop β-acids than with rosin acids (Fig. 20). The necessary concentrations are higher than in case of thermophilic organisms and will only be reached during shock dosages and not on continuous dosing. This is a point for further studies. It was not completed till now, as the work was focused on problems of Austrian factories, such as lowering of lactic acid and NO_2 contents in tower extractors.

4.2.2 **Results from full scale trials**

The first full scale trials with rosin acids were started during the campaign 2000 at Tulln factory. This factory operates one tower extractor, together with three mixers, and the total slicing capacity is above 11,000 t/d, if beet pulp can be pressed well. Dry substance content of pulp is very important for the slicing capacity and it is often low during the first part of the campaign. As lactic acid improves pulp pressing, only just “traces” of biocides are allowed if pulp pressing does not work properly. Nearly no effect is visible during this time, but only 3.5 mg/kg of hop β-acids had a good effect after 20 days of campaign (Fig 21). In the second part of the campaign, when dry substance content of pulp went up to 30% and more, it was possible to lower lactic acid content with 12 and later 18.5 mg/kg of rosin acids to a level of about 400 mg/L during the dosing periods (Fig. 21). The last period was used to take samples for residue studies. The effect of rosin acids is presented with one repetition in the following campaign.

Pulp pressing was bad during the first part of the campaign 2001 and no biocides were allowed, in order to keep the beet slicing capacity. But during the second half of the campaign several alterations with dosing, without dosing and with dosing of BetaStab

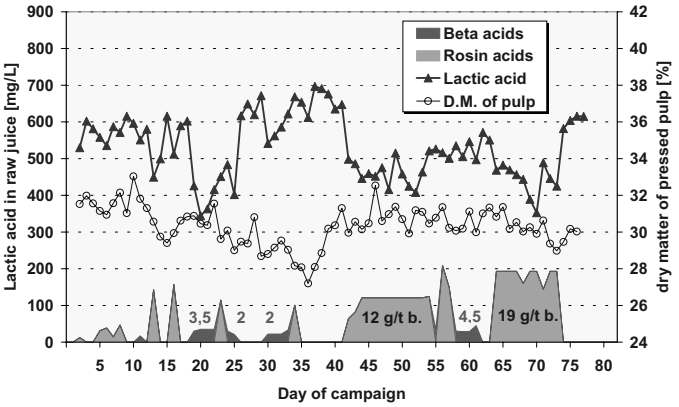

Fig. 21: First full scale application of rosin acids in Tulln in 2000

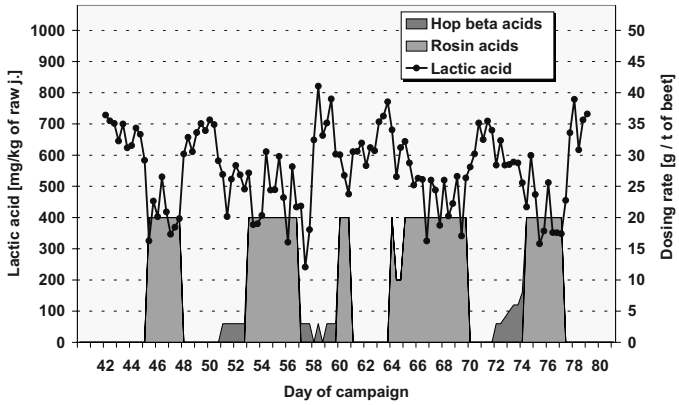

Fig. 22: Full scale application of rosin acids in Tulln in 2001

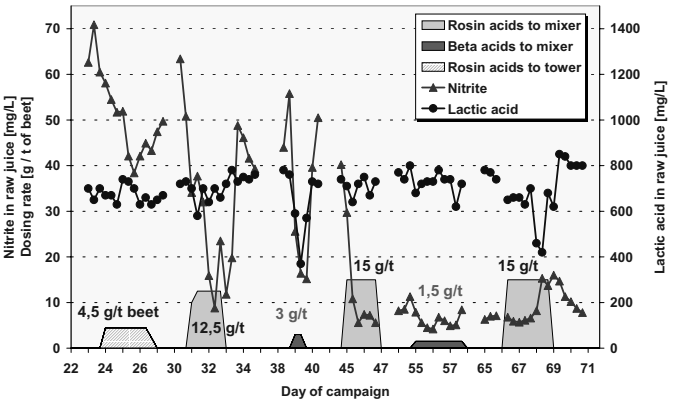

Fig. 23: Nitrite and lactic acid content at Leopoldsdorf factory in 2001

were possible (Fig. 22). Lactic acid dropped to 400 mg/L with 20 mg/kg of rosin acids, but only short effects were reached with 3 mg/kg of hop β-acids. This was in agreement with earlier findings for the Tulln factory, as reported earlier [11]. For a short time 6 mg/kg of hop β-acids were used, which was equal to 20 mg/kg of rosin acids.

During the dosing periods the lactic acid levels slowly decrease. This may be caused by killing of biofilms on inner surfaces of the tower and the three mixers in course of time. As trials require alterations between dosing- and blank-periods for reasons of demonstration, the re-establishment of biofilms during blank periods was allowed for unintentionally. Thus higher lactic acid levels have to be accepted or higher dosing rates were necessary, compared to conditions without trials.

At the Leopoldsdorf sugar factory high levels of NO_2 occured in raw juice for the first time after several years. Addition of rosin acids was started to the two existing towers at the lowest possible position. Due to a dosing failure the dosing rate was very low for rosin acids and no significant effect is visible (Fig. 23). After a blank period the mixer was chosen as new dosing point and dosing was increased to 12,5 mg/kg – with a big effect on NO_2 and no effect on lactic acid contents. After a new blank period 3 mg/kg of hop β-acids were used, with a big effect on both, NO_2 and lactic acid contents. After a further blank period 15 mg/kg of rosin acids were used and the nitrite forming organisms were knocked out for the rest of the campaign.

The lowering of lactic acid contents by addition of 3 mg/kg of hop β-acids had a negative effect on pulp pressing and energy targets of the factory. Therefore, during the next period only 1.5 mg/kg were added and this low dosage did not show any effect on nitrate and lactic acid levels. A last period with 15 mg/kg of rosin acids resulted in a lowering of lactic acid, but the difference to the earlier trial with the same dosing cannot be explained. But the valuable effect of hop β-acids on NO_2 reduction was also confirmed for rosin acids in this factory.

4.2.3 **First results on rosin acids residues**

Table 2 shows some preliminary data on residue in intermediate products. The results are similar to hop β-acids. The rosin acids are found in part in the raw juice and the other part is precipitated and removed with the pulp. Because of the low amount of pressed pulp, compared to raw juice, the concentration is higher. A large amount of rosin acids is removed during juice purification, the rest is found mainly in molasses. As rosin acids are not effective at low temperatures, no danger for molasses fermentation should occur. If amounts in white sugar and retsina wine are compared, the consumption has to be considered. Traces of rosin acids were found in chewing gum with turpene resin declaration on the package, which was probably produced with rosin acid glycerol ester.

Table 2: Rosin acids residues after dosing of 16 mg/kg of beet

Sample	Mean value
Raw juice	3.9 mg/kg
Pulp 30% D.S.	68 mg/kg
Thin juice	0.65 mg/kg
Thick juice	3.8 mg/kg
Molasses	22 mg/kg
White sugar	60 µg/kg
Retsina	first result: 4.5 µg/kg
Chewing gum	first result: 238 µg/kg

5 **Conclusions**

- New biocides of natural origin have been discussed.
- Hop β-acids have shown particular effects since 1994.
- Rosin acids show great promise to become a second natural biocide for the sugar industry.
- Both natural acids show similar good effects on thermophiles, compared to mesophiles.
- Rosin acids cause higher dosing rates and residues, compared to hop β-acids.
- Lactic acid control seems to be stable with rosin acids, even under severe conditions.
- Leuconostoc inhibition by hop β-acids is a question of further studies.

6 Appendix: Details on materials and methods

- Pure strains of *Bacillus stearothermophilus* (now *Geobacillus stearothermophilus*), such as ATCC 12980 = DSM 22, L33-65 = DSM 457, NCA 1503 = DSM 2027 and of *Leuconostoc mesenteroides, subsp. mesenteroides* (ATCC8293 = DSM 20343) have been ordered from the German type culture collection (DSMZ) and activated as recommended in the catalogue of strains [35].
- Deep-frozen raw juice samples from the Tulln factory of Agrana have been used as inoculum for trials with impure laboratory cultures (20 mL/500 mL).
- Medium "VIII" was composed according to *Bartelmus* and *Perschak* [36], but without sugar, having the following composition per Liter: Bacto-peptone 10 g, yeast extract 5 g, meat extract 5 g, $K_2HPO_4 \cdot 3H_2O$ 1,3 g, $MgSO_4 \cdot 7H_2O$ 0,1 g, $FeSO_4 \cdot 7H_2O$ 0,02 g, pH value adjusted to 7. Sucrose (2.5 g/500 mL of culture) was added as sterilized aqueous solution (40%) at the starting time of trials with pure strains.
- MRS medium was used for *Leuconostoc mesenteroides* as recommended in the DSMZ catalogue [35] for a first cultivation to an active culture. pH value was adjusted to 6.5 before inoculation. 10% of sucrose (insterile) was added prior to the aerobic growth phase to enable dextran formation.
- Pure rosin acids for microbiological trials and analytical standards were ordered from Helix-Biotech, New Westminster, Canada.
- Hop β-acids were used as 10% aqueous solution BetaStab10A.
- Colophony, quality WW from Portugal, was used for trials. The material was ground and dissolved in ethanol or dissolved in water by titration with NaOH to sodium rosinate (10.7% rosinate, corresponding to 10% of rosin acids).
- Laboratory trials were carried out in sterile glass vessels (500 mL) with pH value recording and slow magnetic stirring, in order to avoid artificial aeration and to simulate conditions in extraction towers. In addition to the most important information from pH value recording, samples were drawn for measurement of optical density and microscopy.
- Preliminary trials with Leuconostoc were carried out in a shaker at 30 °C in dented Erlenmeyer flasks. The pH value of the medium was adjusted to 6.5 before inoculation. After growing on of the inoculum, 10% sucrose were added prior to the aerobic growth phase (5 h) to enable growth and dextran formation.
- Optical density was measured against air at 600 nm and reported as difference against substrate.
- Bacterial cells were stained by a vital staining method, the Congo red method [34], but with 1 cm^2 film area instead of 10 cm^2, as reported in the original paper.
- Raw juice samples from full scale trials were analyzed for lactic acid by the yellow springs equipment and for nitrite with the colorimetric method, based on sulfanilic acid and α-naphthylamine [2].
- Rosin acid residues were determined with an existing DIONEX system: RP-column Nucleosil C-18 (Macherey-Nagel) with appropriate pre-column. Eluent: acetonitril-water. Gradient elution from 60:40 to 90:10 within 60 min. Flow rate 1 mL/min at 25 °C. UV/VIS detection at 208/245 nm. The isomers were not separated from each other and till now the common peak was calculated as abietic acid. Additionally the proportion between isomers and the separated dehydroabietic acid was used to check the preliminary results.

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References

1 *Pollach, G.* (1995): Verfahren zur Hemmung thermophiler Mikroorganismen in Gegenwart zuckerhaltiger wässriger Medien. EP 0 681 029 A2, Zuckerforschung Tulln GmbH
2 *Pollach, G.; Hein, W.; Hollaus, F.* (1996): Einsatz von Hopfenprodukten als Bakteriostaticum in der Zuckerindustrie. Zuckerind. 121, 919–926
3 *Johnson, H.; Krüger, A.*: Das große Buch vom Wein. Erweiterte Neuausgabe. Gräfe und Unzer Verlag, München (keine Jahresangabe)
4 *Shimwell, J.L.* (1937): On the relation between the staining properties of bacteria and their reaction towards hop antiseptic, Parts 1+2. J. Inst. Brewing 43, 111–118
5 *Hollaus, F.; Hein, W.; Pollach, G.; Scheberl, A.; Messner, P.* (1997): Nitritbildung im Dünnsaftbereich durch Thermus-Arten. Zuckerind. 122, 365–369
6 *Teuber M.; Schmalreck A.F.* (1973): Membrane Leakage in Bacillus subtilis 168, Induced by the Hop Constituents Lupulone, Humulone, Isohumulone and Humulinic Acid. Arch. Mikrobiol. 94, 159–171
7 *Simpson W.J.* (1991): Molecular structure and antibacterial function of hop resin materials. Ph.D. Thesis, Council for National Academic Awards, UK
8 *Hein, W.; Pollach, G.* (1997): Neue Erkenntnisse beim Einsatz von Hopfenprodukten in der Zuckerindustrie. Zuckerind. 122, 940–949
9 *Gudmundson, C.* (1998): Danisco Sugar's experience with hop extracts as alternative to formaldehyd. Presented at the General Ass. of German sugar technologists (VDZ), Dresden, ref. in Zuckerind. 123 (1998) 456
10 *Brons, N.* (1999): Bericht aus der Kampagne 1998 – Rheinisch-Westfälischer Zweigverein. Zuckerind. 124, 368–377
11 *Pollach, G.; Hein, W.; Rösner, G.* (1999): New findings towards solving microbial problems in sugar factories. Zuckerind. 124, 622–637
12 *Pezzi, G.; Segantin, G.* (1999): Hop products as antimicrobial agents in the extraction process. Proc. 21st Gen. Ass. CITS (Antwerp), Verlag Dr. A. Bartens Berlin, 446–449
13 *Bruhns, M.* (2000): Bericht aus der Kampagne 1999 – Rheinisch-Westfälischer Zweigverein. Zuckerind. 125, 305–310
14 *Fowers, M.* (2001): The Bactericidal Effect of Hop Derived β-acids. ASSBT-Proceedings from the 31st Biennial Meeting at Vancouver, BC (28 Feb. –3 March 2001), Section D – Chemistry and Instrumentation, 79–84
15 *Hein, W.; Pollach, G.; Rösner G.* (2002): Studies on microbiological activities during thick juice storage. Zuckerind. 127, 243–257
16 *Unden, G.* (1999): Aerobic Respiration and Regulation of Aerobic/Anaerobic Metabolism. In: *Legeler, J.W.; Drews, G.; Schlegel, H.G.*: Biology of the Prokaryotes. Thieme-Verlag Stuttgart/New York, 261–276
17 *Prohl, C.; Wackwitz, B.; Vlad, D.; Unden, G.* (1998): Fuctional citric acid cycle in an arcA mutant of *Escherichia coli* during growth with nitrate under anoxic conditions. Arch. Microbiol. 170, 1–7
18 *Carruthers, A.; Gallagher, P.J.; Oldfield, J.F.T.* (1958): Nitratreduktion durch thermophile Bakterien in Diffusions-Systemen. Z. Zuckerind. 8, 541–546
19 *Lee, Y-E.; Jain, M.K.; Lee, Ch; Lowe, S.E.; Zeikus, G.* (1993): Taxonomic Distinction of Saccharolytic Thermophilic Anaerobes. Int. J. System. Bacteriol. 43, 41–51
20 *Collins, M.D.; Lawson, P.A.; Willems, A.; Cordoba, J.J.; Fernandez-Garayzabal, J.; Garcia, P; Cai, J.; Hippe, H.; Farrow, J.A.E.* (1994): The Phylogeny of the Genus *Clostridium*: Proposal of Five New Genera and Eleven New Species Combinations. Int. J. Syst. Bacteriol. 44, 812–826
21 *Weinberg, E.D.* (1962): Mechanisms of Action of Antibacterial Substances. J. Soc. Cosmet. Chem. 13, 89–96
22 *Uehara, K.; Ohhata, Y.; Kawabata, A.; Inoue, Y.; Yokogawa, Y; Tsutsumi, Y.* (1992): Dermatologic preparation. EP 0 465 663 A1
23 *Pollach, G.* (2000): Verfahren zur Hemmung von thermophilen Mikroorganismen in zuckerhaltigen Medien. Austrian Patent Application A 847/2000
24 *Pollach, G.; Hein, W.* (2001): Verfahren zur Herstellung von Zucker oder zuckerhaltigen Produkten aus zuckerhaltigen pflanzlichen Rohstoffen. PCT Patent Application WO 01/88205 A1
25 *Peng, G.; Roberts, J.C.* (2000): Solubility and toxicity of resin acids. Wat. Res. 34, 2779–2785
26 *Mohn, W.W.; Wilson, A.E.; Bicho, P.; Moore, E.R.B.* (1999): Physiological and Phylogenetic Diversity of Bacteria Growing on Resin Acids. Syst. Appl. Microbiol. 22, 68–78
27 *Morgan, C.A.; Wyndham, R.C.* (1996): Isolation and characterization of resin acid degrading bacteria found in effluent from a bleached kraft pulp mill. Can. J. Microbiol. 42, 423–430
28 *Bicho, P.A.; Martin, V.; Saddler, J.N.* (1995): Growth, Induction, and Substrate Specifity of Dehydroabietic Acid-Degrading Bacteria Isolated from a Kraft Mill Effluent Enrichment. Appl. Environ. Microbiol. 61, 3245–3250

29 *Fiebach, K.* (1993): Resins, Natural. In: Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., VCH-Verlag Weinheim, Vol. A23, 73–88
30 *Narziß, L.* (1986): Abriß der Bierbrauerei. Enke-Verlag Stuttgart, 283
31 *Potera, C.* (2001): Tree Extract Curbs Foodborne Pathogens. ASM News, 67, Nr. 12, 605–606
32 *Schuster, K.C.* (1994): Kultivierung von Bacillus stearothermophilus PV72 mit definierten Zellwandeigenschaften für biotechnologische Anwendungen von S-Schichten. Doctoral thesis, Technische Universität, Vienna, 24–25
33 *Baier, U.* (1987): Zur Physiologie thermophiler Bacilli. Doctoral thesis, Eidgenössische Technische Hochschule – ETH Zürich, 168–169
34 *Weidenhagen, R.; Gruschkau, H; Gössel, I.* (1964): Eine Schnellmethode zur Infektionskontrolle in Zuckerfabrikssäften. Zucker 17, 574–576
35 DSMZ (2001): Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Catalogue of Strains 2001, 7. ed., 45, 123, 347
36 *Bartelmus, W.; Perschak, F.* (1957): Schnellmethode der Keimzahl-bestimmung in der Zuckerindustrie. Z. Zuckerind. 7, 276–281

Application des acides β du houblon et des acides de colophane dans l'industrie sucrière (Résumé)
En 1994, on a utilisé pour la première fois avec succès des produits extraits du houblon pour lutter contre les bactéries dans les appareils de diffusion des betteraves. C'était un domaine d'application tout nouveau pour les produits du houblon comparativement à leur utilisation traditionnelle en brasserie. Aujourd'hui, on utilise en sucrerie une solution alcaline d'acides β du houblon sous le nom commercial de „BetaStab". Les acides β du houblon se sont avérés très efficaces contre la formation de NO_2 et les infections anaérobies dans les tours d'extraction où l'on tolère souvent intentionnellement une fermentation lactique. Les acides β du houblon se sont montrés en outre efficaces dans le domaine du stockage du jus dense. Parfois, dans le cas de lutte contre l'acide lactique, on a observé une sélection d'organismes moins sensibles et il faut alors utiliser un second désinfectant en alternance avec le houblon. C'est ainsi qu'est née l'idée d'utiliser des acides de colophane comme autre biocide naturel inoffensif.
Ce rapport présente les résultats d'essais de laboratoire, des essais à grande échelle ainsi que les premières études sur les résidus. Les acides de colophane montrent une potentialité à être utilisés en sucrerie, soit en alternance avec les produits du houblon, soit pour formuler de nouveaux produits plus économiques.

El empleo de ácidos-β-de lúpulo y ácidos de resina en la industria azucarera (Resumen)
En el año 1994 se emplearon con éxito por primera vez productos de lúpulo en la lucha contra bacterias presentes en la extracción de remolachas. Esto significa un sector de aplicación completamente nuevo en comparación al empleo tradicional de productos de lúpulo en cervecerías. Actualmente se aplica en fábricas de azúcar una solución alcalina de ácidos-β-de lúpulo con la marca registrada "BetaStab". Acidos-β-de lúpulo son muy efectivos contra la formación de nitrito e infecciones anaerobias en torres de extracción, en las cuales, muchas veces, se opera intencionalmente con fermentaciones de ácido láctico. Además tienen los ácidos-β-de lúpulo buen efecto a nivel del almacenamiento de jugo denso. Algunas veces se puede observar durante la lucha contra ácido láctico una selección de organismos no tan sensitivos lo que requiere una segunda aplicación alternada con productos de lúpulo. De esta necesidad nació la idea de emplear ácidos de resina como un otro biocida natural y no peligroso. Se presentan resultados de endayos de laboratorio, de ensayos llevados a cabo a nivel industrial y de primeros estudios de los residuos. Acidos de resinas son un otro potencial para la industria azucarera y pueden ser empleados tanto alternadamente con productos de lúpulo como también en una creación de productos combinados más económicos.

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