THE USE OF FLUORIDES IN THE MANUFACTURE OF ALCOHOL.

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Dr. Effront's researches on the antiseptic properties of fluorine compounds have lately received important applications in the alcohol industry and a condensed statement upon this subject may be of interest not only to alcohol manufacturers but also to the biological chemist.

It is a known fact that for the transformation of starch into maltose and dextrine by the action of diastase, the most favorable temperature is from 50° to 60° C. At any lower temperature, the action of the diastase becomes slower, while a higher temperature has a destructive effect upon this soluble ferment which at 80° C. completely loses its saccharifying power.

In laboratory experiments where pure starch and pure diastase can be used it is possible to exclude all secondary influences from other ferments. In practice however, conditions are far different; the raw materials (malt, grain, corn, potatoes, etc.), introduce other ferments which have a very disturbing influence. Already during the germination of the grain we notice that beside the useful diastase, a certain quantity of organic acid is developed. But this is only the beginning of the trouble for we see this acidity increasing during all the following operations, from the making of the sweet wort to the end of the alcoholic fermentation. This acidity is due chiefly to the formation of lactic and butyric acids which are the result of the activity of well specified micro-organisms, the lactic and butyric ferments, the latter being more specially known as *Clortridium Cutyricum*. If a small quantity of lactic acid seems to be favorable to a healthy development of yeast cells, a too large quantity of this acid is useless if not harmful, and any quantity of butyric acid is decidedly injurious to the *enzymotic* action of the diastase as well as to the alcoholic fermentation.

Alcohol manufacturers are very cautious to avoid acidification as they know that the yield of alcohol is smaller in proportion to the increasing acidity of their wort or mash.

The ferments which develop these acids are particularly active at a temperature from 50° C. to 60° C. This means that just that temperature which is most favorable for the diastatic action of the malt will also allow the acid ferments to show their harmful action and to develop the largest quantity of lactic and butyric acid. At a higher temperature however, the action of butyric and lactic ferments is paralyzed, and this is the reason why in making sweet wort a temperature is selected whereby the development of acidity is retarded and by which the diastase does not lose too much of its power. But this means a loss of malt on account of the weakening of the power of the diastase contained in it.

The injurious action of this higher temperature on the diastase is not limited to this ; facts have proven that diastase which has been submitted to a high temperature, if only for a moment, has been irremediably injured in regard to its future properties ; it will not keep its enzymotic action as long as it would if it had been kept in healthy conditions. This fact is very important if we recollect that the saccharification of the wort is never complete and that the diastase is expected to continue its action during the whole period of alcoholic fermentation in order to saccharify the last quantities of starch left in the wort. Other means have been tried in order to combat the butyric and lactic baccilli and the use of sulphuric and muriatic acids, sulphites and other antiseptics have been proposed. None of them has proved of great advantage ; they all show an injurious effect upon the diastatic action of the malt, and the activity of the alcohol ferment.

Hydrofluoric acid or soluble fluorides on the contrary, when used in very small doses prevent the development of butyric and lactic acid and are completely harmless to the diastase or to the alcoholic ferment. Furthermore, such small quantities of fluoride instead of injuring the diastase seem to excite its activity and preserve its power for quite a longer period than when used alone. Experiments have shown that by addition of a small quantity of fluoride, diastase could be kept in action during seven days, and at the end of

this period 80% of theoriginal quantity of diastase was still active, while without the use of fluorides the quantity of active diastase is reduced to 12% at the end of the same period.

No other antiseptics or mineral acids show these preserving qualities when added to diastase. Mineral acids, for instance, when used, even in very moderate quantities have killed the whole of the diastase after five days.

A first advantage of the use of small quantities of fluorides is thus to save malt, of which less need be brought in operation, still giving the same result as larger quantities when used without fluorides. This malt will have a more free and more complete action, and this action will be continued for a longer time on account of the antiseptic properties of fluorides, development of organic acids will be prevented and the wort can be kept nearer between 50° C. and 60° C., which is the temperature at which diastase is most active and where its enzymotic powers are best kept up

The addition of fluorides have rendered possible the use of malt of very inferior quality with surprising results.

The same beneficial effect of hydrofluoric acid or its salts is observed during the alcoholic fermentation which thereby becomes more regular and more complete.

The fermentation keeps up with about the same intensity during the whole period until all the saccharine matter has been converted into alcohol. The liquid finally proves to be far less acid than if no fluoride is used. The practical result of all this, is a larger yield of alcohol and a better product. If small quantities of fluoride have a beneficial effect on diastase and alcoholic ferment, the contrary result is obtained if larger quantities of fluorine compounds are used. An addition of 25 grammes of commercial hydrofluoric acid (30%) per hectolitre of wort proved very injurious to the action of the diastase even at temperatures as low as 30° C. At higher temperatures, 55° C. for instance, this injurious effect was already produced by 6 or 7 grammes of hydrofluoric acid per hectolitre. Very concentrated or very acid worts have proved especially sensitive to large quantities of hydrofluoric acid.

The same bad effects of an excess of hydrofluoric acid are shown in the alcoholic fermentation which in some cases may be entirely

stopped by it. Soluble fluorides however, even when used a little in excess, are less violent in regard to this. All this shows that if we have in fluorides a sure and powerful destroyer of useless ferments, this agent should be used judiciously as any excess of it may prove detrimental to the expected results.

It would be out of the question to give a general recipe for the quantities of hydrofluoric acid or fluorides which should be used, this depends on too many conditions such as quality of material, temperature of mashing, concentration or acidity of the wort. For instance, quantities of fluoride which may be advantageous in a wort of average concentration and at a low temperature may become injurious in a more concentrated wort, or if kept at a higher temperature.

In every instance where the fluoride process is introduced in a distillery, some preliminary testing has to be done in regard to this before the best effects are obtained.

The destructive action of fluorides on butyric baccilli in the mash can be easily observed by the aid of the microscope. (See *Das Flüssäure Verfahren in die Spiritusfabrikation.* by Prof. Dr. Maerker.—Berlin, Verlag von Paul Parey.)

The chemical effect of fluorides is better demonstrated by the following experiment: (See Soxhlet. *Zeitschrift des Landwirths-Vereins in Bayern,* July, 1890) 1000 grammes of crushed corn were boiled with four litres water and the resulting starch heated in a digestor(autoclave) at 3 atm. pressure during one hour. The resulting liquid was cooled off to 50° C. and then 80 grammes of dry malt were added and the saccharification kept on at this temperature for about 30 minutes. The resulting sweet wort was divided into two equal portions. To one portion 150 milligrammes of ammonium fluoride were added (corresponding to 6 grammes per hectolitre) and the other portion was left as it was. Both portions were kept constantly at 30° C. After two days the wort without fluoride had considerably thickened and was full of gas bubbles while the other portion which contained fluoride remained entirely clear and liquid.

A comparative analytical examination of both liquids showed :

	No fluoride.	Fluoride.
Degrees saccharometer	13.30	13.60
Maltose	10.13	11.61
Dextrine	2.07	1.07
Acidity (Normal soda solution per 100 c. c. wort)	8.15	3.9

In another series of experiments wort was prepared in the same way, but after being cooled off to 30° C., 3 grammes of yeast per liter were added. This mixture was put in several bottles which were provided with a hydraulic stopper and which could be kept at a temperature of 30°. To the contents of some of these bottles a previous addition of fluoride had been made in quantities equivalent to 6 grammes per hectolitre of wort. After three days, fermentation was complete in all the bottles and the comparative analysis showed:

	No fluoride.	Fluoride. (6 gr. per hectolitre)
Alcohol	6.4	7.2
Acidity (Normal soda per 100 c. c.)	5.6	4.0

The proportion of alcohol in the above results is about 12.5% higher in the sample where fluoride has been used. (See also Effront. *Action des acides mineraux dans la saccharification par le malt et la fermentation des matiéres amylacées.—Moniteur Scientifique* 1890.) (Maerker, *Flussäure verfahren, loc. cit.*)

The specific action of fluorides on the development and activity of yeast cells has been the object of other interesting experiments. (See Effront. *Moniteur Scientifique.* November 1891.—Also Maerker, loc. cit.) An addition of small quantities of fluoride say in 30 milligrammes per 100 c. c., is favorable to the development or increase of the number of yeast cells. Larger quantities (70 to 150 mgs.) decrease the formation of new yeast cells and when as much as 250 to 300 mgrms. per 100 c. c. are used, the development of new yeast cells is practically interrupted. But even then when such large quantities of fluoride are used, the ferment itself is not killed, it

has merely suspended the reproductive faculties of the yeast cells, while their alcohol making action is still existant but with lessened activity. Furthermore if such yeast heated previously with relatively large quantities of fluoride is brought into fresh wort containing no fluoride it will at once resume its full vital properties, new yeast cells will be produced and the alcoholic fermentation will be very energetic.

In some experiments small quantities of yeast (0.25 to 0.25 grm. per 500 c. c.) were added to sweet wort made with corn and left to develop with different quantities of fluoride, and afterwards the relative number of yeast cells was determined in each of the samples with the following results:

	Acidity (c. c. normal soda).	Relative number of yeast cells.
No fluoride	1.10	331
0.02 Amm. fluor.	0.80	449
0.07 "	0.90	381
0.15 "	0.70	208

A certain quantity of each of these samples has been added to a fresh wort containing no fluoride (500 c. c. of each sample to 1 kilogr. of wort, 17.4 Sacch.) and after the fermentation was finished the quantity of alcohol produced by the sample was

	Alcohol.
No fluoride	4.1%
0.02 grm. amm. fluor	7.1
0.07 "	8.4 (!)
0.15 "	8.5 (!)

The above results show clearly that even by starting with a smaller number of yeast cells, more alcohol has been produced and this is on account of the increased vital powers of these yeast cells after they have been under influence of fluoride.

The specific action of fluorides is not the same on all varieties of yeast. Four kinds of yeast have been especially examined in this direction.

Saccharomyces Cerevisiæ.
" *Pastorianus I.*
" *Carlsberg.*
" *Bourton.*

By addition of 100 mgrms. of fluoride to 100 c. c. of liquid the following results have been observed:

	Decrease of development of yeast cells by addition of 100 mgrms. fluoride.	Decrease in production of alcohol by addition of 100 mgrms. fluoride.
S. Pastorianus	34%	41%
— *Bourton*	31%	14%
— *Carlsberg*	25%	13%
— *Cerevisiæ*	9%	4%

This shows that *S. Pastorianus* is most sensitive to an addition of 100 mgrms. fluoride, the quantity of alcohol decreases to 41% of what can be produced without fluoride. *S. Bourton* and *S. Carlsberg* are less sensitive but the most resisting of all is *S. Cerevisiæ*. By addition of 300 mgrms. of fluoride the production of new yeast cells is nearly completely stopped in the four varieties but even then *S. Cerevisiæ* gives about three times as much alcohol as *S. Pastorianus* (See table.)

Number of sample.	Name of yeast.	Fluoride per 100 c. c. mgrms.	Number of yeast cells.	Alcohol %
1	*Pastorianus I*	0	23	7.3
2		100	15	4.3
3		150	8	2.6
4		200	7	2.4
5		300	2	1.7
6	*Bourton*	0	32	7.9
7		100	22	6.8
8		150	20	5.8
9		200	10	4.1
10		300	3	4.0
11	*Carlsberg*	0	16	6.8
12		100	12	5.9
13		150	10	5.6
14		200	6	5.2
15		300	2	2.3
16	*Cerevisiæ*	0	22	7.1
17		100	20	6.8
18		150	11	5.2
19		200	4	4.9
20		300	2	4.5

The difference in the resisting power of these four varieties of yeast to the action of fluorides can be used to separate one from another. For instance, if a mixture of equal parts of *S. Pastorianus* and *S. Bourton* (whose cells are easily to be distinguished under the microscope) is kept during 72 hours in a wort containing 300 mgrms. of fluoride per 100 c. c. and afterwards transferred to a wort containing no fluoride where they are allowed to ferment during 48 hours, and if these operations are repeated three times, it is possible to eliminate entirely all yeast cells of the *Pastorianus* variety.

It is to be noted as an important fact that yeast can be kept for more than six months with an addition of 200 to 300 mgrms. of fluoride per 100 c. c. Even after this time the yeast cells have proved to be in an excellent state of preservation and when brought afterwards in a wort without fluoride they developed rapidly and produced a splendid fermentation.

The practical results which have been obtained in distilleries, after Effront's process have fully demonstrated the remarkably useful properties of fluorides. In Europe nearly all important distilleries have adopted the process and their reports have been very favorable. In general an increase from 8 to 10% in the yield on alcohol has been obtained, and a more steady and more complete fermentation has been noticed. The production of lactic and butyric acid has been reduced to a minimum. The quality of alcohol has also improved and this is probably due to the prevention of secondary fermentations. The odor and taste of such alcohol is very similar to that of ordinary alcohol that has been filtered over charcoal.

From the beginning when the fluoride process was introduced into distilleries special experiments have been made in order to see if the presence of small quantities of these salts in the slops or wash did not have unhealthy effect on the cattle using such food. Dr. Tappeiner (*Zeitschrift des Landwirth's Vereins in Bayern*, 1890) has published some interesting laboratory experiments which show clearly that not the smallest difference has been observed in the use of such slops, either in the composition of the milk nor the general conditions of the animals fed upon it.

Since then practice has entirely confirmed these conclusions. The slops produced from distilleries working with fluoride is far less acid than usual and is remarkable for its keeping qualities. While, usually, slop becomes acid very quickly, it will, on the contrary, keep for weeks without much alteration if fluorides are used. Its taste is sweeter, also, and this is probably the reason why in some cases where the fluoride process has been introduced, it took the cattle a few days before being accustomed to this taste. It should be reported however that in one case when the slop was used over again in the mashing process the fluorides seemed to accumulate in it and therefore it is thought advisable not to use the same slop more than twice if it has to be used afterward for feeding purposes.