A C T A A L I M E N T A R I A P O L O N I C A
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# STABILIZATION OF APPLE WINES CONTAINING GELATINIZED STARCH AND DEXTRIN

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A method of the stabilisation of apple wines containing gelatinized starch and dextrin has been developed. The method depends on the hydrolysis of these ingredients with amylolytic enzymes contained in "Pektopol P" at the storage cellar temperature of approximately 15°C for one week, followed by the inactivation of introduced enzymes by short pasteurisation at 87-90°C.

## INTRODUCTION

Starch accumulates in apples during their growth, in the ripening period it is hydrolysed to glucose. In the late-ripening apples Bigelow and Gore found 3.7% starch and 5.7% monosaccharides in the end of July, and 0.4% starch and 9.9% monosaccharides in the end of October [1].

Cerievitinov, examining apples from the same tree stated that the contents of starch continuously decreased in the successive apple ripening months [2].

From what has been said it results the many late-ripening apple warieties can contain great amounts of starch in the technical maturity period, at the moment of being processed into must and wine.

In the past few years Polish fruit growers switched over to the cultivation of apple-trees giving late-ripening fruit. Besides, must is produced from fallen apples in the second half of August. Thus, apple pulp may sometimes contain considerable amounts of starch which is forced, during pressing, to the must.

Apple starch, after gelatinization at elevated temperatures becomes responsible for many technological problems especially in the production of apple juices [3], apple concentrates [4] and pectine from apple cake [5].

Sometimes the fruit wines industry encountres problems with cleating and filtering of apple wines and with the lack of physico-chemical stability of these wines due to the gelatinized starch and dextrin left in them as the starch hydrolysis products [8].

These were the reasons that induced the authors of undertake the investigations being reported in this paper. The investigations embraced:

1) external look and temperature parameters gelatinization and dissolution of apple starch obtained from industrial quality apples from domestic plantations, collected in the period from September 1 to 15, 1975,

2) method of stabilisation of apple wines containing gelatinized starch,

3) methods of preventing the passage of starch to wines and turning into gelatinized.

## MATERIALS AND RAW MATERIALS

The following materials were used for investigation:

— apple starch obtained from mixed-variety apples collected between September 1-15, 1975 for use in the industrial production of must,

— soluble starch, p.a.,

— pasteurised apple must from industrial-quality apples of mixed varieties,

— apple wine obtained from pasteurised must,

— "Pektopol P" — a pectolytic preparation from the Vegetable and Fruit Industry Plant at Jasło, of an activity of 40,500°PM and optimum working operation temperature of 55°C.

## METHODS

The Williams et coll. [6] colorimetric method used to determine the content of starch in musts and wines depended on the measurement of the intensity of the blue coloration which it gives with iodine at the light wavelength of $\lambda = 625$ nm.

*Separation gelatinized starch or dextrins from apple must and wine*

10 $cm^3$ of must or wine were poured into a 50 $cm^3$ centrifugal test-tube, then 30 $cm^3$ of methyl alcohol of 96% by volume were added. The content was mixed and placed for 5 minutes in a water bath of a temperature of 80°C. After the elapse of this time the content of the test-tube was cooled and the precipitated starch was centrifuged at 4000 g for 5 minutes. The liquid collected on top of the sediment was poured down,

and the sediment was dissolved in 2 $cm^3$ of 0.5 n KOH. After dissolving the solution was neutralised with 10 $cm^3$ of 0.1 n HCl, poured into 50 $cm^3$ lask, and the starch content was determined by the method of Williams and coll.

*Separation of starch from apple pulp*

25 g of apple pulp were weighed and mixed thoroughly in a mixer with 50 $cm^3$ of water. Then the starch was washed out from the pulp on a fine sieve. The separated starch was washed several times with water and centrifuged at 2000 g.

From the washed-out starch a suspension was prepared concentrated so that some 20 mg starch were present in the volume taken for determination. The starch suspension sample was centrifuged at 4000 g for 2 minutes. The solution collected on top of the sediment was poured off and the centrifuged starch was dissolved in 5 $cm^3$ of 5 n KOH and, afterwards, it was centrifuged once again at approximately 4000 g.

The solution collected on top of the sediment was poured into a 100 $cm^3$ flask and the sediment was dissolved once again in 5 $cm^3$ of 0.5 n KOH. Then the procedure was as before. The sediment left after starch centrifuging was washed twice with 10 $cm^3$ volumes of distilled water, and centrifuged. The eluates from washing were mixed with starch solution in a 100 $cm^3$ flask. The contents was neutralised with 0.1 n of HCl solution (50 $cm^3$) and filled up to the mark. 5 to 30 $cm^3$ of this solution (depending of starch concentration) were used to determine starch content according the method of Williams et coll.

*Adding of soluble starch to the solution*

2% water solution of apple starch were used. The solution was heated for 5 minutes at 40-60°C, and for 2 minutes at 65-100°C. After cooling the sample was centrifuged for 2 minutes at 2000 g. Then 5 $cm^3$ of the liquid were taken, 1 drop of 0.02 n of iodine solution was added and coloration was determined. Additionally the content of extract and viscosity were determined for the contrifuged solution. Temperature of apple starch dissolving and mashing was determined from the colouration with iodine and viscosity.

*Detecting of dextrins*

Dextrins were detected in the wine by their precipitation from the wine with ethyl alcohol at 80-85°C for 5 minutes. The precipitate was centrifuged at 4000 g, then it was dissolved in water, and the presence of dextrins was detected by iodine reaction, This method makes it possible

to detect in the wine trace quantities of dextrins which become coloured with iodine.

Sugars were determined by the Luff-Schoorl method [10]. The extract was determined using Abbe's refractometer [11], whereas viscosity was determined in the Höppler viscometer [12].

## RESULTS AND DISCUSSION

The shape and size of apple starch grains was determined with the microscopic photography technique. The apple starch grains were found to be round, of diameters from 2 to 15 μ, fine grains with diameters up to 5 μ accounting for approximately 80% of all starch. The domination od the fine grained fraction produces difficulties with its sedimentation which can last even several days.

In continuous production processes grained starch can quickly be separated from apple must by its centrifuging or filtering through diatomaceous earth.

From results presented in Table 1 it can bee seen that the apple starch amylose begins to dissolve at 55°C, whereas starch is started to gelatinized up from a temperature of 90°C.

The results obtained in this part of the work confirm the results

Table 1. Temperature of apple starch dissolution and gelatinization

| Temp. °C | | Centrifuged starch solution | | |
|---|---|---|---|---|
| | coloration with iodine | extract % refr. | viscosity cp | remarks |
| 40 | no coloration | | | |
| 45 | no coloration | | | starch does not dissolve |
| 50 | no coloration | | | |
| 55 | light blue | | | |
| 60 | blue | 0.1 | 0.123 | |
| 65 | blue | | | |
| 70 | blue | 0.1 | 1.234 | |
| 75 | intensively blue | | | starch dissolves |
| 80 | intensively blue | 0.3 | 1.345 | |
| 85 | intensively blue | | | |
| 90 | intensively blue | 0.5 | 1.453 | |
| 95 | | 0.7 | 1.587 | starch gelatinized |
| 100 | | 0.9 | 1.931 | |
| 120 | | 1.4 | 3.169 | heated in autoclave for 20 min. |

obtained by Krebs' investigations into the physico-chemical properties of apple starch [3], but for the samples under test the share of the finest-grained starch (grain diameter 2-5 μ) was high, reaching 80% of its total amount.

Apple wine may contain gelatinized starch only when the must or wine containing grained starch was exposed to the action of elevated temperature, e.g. of 60°C. This takes place most frequently when one pasteurises the must from which grained starch was not only completely separated by centrifuging or sedimentation, or when one pasteuries apple wine with the post-fermentation sediments containing grained starch not completely separated from it. Besides, sediment is, as a rule, precipitated after some time from the wine obtained from concentrates containing gelatinized starch or fragments of incompletely swollen starch or dextrin after being bottled, in spite of complete clarity during bottling [8].

The content of gelatinized starch in the wine, in an amount of a few mg/l results in the wine loosing its physico-chemical stability after many months, because dissolved and gelatinized starch is slowly retrograding, aspecially after having been cooled, giving long-lasting turbidity. A similar phenomenon can be caused by the dextrins, the starch hydrolysis products.

During the tests of their physico-chemical stability the apple wines containing dextrin become opaque after cooling but they get clear again after heating them to the room temperature.

Dextrins in apple wine behave in the same way as reversible coloids.

The detection of the presence of gelatinized starch in the wine by direct iodine reaction is not difficult at all. On the other hand, it is rather difficult to detect in the wine the presence of dextrins by direct iodine reaction because of the fact that the dextrin-iodine colour is masked by the natural colour of the wine [8]. Usually it is impossible to detect in the wine the presence of little amounts of low-molecular dextrins of the oreder of several mg/l by the direct iodine reaction.

Krebs [3] and Gramp [4] in their investigation hydrolysed the gelatinized starch in apple juice with amylolytic enzyme preparations, at elevated temperatures.

The usefulness of cooling for the removal of gelatinized starch and dextrins from the wines was investigated as one of the simpler wine stabilisation methods. The wine was cooled at -3 to -4°C for 3 days and filtering was carried out at the temperature of cooling. After this cooling time starch and dextrins can be separated completely from the wine.

The method was, however, evaluated as not useful because of the fast clogging of the filters and the great technical difficulties resulting from this fact, as well as because of a high consumption of the filtering mass. In further investigations it was found that the pectolytic enzyme prepa-

ration "Pektopol P" has high amylolytic ability. This fact has been confirmed by the results of the hydrolysis of the potato starch glue containing 20 mg/l of starch D.M. The same starch hydrolysis parameters were used as in depectinisation i.e.: pH 3.2, temperature 55°C, time 2 hours, preparation dose 2 g/l [7]. The results obtained are specified in Table 2.

Table 2. Amylolytic ability of „Pektopol P"

| Specification | Content of sugars in terms of glucose mg/l |
|---|---|
| Sugars after hydrolysis with „Pektopol P" of gelatinized starch (lask of reaction with iodine) | 15 800 |
| Sugars after supplementary acid hydrolysis of dextrins by Fehlenberg method (9) | 20 832 |
| Sugars contained in „Pektopol P" added, kept for two hours at 55°C | 248 |
| Gelatinized starch without „Pektopol P" | not present |

From the data contained in this Table it results that gelatinized starch is mainly hydrolysed to glucose. The hydrolysis of the gelatinized starch with "Pektopol P" offers a possibility of using it for the stabilisation of apple wines containing dissolved starch.

In order to check this assumption the authors tried to stabilise the wine containing gelatinized starch in an amount of 25 mg/l using "Pektopol P". O.25 g/l of the preparation was used, and the following temperatures: a) 55°C, b) storage cellar temperature (15-17°C).

It was found that the apple wines, hot stabilised (55°C) with this preparation, already after 10 minutes do not show the presence of gelatinized starch or dextrin in the iodine reaction. In stability tests these wines behave as physicochemically stable. It was found, however, that during the hot stabilisation of apple wines with "Pektopol P" their quality is considerably reduced, from 15 points to only 12 in the 20-point scale.

This fact excludes the possibility of hot stabilisation with "Pektopol P" of apple wines containing gelatinized starch. In this connection further stabilisation tests were conducted at the storage cellar temperature (approx. 15°C). The dose of "Pektopol P" was determined in the course of laboratory tests using increasing amounts of this preparation, i.e. 0.05, 0.01, 0.2, 0.3 and 0.4 g/l.

After one week all the samples with a "Pektopol P" dose of 0.1 g/l and more did not show any presence of starch or dextrins. The sample

with a 0.05 g/l dose of "Pektopol P" showed the presence of low-molecular dextrins.

Industrial scale tests of the stabilisation of wines containing gelatinized starch in an amount of 25 mg/l were also carried out using a 0.25 g/l dose of "Pektopol P".

The wines contained 100-150 mg $SO_2$ (total) including 10-20 mg free $SO_2$ per litre which protected them against the activity of oxidases present in the preparation. After one week of the activity of the enzymes, the wines, in the iodine reaction, did not show any presence of starch or dextrins and they also featured a high physico-chemical stability.

For a effective inactivation of the oxidases the flash pasteurisation of stabilised wines at 87-90°C was employed. The wines stabilised by this method maintained their quality. It is necessary to prevent the starch from getting gelatinized in the must or wine. Thus, before heating them above 55°C the grained starch should be separated from the must by centrifuging or filtering through diatomaceous earch. Wine sedimentation and accurate removal from above the sediment may also be used.

## CONCLUSIONS

1. Apple wines containing gelatinizes starch and dextrins may be stabilised with enzymatic hydrolysis using amylolytic enzymes of "Pektopol P", at the storage cellar temperature for 1 week, with subsequent inactivation of the introduced enzymes by means of short pasteurization at 87-90°C. The dose of the preparation is determined during the initial tests.

Stabilisation by cooling at the temperature of -3 to -4°C for 3-4 days, with filtering at this temperature was assessed as less useful because of difficulties which it created during the filtering process.

2. To determine the content of gelatinized starch in wines and musts it is necessary to preliminarily precipitate it with ethanol by the hot method (80°C) and to separate it from the solution.

3. To avoid the loss of apple wines stability, grained starch should be separate before pasteurisation from must or wines by centrifuging, filtering or sedimentation. The share of fine grains of diameters from 2 to 5 μ in apple starch amounts to some 80%, which is the cause of the slow course of sedimentation.

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## STBILIZACJA WIN JABŁKOWYCH ZAWIERAJĄCYCH SKLEIKOWANĄ SKROBIĘ

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Streszczenie

W pracy badano przyczyny braku stabilności fizykochemicznej win jabłkowych, jaka niekiedy występuje w produkcji przemysłowej. Stwierdzono, że częstą przyczyną braku stabilności win jabłkowych jest obecność w nich skleikowanej skrobi i dekstryn. W związku z tym w pracy badano: wygląd, uziarnienie i niektóre właściwości fizyczne skrobi z jabłek otrzymanych w polskich warunkach klimatycznych, adaptowano metody do oznaczania skrobi, opracowano metody stabilizacji win jabłkowych zawierających skleikowaną skrobię i dekstryny.

Stwierdzono, że skrobia z badanych jabłek posiada średnicę ziaren 1-15 μ, początkową temperaturę rozpuszczania amylozy 50-55°C, początkową temperaturę kleikowania ok. 90°C. Udział ziaren drobnych o średnicy 2-5 μ wynosi ok. 80%.

W pracy stwierdzono przydatność zmodyfikowanej przez autorów jodometrycznej metody Williamsa i współpracowników do oznaczania skrobi w jabłkach oraz moszczach i winach. Przy oznaczaniu zawartości skleikowanej skrobi w moszczach i winach konieczne jest jej wstępne wytrącenie etanolem na gorąco (80°C) i wydzielenie z roztworu. Przy oznaczaniu skrobi (ziarnistej) w miazdze jabłkowej, moszczach, wymagane jest wstępne wydzielenie jej drogą wymywania i frakcjonowanego wirowania. Wina jabłkowe zawierające skleikowaną skrobię i dekstryny można stabilizować hydrolizą enzymatyczną wykorzystując enzymy amylolityczne „Pektopolu P", w temperaturze leżakowania w ciągu 1 tygodnia z późniejszą inaktywacją wprowadzonych enzymów, pasteryzacją momentalną w temperaturze 87-90°C. Dawkę preparatu ustalano w próbach wstępnych. W warunkach przemysłowych do stabilizacji wina stosowano dawkę „Pektopolu P" 0,25 g/l. Stabilizacja na ciepło tym preparatem pogarsza jakość win. Stabilizację drogą wychładzania w temperaturze -3 do -4°C w czasie 3 do 4 dni z filtracją w tej temperaturze oceniono jako mniej przydatną ze względu na trudności w procesie filtracji.