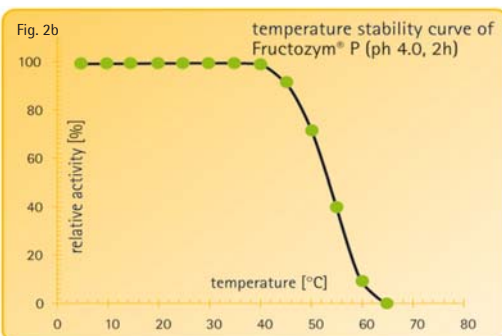
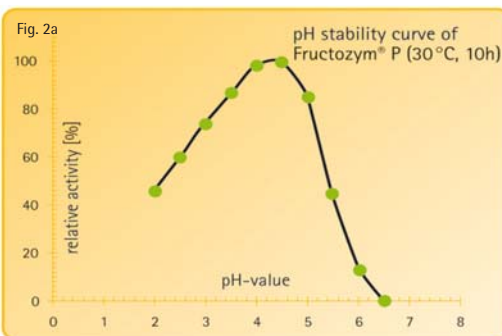
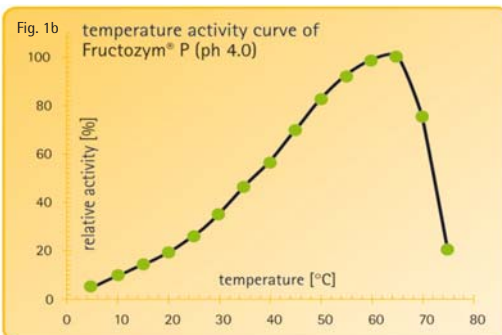
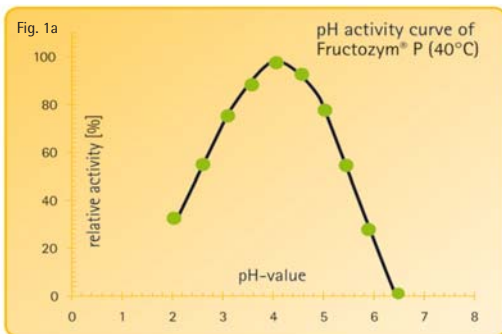


ENZYME PREPARATIONS FOR THE MANUFACTURE OF FRUIT JUICE

FROM THE POINT OF VIEW OF THE APPLICATION TECHNOLOGY

German Hasselbeck



CHARACTERIZATION OF ENZYMES

In enzymology, for enzyme preparation characterization, among others, the activity of the enzymes as a function of pH-value and temperature of the reaction, is determined in the respective substrate and graphed. In practice these parameters are important for the user to achieve maximal efficiency of the enzyme in the course of the enzymatic reaction (Fig. 1a & 1b). Usually the pH-value as well as the temperature are adjusted to the respective activity optimum as this is possible, for instance, in starch hydrolysis with α -amylases and glucoamylases. For the application of enzymes in fruit juice making the possibilities for adjustment are limited to the temperature range since the pH-value of the juice is a natural constant and cannot be changed. For pectinases this means that the optimal reaction conditions with regard to the pH-value are achieved only in exceptional cases; in most cases the pH-values of the fruit juices are lower than the activity optimum of pH 4.0. Most probably the performance of the pectinases is then suboptimal. With regard to the reaction temperature large enterprises are in a position to fully utilize the activity optimum while small enterprises have to live with activity losses which are, with 20 % residual activity at usual European autumn temperatures, more significant. But reduced activities can be compensated by higher dosages and are mostly already taken into consideration in the application instructions of the producers with recommended higher dosages.

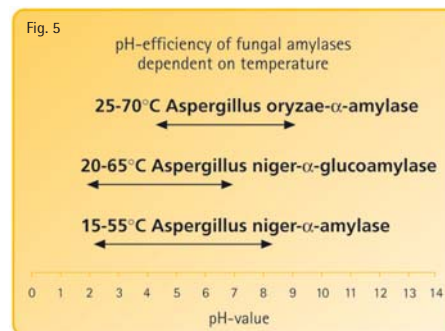
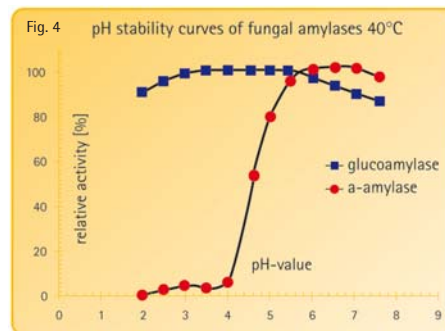
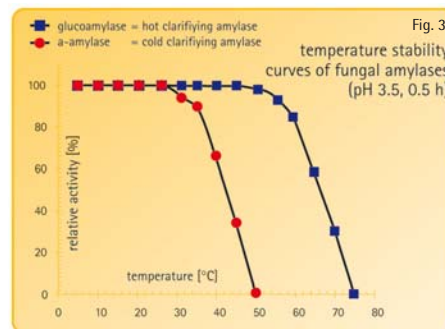
Principally in enzymology not only activity characteristics, but also the stability characteristics of an enzyme are determined and graphed. The dependence of pH-value and temperature is equally covered (Fig. 2a & 2b). The data concerning the activity as well as the data concerning the stability reflect as such the profile of action of an enzyme, when combined the efficiency of an enzyme is shown. When interpreting the data correspondingly the user is able to apply the "technical aid" enzyme under consideration of the reaction conditions economically with regard to reaction time or processing time. We will use our products – the pectinases Fructozym P, Fructozym Color as well as Fructozym P-6L – and selected examples of fruit juice making as a model to explain how the enzyme dosage in the treatment of mash or juice can be adjusted optimally to the operation conditions, i.e. temperature and processing time, for every fruit and its individual, special pH-value.

CLARIFICATION OF APPLE JUICE WITH FRUCTOZYM P

The enzymatic clarification of apple juice is carried through for microbiological reasons either at temperatures around or below 20 °C or – in connection with hot clarification – at temperatures between 50 °C and 55 °C, in this case usually with dearomatised semi-concentrated juice in concentrate making. The pH-activity-optimum of Fructozym P is in the pH-range between 3.5 and 4.5, which is typical for the fungal enzymes, that means, it is close to the usual pH-value of apple juice. In most of the commercial pectinases the temperature activity optimum is in the range between 50 °C and 60 °C. The temperature optimum of Fructozym P is with 65 °C exceptionally high. At their respective activity optimum, enzymes have the highest conversion rate. At lower temperatures the conversion rate drops rapidly. Juice pectinases are sufficiently effective to a temperature of 15 °C. At lower temperatures the application of enzymes is no longer profitable, yet, exceptions prove the rule. At temperatures above the activity optimum the enzymes become rapidly inactivated, i.e. in case of Fructozym P at and above 65 °C. The difference with regard to pH-dependent conversion rate is not only that the rate drops – seen from the pH-optimum – with decreasing or increasing values but the enzyme is inactivated at the same time.

As mentioned before the reaction rate of an enzyme alone does not provide much information on the conversion capacity because for a determination of the capacity the factor time – or better the reaction duration – has to be taken into consideration too. To determine the influence of the reaction parameter "time", the stability characteristics of Fructozym P are included in addition (Fig. 2a & 2b). The pH-stability optimum almost coincides with the pH-activity optimum (Fig. 1a), i.e. ranges between pH 3.5 and 4.5. This explains why the enzyme is inactivated when the pH-value differs from the pH-optimum. The temperature-stability-optimum deviates significantly from the respective activity optimum (Fig. 1b). Under the pH-conditions of apple juice, which are almost optimal for fungal pectinases, Fructozym P is reliably stable at temperatures up to 40 °C but then the activity rapidly declines with higher temperatures. At the temperature optimum of 65 °C Fructozym P is at pH 4.0 completely inactivated after merely 2 hours. This explains why for hot enzymatization a temperature of 50 °C is recommended. Here the stability of the enzyme is, with 70 % of the initial activity, sufficient for a complete pectin degradation. The enzyme is inactivated after about 6 hours. For cold enzymatization at temperatures of around 20 °C the applied reaction times are much longer (4-6 hours, often even overnight) and/or higher enzyme dosages are necessary.

While the current commercial pectinases all have more or less comparable activity characteristics, the fungal amylases, which are employed in fruit juice making for starch degradation, are markedly different in their "temperature dependent acid stability". A distinction is made between the so-called hot clarification amylases and the cold clarification amylases. This means that, contrary to the hot clarification amylases, the cold clarification amylases are not suited for the clarification of pome fruit juices at high temperatures ($t > 35\text{ °C}$) due to their high temperature dependent acid sensitivity (Fig. 3 and 4). Because of the problem of cloudiness (filamentous cloudiness), arising from hot clarification amylases of the HT-



type, the application of cold enzymatization is still up-to-date and important. However, for some time already acid stable α -amylases derived from *Aspergillus niger* as, for instance, Fructamyl FHT are available, which do not cause protein haze (Fig. 5).

BERRY MASH ENZYMATIZATION

In the production of coloured juices mash enzymatization is usually carried through with pectinases at a temperature of 50 °C. The processing time varies from fruit variety to fruit variety and depends on the time schedule of the processing plant. Some processing plants have 1 hour time before the black currant mash is operated on the

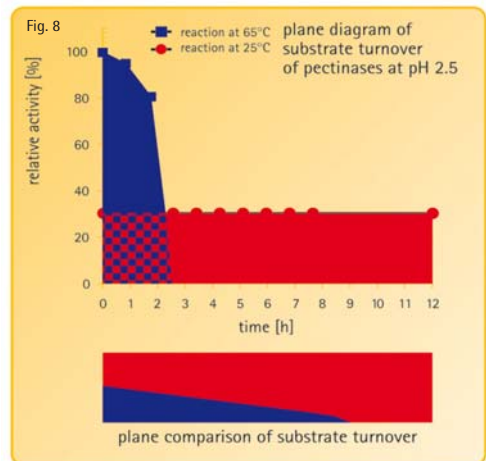
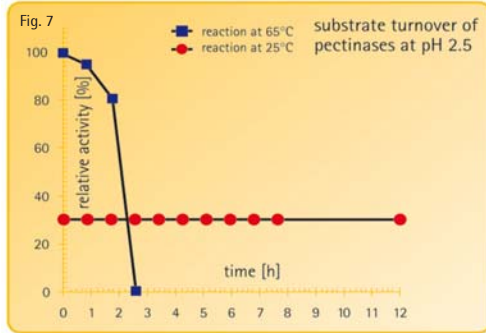
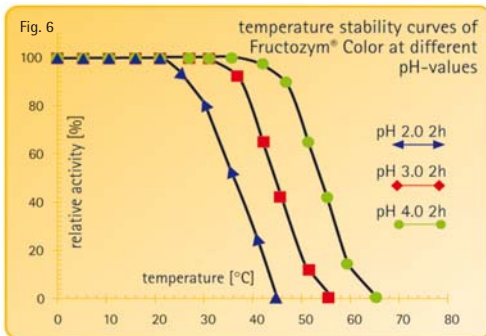


Fig. 9A pH-value dependent maximum temperature for enzymatisation of juices using Fructozym® pectinases under consideration of 100% enzyme stability

pH-value	maximum enzymatisation temperatur
pH 4.5	45°C
pH 4.0	45°C
pH 3.4	40°C
pH 2.9	35°C
pH 2.5	30°C
pH 2.0	20°C

Fig. 9B Fruit dependent optimum temperature for enzymatisation of juices using Fructozym® pectinases under consideration of 100% enzyme stability

fruit juice	optimum enzymatisation temperatures
pear juice	55°C
appel juice	50°C
black currant juice	45°C
cranberry juice	40°C
lemon juice	25°C
lime juice	20°C

concentrate plant, others have an entire day until bottling. In the first case extremely high dosages have to be employed, in the latter case low enzyme dosages are sufficient for a complete pectin degradation. Taking into consideration the pH-dependent temperature stability of pectinases (shown at the example of Fructozym Color – see Fig. 6), the first plant can easily process currant mash at the average currant pH-value of approx. 3.3 at the usual processing temperature of 50 °C. Within one hour not much pectinase is inactivated. But the second plant can save large enzyme quantities by lowering the temperature to 40 °C to maintain a long-lasting stability of the pectinase activity. Whether this is possible or not depends on the microbiological preconditions. If the pH-values are too high, the risk of microbial spoilage arises. In principle at lower pH-values the risk of microbial damage is reduced, the appropriate hygienic measures provided. When processing cranberries with their usual pH-value around or slightly below pH 2.9 the risk of microbial spoilage is so low that – from the economic viewpoint – it makes sense to aim in mash enzymatization at temperatures around 40 °C to avoid too high activity losses.

PRODUCTION OF CLEAR LEMON JUICE WITH FRUCTOZYM P-6L

In processing fruits with pH-values around pH 2.5 the inactivation of the enzymes sets in very quickly, even with acid stable pectinases as for instance Fructozym P-6L, if the processing temperature is above 25 °C. Since microbiological risks at such low pH-values can be almost excluded in hygienic, properly working plants, longer reaction times do not pose any problems. It is advisable to allow reaction times of 12-20 hours. Then pectin degradation with economical dosages is possible. The temperature dependent substrate conversion with Fructozym P-6L at pH 2.5 clearly shows the advantages of a temperature adaptation. This is illustrated in Fig. 7 & 8 which compares the conversion as an area diagram under the respective curves.

CONCLUSION:

FRUIT JUICE MAKING UNDER CONSIDERATION OF ACTIVITY AND STABILITY OF THE PECTINASES

Since at higher temperatures the pH-dependent enzyme activation proceeds faster the processing temperature for the pectin degradation in the course of juice making has to be adjusted to the pH-value of the juice. The maximal enzymatization temperature of the Fructozym pectinases can be defined hereby for different pH-values in such a way that the activity in long-term reactions is still within the range of 100 %, furthermore the diagram can illustrate the best processing temperature (with regard to enzymatization technique) for different fruits or fruit juices under consideration of the fastest conversion rate and with sufficient enzyme stability at the same time (Fig. 9a & 9b). Yet in practical operation often higher temperatures are employed aiming at a quicker completion of pectin degradation. In these cases the enzyme losses resulting from thermal inactivation have to be compensated by higher dosages.

EFFECT OF THE PECTINASE FRACTIONS
PECTINESTERASE AND POLYGALACTURONASE IN
PECTIN HYDROLYSIS WITH COMMERCIAL
PECTINASES

Besides the pectinase main activities, technical pectinase preparations contain numerous cellulytic, hemicellulytic and proteolytic side activities in different concentrations, depending on the production strain and production method of the enzymes. But also the pectinase activity as such is not an isolated activity. The pectin degrading effect of pectinase preparations is indeed based not only on the effect of an individual „pectinase enzyme“ but on the combined effect of several individual activities each of which performs a different reaction in the pectin molecule (Fig.10). Four pectinase fractions are distinguished which contribute to the degradation of the dissolved pectin (hydropectin): pectinesterase (PE, PME), polygalacturonase, pectin lyase and pectate lyase. Sometimes they are even also divided into endo- and exo-enzymes. In addition to this there are pectinase activities which attack high molecular undissolved pectin (protopectin) and convert this into soluble pectin. Such pectinases are called maceration enzymes. Their individual pectinolytic activities are not yet completely analysed. In any case they contain an endo-polygalacturonase, other possible activities are named pectin glycosidase, protopectinase or macerases or they are at least claimed to exist. But pectin is not a homogeneous substance with a uniform molecule structure, it is rather composed of heterogeneous molecules (Fig. 11). Therefore the conversion of the protopectin into hydropectin might as well be based on the effect of non-pectinolytic enzyme activities. Possible

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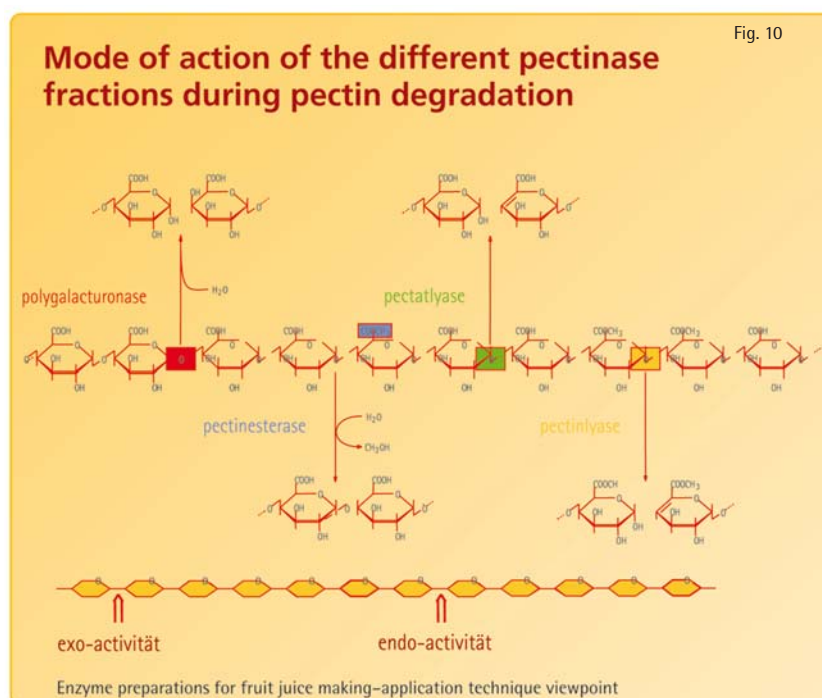
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“candidates” are hemicellulases and/or proteinases or possibly some kind of “pectinsolubilase” which is comparable to the glucansolubilase in malt from barley. Due to their effect on the „hairy regions” of the pectin molecule meanwhile also some of the hemicellulases are associated with pectinases as, for instance, acetylesterase, rhamnogalacturonase and arabinogalactanase.

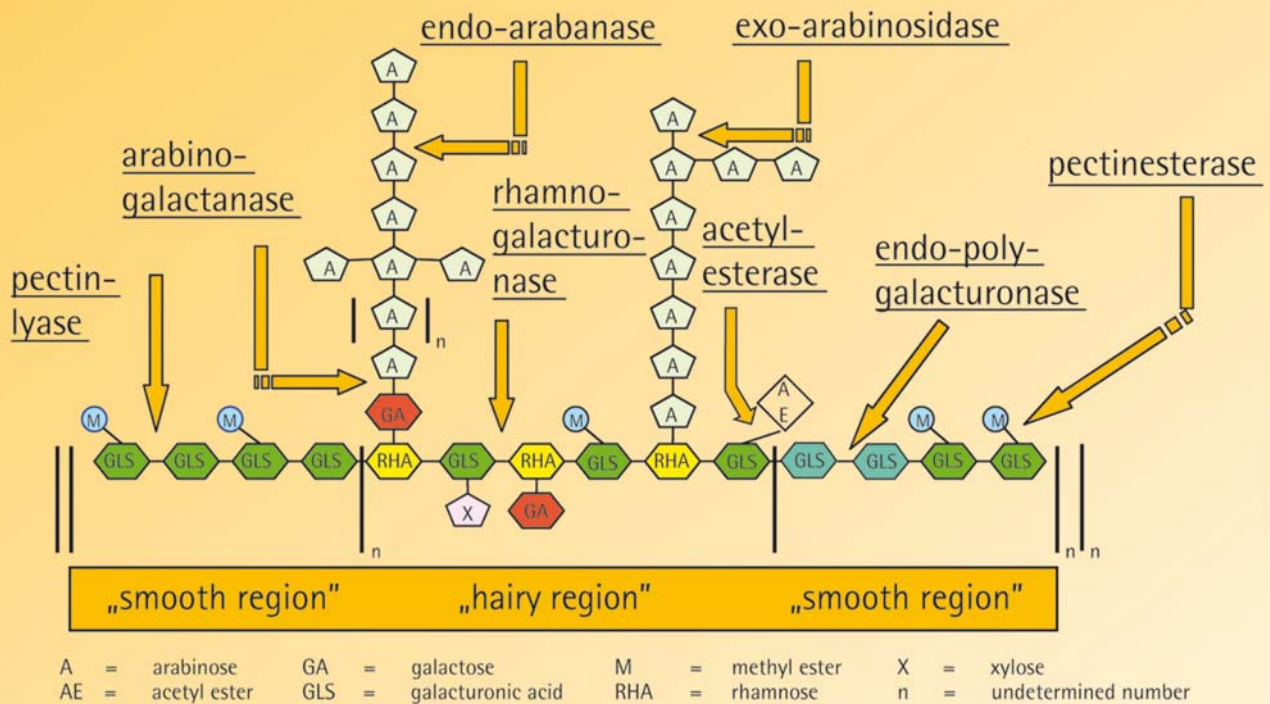


ENZYMATIZATION OF APPLE MASH

Pome fruit mashes from fresh fruits can easily be extracted even without pre-treatment. Mashes from fruits in a late stage of ripeness or stored fruits tend to become pulpy and juice extraction yields only little juice. It is possible to increase juice yield and press capacity and to shorten pressing times by employing tailored “apple mash pectinase preparations” as, for instance, Fructozym MA. By their matched composition of pectinolytic single activities mainly the hydropectin is attacked, which, by its elec-

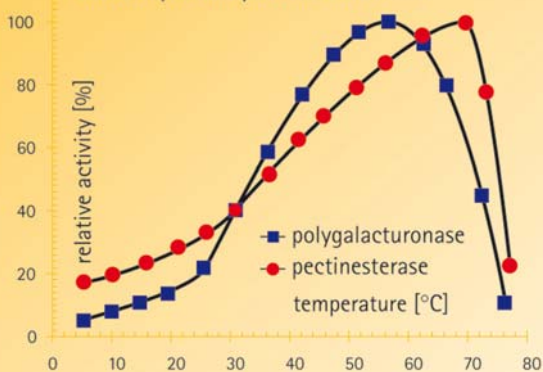
Pectin molecule and required enzyme activities

Fig. 11



temperature activity curves of the most important pectinase fractions

Fig. 12



tro-chemical forces, exerts a binding effect between the solid and the liquid phase. As a result of the degradation of the hydropectins the juice can better drain off the mash. The effect of Fructozym MA and Fructozym MA-X-PRESS is then based on the deesterification of the hydropectin by the pectinase fraction pectinesterase. This deesterification is carried through as quickly as possible and to the largest possible extent, so that the polygalacturonase can effect a rapid viscosity lowering. For that purpose primarily the pectinesterase must react and the polygalacturonase only as far as necessary so that pulpiness is prevented and the protopectin of the marc remains almost unaffected. This is achieved by an enzyme which is designed correspondingly and which has a high

content of pectinesterase and a reduced content of polygalacturonase. The desired effect is furthermore supported by the different activity characteristics of the two pectinase fractions pectinesterase and polygalacturonase because in case of lower reaction temperatures the pectinesterase reacts by far more rapidly than the endo-polygalacturonase (Fig. 12). The efficiency of the Fructozym apple mash enzymes with regard to juice extraction is the more specific the lower the processing temperatures are. Therefore the enzyme producers recommend to carry out apple mash enzymatization at temperatures around or below 25 °C, whenever possible. However, due to the reduced total conversion capacity resulting from the lower temperatures, higher dosages might be required. After mash enzymatization with Fructozym apple mash enzymes the hydropectin is converted to such a degree that the sediment stabilizing effect of the pectin is generally counterbalanced conversion requires an easily modified mode of operation due to the changed filtration characteristics.

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