

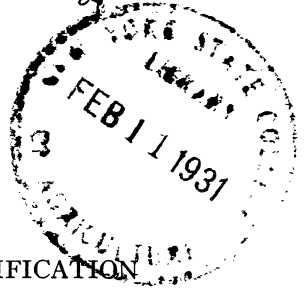
9448
Ha Ar

BULLETIN No. 589

DECEMBER, 1930

New York State Agricultural Experiment Station

Geneva, N. Y.



A NEW METHOD FOR ENZYMIC CLARIFICATION OF UNFERMENTED APPLE JUICE

ZOLTAN I. KERTESZ



PUBLISHED BY THE STATION
UNDER AUTHORITY OF CORNELL UNIVERSITY

CORNELL UNIVERSITY
NEW YORK STATE AGRICULTURAL EXPERIMENT
STATION, GENEVA, N. Y.

STATION STAFF

ULYSSES P. HEDRICK, Sc.D., *Director.*

- ROBERT S. BREED, Ph.D.,
GEORGE J. HUCKER, Ph.D.,
Chiefs in Research (Bacteriology).
- HAROLD J. CONN, Ph.D.,
Chief in Research (Soil Bacteriology).
- CARL S. PEDERSON, Ph.D.,
Associate in Research (Bacteriology).
- P. ARNE HANSEN, B.S.,
Assistant in Research (Bacteriology).
- FRED C. STEWART, M.S.,
MANCEL T. MUNN, M.S.,
Chiefs in Research (Botany).
- MARY E. WOODBRIDGE, M.S.,
OLIVE HOEFLE SIPPLE, B.S.,
Assistants in Research (Botany).
- WALTER O. GLOYER, M.A.,
W. HOWARD RANKIN, Ph.D.,
EDWARD E. CLAYTON, Ph.D., (Riverhead),
JAMES G. HORSFALL, Ph.D.,
JAMES M. HAMILTON, Ph.D.,
Associates in Research (Plant Pathology).
- DWIGHT C. CARPENTER, Ph.D.,
ARTHUR W. CLARK, B.S.,
LEON R. STREETER, M.S.,
Chiefs in Research (Chemistry).
- MORGAN P. SWEENEY, A.M.,
EDMUND L. GREEN, M.S.,
ZOLTAN I. KERTESZ, Ph.D.,
Associates in Research (Chemistry).
- WILLIAM F. WALSH, B.S.,
FRANK KOKOSKI, B.S.,
FLOYD E. LOVELACE, A.B.,
GEORGE W. PEARCE, M.S.,
JOHN J. KUCERA, Ph.D.,
Assistants in Research (Chemistry).
- ARTHUR C. DAHLBERG, Ph.D.,
Chief in Research (Dairying).
- J. COURTENAY HENING, M.S.,
Associate in Research (Dairying).
- JULIUS C. MARQUARDT, M.S.,
Assistant in Research (Dairying).
- HERBERT L. DURHAM,
Dairy Technologist.
- PERCIVAL J. PARROTT, M.A.,
Vice-Director; Chief in Research (Entomology).
- HUGH GLASGOW, Ph.D.,
PAUL J. CHAPMAN, Ph.D.,
Chiefs in Research (Entomology).
- FRED Z. HARTZELL, M.A.,
HUGH C. HUCKETT, Ph.D. (Riverhead),
FREDERICK G. MUNDINGER, M.S.,
(Poughkeepsie),
S. WILLARD HARMAN, M.S.,
DERRILL M. DANIEL, M.S.,
G. E. R. HERVEY, Ph.D.,
Associates in Research (Entomology).
- FOSTER L. GAMBRELL, Ph.D.,
Assistant in Research (Entomology).
- RICHARD WELLINGTON, M.S.,
HAROLD B. TUKEY, M.S.,
REGINALD C. COLLISON, M.S.,
Chiefs in Research (Pomology).
- FRED E. GLADWIN, B.S. (Fredonia),
GEORGE H. HOWE, B.S.,
GLEN P. VAN ESELTINE, A.B.,
LESTER C. ANDERSON, B.S. (Hudson),
GEORGE L. SLATE, M.S.,
BERNARD R. NEBEL, Ph.D.,
Associates in Research (Pomology).
- OLAV EINSET, M.S.,
LEWIS M. VAN ALSTYNE, B.S.,
KARL BRASE,
JAMES D. HARLAN, B.S.,
Assistants in Research (Pomology).
- CHARLES B. SAYRE, M.S.,
Chief in Research (Vegetable Crops).
- WILLIAM T. TAPLEY, M.S.,
Associate in Research (Vegetable Crops).
- WALTER D. ENZIE, B.S.,
PARKS V. TRAPHAGEN,
Assistants in Research (Vegetable Crops).
- PATRICK H. CORCORAN, *Agriculturist.*
- JAMES D. LUCKETT, M.S., *Editor.*
- HERMANN O. JAHN, *Florist.*
- MARJORIE B. ROGERS, *Librarian.*
- JAMES S. LAWSON, Phm.B.,
Museum Preparator.

A NEW METHOD FOR ENZYMIC CLARIFICATION OF UNFERMENTED APPLE JUICE

ZOLTAN I. KERTESZ

ABSTRACT

An enzyme decomposing soluble pectin is proposed as an agent for assisting in the clarification of apple cider. This enzyme acts on pectin and produces no changes in starches or proteins. During the decomposition of the pectin of the apple juice by the enzyme, some insoluble materials are formed. These insoluble substances, together with other substances responsible for the cloudiness of cider, are easily removed by filtration or centrifugation, leaving a crystal clear product which may be pasteurized and bottled within 24 hours after pressing the cider. The product is very palatable and possesses no "cooked" taste.

INTRODUCTION

It is estimated that more than 5,000,000,000 bottles of various kinds of soft drinks are consumed annually in the United States. The major proportion of unfermented beverages represents synthetic drinks, artificially colored and flavored, but there is undoubtedly an opportunity for a greatly increased production of pure fruit beverages.

Unfermented apple juice, or sweet cider, is made and consumed in greater quantities in the United States than any other beverage juice. The wide-spread character of cider making as an outlet for surplus apples is evidenced by the fact that the average annual production of cider is somewhere in the neighborhood of 100 gallons per farm.

Sweet cider is largely a seasonal product, made only during the period immediately following the apple harvest. After the close of the pressing season, it practically disappears from the market and is almost unobtainable except for small quantities sold by soda fountains and drug stores. For this reason there is a large consumption of synthetic drinks even in apple-growing districts.

This situation is due to the fact that comparatively few small-scale producers of apple cider practice the bottling and pasteurizing of their product, because of the complicated and time-consuming processes in-

volved. The fresh juice can be sold easily without any treatment, but the bottled product must be filtered and clarified. If cider is placed directly in containers and pasteurized, it throws down more or less of a precipitate, but the juice remains turbid and has a cooked taste. Bottled cider should be clear and there should be no sediment.

Filtration and clarification generally take much time and also necessitate the use of expensive apparatus. Therefore, a study was undertaken at this Station on the clarification of fruit juices, and a new method is presented here for the simple and rapid clarification of unfermented apple cider by means of an enzyme.

METHODS OF CLARIFYING CIDER

The influence of the variety of apple used (1)¹ and of the method of pressing (2) on the clarification of cider will not be discussed here, for they are dealt with in a very extended literature on the subject. Previous methods used for the clarification of cider are effective only after sedimentation (3). For this reason, the cider is stored in barrels or tanks for 10 to 15 hours, or longer, and the clear juice siphoned off without disturbing the sediment. During this period the cider must be kept cool or fermentation will start. This manipulation not only makes necessary a cool place near the cider press where the temperature will remain below 50° F (10° C), but it also requires much storage space and labor. Also, if over-ripe fruit is used for cider, sedimentation may require 24 to 72 hours. In the new method proposed here, less than 24 hours are needed for storage and for manipulation, after which the cider can be bottled and consumed.

Methods used for the clarification of cider include the following: Heat; centrifuging; filtration thru paper or wood-pulp; removal of the colloids by adsorption upon insoluble, chemically inert adsorbing agents, such as Spanish clay, etc.; the formation of a precipitate which "envelops" and carries down suspended material; and finally, enzymes. In this bulletin only the use of enzymes will be discussed.

CLARIFYING CIDER WITH AN ENZYME

The clarification of fruit juices with an enzyme was first undertaken by Jokichi Takamine, who had some eight patents on manufacturing enzymes, mostly from molds or other micro-organisms. His "Taka-diastase" is widely known and his "Polyzyme" has been in use since

¹ Refers to Literature Cited, page 10.



FIG. 1.—SAMPLES OF APPLE CIDER BEFORE AND AFTER CLARIFICATION. From left to right, fresh cider from McIntosh, fresh cider from Rome Beauty, clarified McIntosh cider, and clarified Rome Beauty cider. Note how the newspaper advertisements can be read thru the clarified cider. This cider was clarified and bottled within 16 hours after pressing.

1913, with a steadily increasing demand. “Polyzyme” is three to five times stronger in its starch-converting power than ordinary malt extract. Besides the starch-converting it has also milk-coagulating and fat-splitting properties, and decomposes proteins. “Oryzime” is the trade name of another concentrated enzyme extract and “Protozyme” is a dried culture containing the enzymes (4).

All of these enzymes are used in the clarification of fruit juices. During their action materials occurring in the juices, such as starches and proteins, are converted into simpler compounds and lose their colloidal properties (5). After treatment with one of these enzymes, cider is ready for the filter press. There are a great many types of filters on the market, but they will not be discussed here.

Besides the decomposition of starch and proteins, the treatment of a fruit juice with an enzyme also helps in the filtration. Sometimes a fruit juice may be too turbid for filtration, but when treated with Protozyme, for example, filtration (6) is materially accelerated.

In the Wallerstein process (3, page 3) a protein-splitting enzyme, which may be pepsin, papain, or bromelin, is added to remove the proteins which are present as reversible colloids. The protein-tannin compound is attacked and converted into simpler completely soluble products.

In general, the enzymes used for the clarification of apple juice act on the starches and proteins, but French cider makers lay much stress upon the coagulation of the pectins of apple cider. This is done immediately after pressing and is thought to be a very essential step. According to French writers, apples from different districts of France, and also different varieties, show differences in the readiness with which coagulation of pectin is obtainable. The maturity of the fruit also plays an important rôle in the coagulation of the pectin.

Pectase, the enzyme coagulating pectin, is present in the apples, but acts very slowly at 39° F (4° C). At higher temperatures alcoholic fermentation is not prevented and usually proceeds very rapidly. It is possible to produce almost instantaneous coagulation of pectin by chemical treatments outlined by French writers, but these can not be recommended since they seriously detract from the beverage quality of the juice (3, page 5).

CHARACTERISTICS OF THE NEW ENZYME

Fruit juices generally contain pectic materials. The pectin content of apple juice is around 0.2 to 0.4 per cent. The cloudiness of cider is partly due to the pectin content, since colloidal materials, such as pectin, hold other substances in solution which would not stay in solution without them. The principle involved in the clarification of these juices is to convert the colloidal substances into particles which are sufficiently fine to form true solutions. In the present case, if the pectin is decomposed, it does form some insoluble material (about 10 per cent of the pectin), but the rest loses its colloidal properties completely. Passing into a true solution, the pectin loses its protective action on other substances and they precipitate out, together with the insoluble material formed.

According to the literature on pectic enzymes (7), this action is closest to the effect of pectinase. This enzyme is supposed to decompose soluble pectin accompanied by the formation of some reducing materials, especially sugars. There is little known about the changes caused by this enzyme in the pectin molecule. The reason for this is that the

structure of the pectic materials has been determined only in recent years.

The enzyme used in the clarification of cider is produced by a number of micro-organisms (8). The mold fungus *Penicillium glaucum* has been found to be an excellent source of this enzyme when grown on the proper nutrient medium. The enzyme can be prepared in liquid or solid form. In the experiments described later, a very active liquid enzyme preparation was used.

This enzyme also decomposes pure pectin solutions. In this case the optimal acidity has been found to be pH 3.0 to 3.5 and the optimal temperature around 104° F (40° C). If heated to 131° F (55° C) for 30 minutes the enzyme is completely inactivated. The preparations used did not display any starch-converting activity. The experiments performed with this enzyme on pectin solutions will be discussed in a later bulletin.

HOW TO USE THE ENZYME

The enzyme proposed for the clarification of apple juice should be used as follows: After pressing, the juice should be filtered thru a cloth which is open enough in texture to permit the juice to run thru quite readily. The use of closely woven cloths in an effort to remove finer particles is useless as they clog very quickly. The juice should be placed in clean containers of any size and the enzyme added immediately without allowing any time for sedimentation, at the rate of about 0.5 per cent by volume. The cider-enzyme mixture should be stirred thoroly and then allowed to remain undisturbed until the action of the enzyme is completed.

The natural acidity of apple juice is generally not very far from that best suited to the action of the enzyme, therefore no attention need be given to the matter of acidity of the juice. The temperature should be kept rather low, altho it is not necessary to store the cider-enzyme mixture at a temperature below 50° F (10° C). The duration of the clarification process should be so regulated that there will be no danger of alcoholic fermentation. This can be easily accomplished by the use of a proper quantity of the enzyme.

In the experiment which will be discussed later as an example of clarification, 0.5 per cent enzyme solution was used and 13 hours were allowed for the clarification. This enzyme preparation had not been concentrated by any of the methods which will be described later by

Willaman and Kertesz, whereby preparations five times as strong have been produced. It should be stated, that the amount of enzyme to be added depends on the activity of the enzyme, and that the time needed for clarification varies inversely with it. In other words, clarification can be easily finished within 10 to 15 hours with a sufficiently strong enzyme solution. When a sample taken from the mixture shows an increase in the size of the particles which formerly have been colloidal and when sedimentation starts, the work of the enzyme is completed. After allowing 1 hour more for completion of the action of the enzyme, the cider should be centrifuged or filtered. The treated cider can be filtered without difficulty, but centrifugation works in this case much better and quicker than filtration. After centrifuging for a certain period, depending on the cider and the centrifuge speed, the perfectly clear cider is obtained and may be bottled.

For preserving, several methods may be used, but the simplest is pasteurization. Since all the colloidal materials are removed from the juice, no precipitates occur during pasteurization and there is no "cooked taste" afterwards. Benzoate can be used for preserving cider, but it is not recommended (9). Sterilization can also be done by filtration, and if the centrifuged clarified juice is used there is no danger of choking the filters.

CHANGES PRODUCED BY CLARIFICATION IN THE COMPOSITION OF CIDER

The changes observed during the clarification of cider may be noted from the description of one experiment.

Around 20 pounds of ripe Rome Beauty apples were washed, run thru a meat chopper, and pressed in a laboratory hand press. During the pressing, regular press-cloth was used. About 1½ gallons of cider of a good quality was obtained. After filtration thru another press-cloth the juice looked rather turbid and contained considerable quantities of finely divided pomace. After pressing and refiltering, 0.5 per cent of enzyme solution was added. The juice was then stirred thoroly and kept at room temperature of about 77° F (25° C).

Within the next 10 hours no change could be observed in the appearance of the cider. But after the eleventh hour it was more turbid and cloudier than before and by the twelfth hour the formation of a sediment could be observed. A sample drawn off and centrifuged for 10 minutes was perfectly clear and had a dark yellow color.

Immediately after pressing and filtering and before the addition of the enzyme, duplicate samples were taken for the determination of the pectic materials as "alcohol precipitate" (10). At the twelfth hour of the experiment other samples of the same size were taken. These samples included all the turbid materials present in the juice. Finally, samples were taken from the centrifuged clear cider. In Table 1 is shown the amount of pectin in the juice at these different times of sampling.

TABLE 1.—CHANGES IN THE PECTIN CONTENT OF CIDER DURING CLARIFICATION.

TIME OF SAMPLING	GRAMS OF PECTIN IN 100 CC OF JUICE*
Fresh cider	0.1840
Just after formation of precipitate	0.0568
After centrifuging	0.0560

* Average of duplicate samples.

It will be seen that the pectin content of the juice when the sediment is formed is practically the same as that of the centrifugated juice. The low pectin content found in the cider after clarification may be due to the imperfect decomposition of pectins by the enzyme or to some other materials which are precipitated by the alcohol and can be redissolved in water. The pectin content of the original juice is rather low, according to the literature (11), but in case of a higher pectin content the process should work even better.

When both original and clarified cider were filtered thru filter paper, the cloudy juice went thru very slowly from the beginning and soon the filtration came to a halt. However, from 120 cc of clear cider poured on a filter paper, 100 cc ran thru within 3 minutes. Attempts were made to filter the juice after the action of the enzyme without centrifuging first and even in this case the juice could be filtered at a satisfactory speed and the precipitate failed to choke the filter paper.

The samples in this experiment were bottled after centrifuging, and were heated for 20 minutes to 168.8° F (76°C). They did not show any trace of precipitate, and their taste was just as satisfactory as that of the original juice and had no "cooked" flavor. No precipitate was observed in the bottles during storage.

As with any enzymic process, all the conditions of temperature, amount of enzyme, and duration of the clarification process should be carefully controlled. If this enzyme and the method described here are used in a proper way, clear cider may be produced, bottled, and consumed within 24 hours after pressing.

LITERATURE CITED

- 1 Caldwell, Joseph S. Chemical composition of American-grown French cider apples and other apples of like character. *Jour. Agr. Res.*, **36**, 391. 1928.
- 2 —————. Farm manufacture of unfermented apple juice. *United States Dept. Agr. Farmers Bul. No. 1264*. 1922. (Revised 1928.)
- 3 —————. Studies in the clarification of unfermented fruit juices. *United States Dept. Agr. Bul. No. 1025*. 1922. (Revised 1928.)
- 4 Daughters, Milo L. The clarification and filtration of fruit juices. *Canning Age*, May, 1927.
- 5 Waksman, S. A. The use of enzymes in the clarification of fruit juices. *The Canner*, April 25, 1922.
- 6 Mehlitz, Alfred. Ein neues Verfahren zur Herstellung von Fruchtsäften, Süssmosten und dergleichen. *Die Konserven-Industrie*, Nos. 19 und 20, May, 1930.
- 7 Willaman, John J. Enzymic relations of pectin. *Minnesota Studies in Biol. Sci.*, No. 6. 1927.
- 8 Willaman, John J., and Kertesz, Z. I. Unpublished work.
- 9 Irish, John H. Fruit juices and fruit beverages. *Fruit Prod. Jour. and Amer. Vinegar Ind.*, **8**, 12. 1928.
- 10 Wichman, H. J. Report on determination of pectin in fruit and fruit products. *Jour. Assoc. Off. Agr. Chem.*, **7**, 107. 1923-24.
- 11 Eckart, Hanns. Untersuchungen von Apfelsäften und Pektinerzeugnissen des Handels. *Zeit. Unters. Nahrungs - u. Genussmittel*, **50**, 405. 1925.