# Studies of stored run-offs from raw cane sugar refining

## Untersuchungen von gelagerten Abläufen aus der Rohrrohzuckerraffination

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Results of microbiological and physical/chemical investigations of stored run-offs from several East German sugar factories are discussed. After testing run-offs as affination syrup, 1st remelt run-off syrup, and 2nd remelt run-off syrup from raw cane sugar refining only 2nd remelt run-off syrup was suitable for storage. This run-off was stored with 70%, 75% and 80% dry substance content, pH value 6 and 9 and at 5 °C and 20 °C in preliminary tests. Changes in microbial count and in dry substance, sucrose, and reducing sugars were studied. At the same time the water activity $a_w$ was measured. The difference of $a_w$ in run-offs with 70% and 75% ds is significant, whereas in run-offs with 75% and 80% ds it is very small. A series of tests was carried out with 75% ds and at 20 °C. Samples were stored up to 180 days according to a programme at pH 6 and 9 with and without inoculation, with and without formaldehyde addition as a disinfectant. The conclusion is that 2nd remelt run-off syrup is suitable for storage over a period of 180 days at 20 °C, if minimum dry substance content is 75 % and formaldehyde is added at fortnightly intervals and in doses of 15 $cm^3/m^2$ run-off surface. For these conditions a minimum pH 6 is sufficient. If formaldehyde has to be avoided, alkali addition is recommended to raise the pH to 9. The total microbial count in run-off should not exceed $10^3$ CFU/g before storage. After 120 days microbial count should be checked every 20 days as precaution.

Es werden die Ergebnisse mikrobiologischer und chemisch-physikalischer Untersuchungen über die Lagerfähigkeit von Abläufen aus mehreren ostdeutschen Zuckerfabriken diskutiert. Die aus der Rohrrohzuckerumarbeitung stammenden Produkte Affinationsablauf, Ablauf B und Ablauf C erwiesen sich bis auf Ablauf C als nicht lagerfähig. Dieser Ablauf wurde in Vorversuchen mit 70 %, 75 % und 80 % Trockensubstanz, pH-Werten von 6 und 9 sowie bei Temperaturen von 5 °C und 20 °C eingelagert, auf Veränderungen im Keimgehalt, im Gehalt an Trockensubstanz, Saccharose und reduzierenden Substanzen untersucht. Gleichzeitig wurde der $a_w$-Wert gemessen und festgestellt, daß die Differenzen der $a_w$-Werte bei 75 % und 80 % Trockensubstanz deutlich geringer sind als bei 70 % und 75 % TS. Die Hauptversuche wurden bei 75 % TS und 20 °C durchgeführt. Die Proben wurden nach einem Schema bei pH-Werten von 6 und 9 beimpft und unbeimpft sowie mit und ohne Formaldehyd-Behandlung bis zu 180 Tagen gelagert. Nach den vorliegenden Ergebnissen ist Ablauf C bei 20 °C über einen Zeitraum von 180 Tagen lagerfähig, wenn der Trockensubstanzgehalt mindestens 75 % beträgt und Formaldehyd im Abstand von 14 Tagen in einer Menge von 15 $cm^3/m^2$ Oberfläche zugegeben wird. In diesem Fall reicht ein pH-Wert von mindestens 6 aus. Ohne Formaldehyd-Behandlung wird die Alkalisierung auf pH = 9 empfohlen und ein Gesamtkeimgehalt des Ablaufes bei Einlagerung von maximal $10^3$ pro Gramm Ablauf gefordert. Nach 120 Tagen Lagerung sollte der Keimgehalt im Abstand von 20 Tagen kontrolliert werden.

## 1 Introduction

At the Faculty of Food Technology of the Humboldt-University in Berlin physical/chemical and microbiological parameters of raw cane sugar and affined sugar are investigated [1, 2]. Research showed that raw cane sugar, if stored under certain conditions is a good culture medium for growing microorganisms, especially osmophilic yeasts. Moreover, experiments established the considerable decrease in microbial count of raw cane sugar by affination. Further investigations showed that the affination syrup is heavily contaminated with microorganisms from raw cane sugar [3]. Microbiological control in the manufacture of raw cane sugar and across a refinery were subjects of two papers in the Workshop on Raw Sugar Quality, held in 1986 [4, 5].

The following research projects were concerned with specific metabolism performance of osmophilic yeasts and process optimization for raw cane sugar refining [6, 7]. The main task of these studies was to test the conditions for delivery of run-offs instead of refinery molasses to the fermentation industry. Whereas *Smith* and *Cazalet* [8] had reported on stored A and B molasses from cane sugar factories the purpose of our work was to test run offs from raw cane sugar processing. The reason for run-off supply and a process flow diagram of a sugar refinery was previously presented in another publication [9]. So raw cane sugar from Cuba has been refined subsequent to sugar beet campaign in East German sugar factories since 1956. About 250,000 metric tonnes of raw cane sugar have been imported, approx. 30,000 metric tonnes have been processed for fodder yeast, and the major part used for refining in 1989. Because a deficit of sugar containing raw materials existed in the fermentation industry of the former GDR, surplus refined sugar has been lessened by delivery of run-offs with higher sucrose contents.

Further goals were the shortening of the remelt process in the refinery in order to increase the daily melt rate, to decrease the sucrose losses, and to save energy.

Because run-offs are produced during the campaign and the processing is done in the fermentation industry all the year round, investigations were aimed at keeping quality in storage. Experiences drawn from thick juice storage were utilized. But it was unsuitable to transfer these results to run-off storage unindiscriminately because, compared with thick juice from sugar beet, syrups from raw cane sugar refining differ markedly in purity, content of reducing sugars, pH value, and initial microbial count, the last due to raw sugar storage in the open air. Therefore, this paper deals with results of physical/chemical and microbiological studies of stored run-offs from raw cane sugar processing.

## 2 Materials and methods

### 2.1 Materials

Various run-offs from different East German sugar factories were investigated. Affination syrup, 1st remelt run-off syrup, and 2nd remelt run-off syrup formed part of studied run-offs. The process flow diagram (Fig. 1) shows the sampling points for the syrups used (broken lines). In the course of initial investigations it was established that the affination syrup and 1st remelt run-off syrup were not suited for long storage for various reasons. It was decided to use only 2nd remelt run-off syrup for further investigations. For main test series Güstrow sugar factory had placed at our disposal 2nd remelt run-off syrup with the following parameters in original condition:

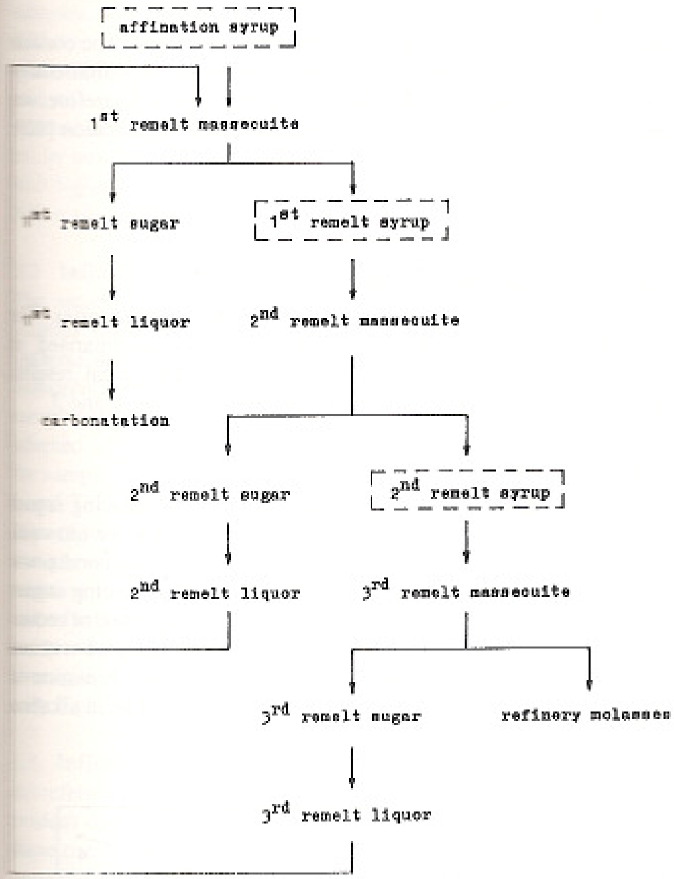


Fig. 1: Remelt process with delivery of different run-offs

| | |
|---|---|
| dry substance (ds) | 83.0 % |
| purity | 68.8 % |
| reducing sugars (rs) | 10.59 g/100 g ds |
| pH value | 6.4 |

### 2.2 Preliminary test series

During the preliminary experiments various dry substance contents, temperatures and pH values were used. The run-offs were prepared at 70%, 75% and 80% ds and pH 6 and 9. The storage was carried out at 5 °C and 20 °C. The syrups with 75% and 80% ds were shown to be the most suitable for storage, independent of their pH values. The lower temperature of 5 °C had a positive effect on the storage results. With respect to practical application samples were stored at 20 °C in main test series. Due to optimal growing conditions at higher temperatures for most of the microorganisms (mesophilic) it is necessary to keep run-offs at these temperatures.

In addition to chemical/physical investigations, samples were tested microbiologically. It was concluded that the storage of the run-offs is affected by the development of the microorganisms which they contain. In order to multiply, microorganisms need appropriate temperatures and pH values, nutrients and oxygen, but mainly water. It is not water content as measured by the dry substance of a syrup, which determines what microorganisms can use for their development, but rather the water activity ($a_w$) [10]. For that reason water activity was determined in run-off storage (Fig. 2). As can be seen in Table 1, microorganisms are able to grow at the listed water activities or they can induce undesirable changes in substrates by metabolic activities.

At the beginning of storage run-offs with 70% ds and $a_w$ 0.8512 are not preserved from deterioration. Microorganisms are also capable of attacking syrups with 75% and 80% ds, provided that the dilution occurs on run-off surface in large-scale storage. There is a marked difference in water activity values between run-offs of 70% and 75% ds but between 75% and 80% ds the difference is very small (Tab. 2).

The water activities of syrups with 75% and 80% ds only differ sligthly, the conditions of development for microorganisms are almost identical (Fig. 3). Subsequently, and to avoid crystallization at 80% ds the main test series was carried out on a run-off of 75% ds.

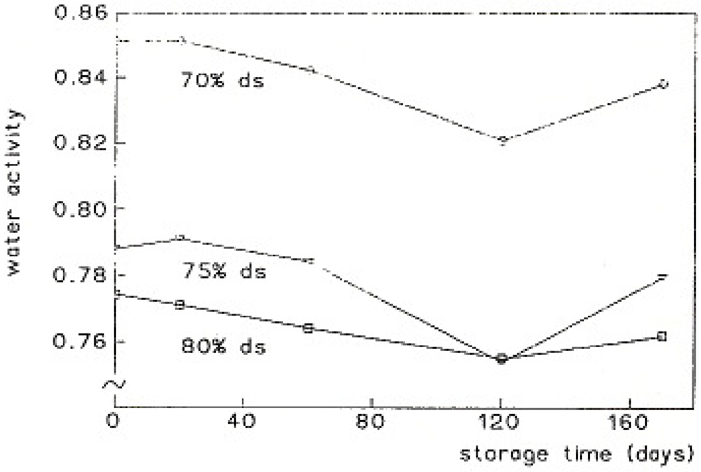


Fig. 2: Development of water activity during storage of syrups

**Table 1:** Influence of wateractivity ($a_w$) on microbial growth [11]

| water activity ($a_w$) | microorganisms which were inhibited |
|---|---|
| 1.00 … 0.95 | gramnegative bacteria, some yeasts |
| 0.95 … 0.91 | most cocci, *Lactobacillus*, some fungi |
| 0.91 … 0.87 | most yeasts |
| 0.87 … 0.80 | most fungi, *Staphylococcus aureus* |
| 0.80 … 0.75 | most halophilic bacteria |
| 0.65 … 0.60 | osmophilic yeasts |

**Table 2:** Water activity and dry substance of run-offs of raw cane sugar refining

| ds in % | 70 | 75 | 80 |
|---|---|---|---|
| $a_w$ | 0.8512 | 0.7880 | 0.7740 |
| difference of ds | | 5 | 5 |
| difference of $a_w$ | | 0.0632 | 0.0140 |

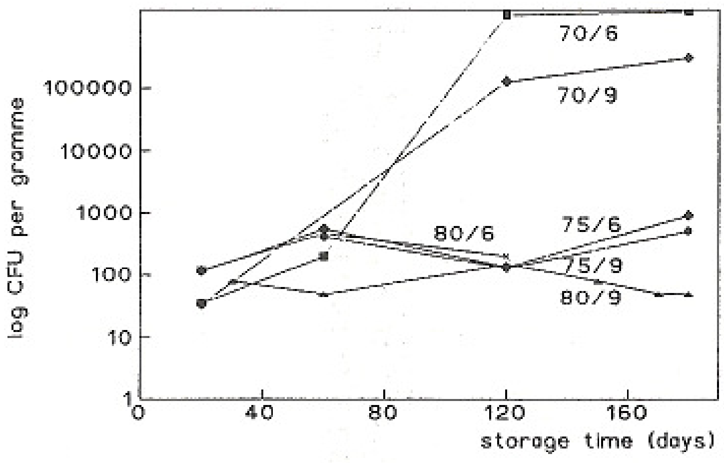


Fig. 3: Development of the CFU during storage of run-offs, $t$ = 20 °C

### 2.3 Main test series

#### 2.3.1 Storage conditions

2nd remelt run-off syrup was prepared at 75% ds. The storage temperature was adjusted to 20 °C. The quality criteria and modifications of test conditions are shown in Table 3.

Table 3: Programme for main test series

| Sample No. | pH value | inoculation | formaldehyde treatment |
|---|---|---|---|
| 1 | 6 | – | – |
| 2 | 6 | – | x |
| 3 | 6 | x | – |
| 4 | 6 | x | x |
| 5 | 9 | – | – |
| 6 | 9 | – | x |
| 7 | 9 | x | – |
| 8 | 9 | x | x |

Microorganisms were inoculated in the run-offs by means of 1 g soil/l run-off. This soil was collected next to a molasses tank and includes:

$123 \cdot 10^6$ spore forming bacteria,
$61 \cdot 10^5$ yeasts,
$45 \cdot 10^2$ osmophilic yeasts.

The formaldehyde treatment was repeated once a fortnight in a dosage of 15 $cm^3/m^2$ substrate surface. For every sample a parallel sample was stored. Changes of quality parameters were controlled at intervals of 30 days.

2.3.2 Microbiological test methods

Investigations were carried out for bacteria (nutrition agar, 30 °C; 48 h), yeasts and mould fungi (Sabouraud-glucose-agar, 25 °C; 48 h) and osmophilic yeasts (Sabouraud-agar with 50% w/w glucose, 25 °C; 72 h).

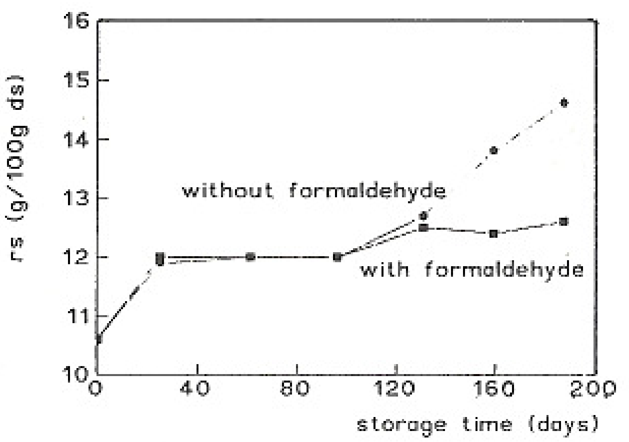


Fig. 4: Development of reducing sugars in 2nd remelt run-off syrup; 75% ds; pH 6; 20 °C

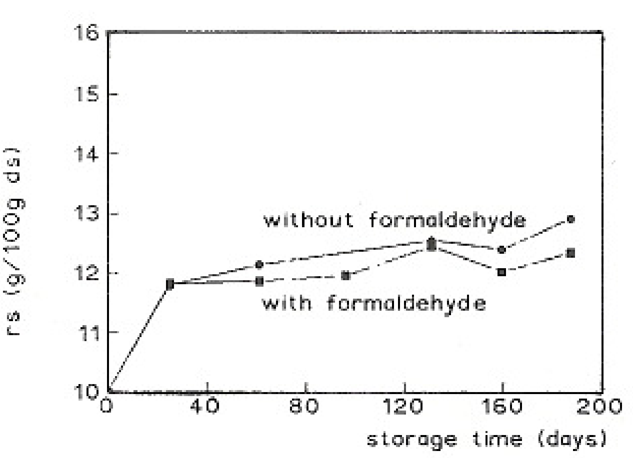


Fig. 5: Development of reducing sugars in 2nd remelt run-off syrup; 75% ds; pH 9; 20 °C

2.3.3 Analytical methods

Refractometric determination of dry substance was used. The content of reducing sugars was measured by this method with 3.5-dinitrosalycilic acid; sucrose was checked by direct polarization. Therefore, sucrose content was corrected by means of the following equation [12]:

$$s_{cor} = s_{pol} + 0.3102 \cdot rs.$$

## 3 Results and discussion

The evaluation of experiments is restricted to the main test series. The chemical/physical and microbiological results are summarised in Figures 4–7. Figures 4 and 5 show the chemical/physical results, whereas microbiological results are illustrated in Figures 6 and 7.

### 3.1 Influence of the storage time

As can be seen in Figures 4 and 5 the first rapid rise of reducing sugars content happened within 30 days. This increase seems to be unavoidable and averages 1.56 g/100 g ds, independent of storage conditions. If run-offs are treated with formaldehyde, 75% of the reducing sugars are formed within the first 30 days. A second steep increase of reducing sugars is obvious after 120 days the in case of samples without formaldehyde. This development depends on pH-value. Formation of reducing sugars was rather accelerated in tests at pH 6 than in alkaline

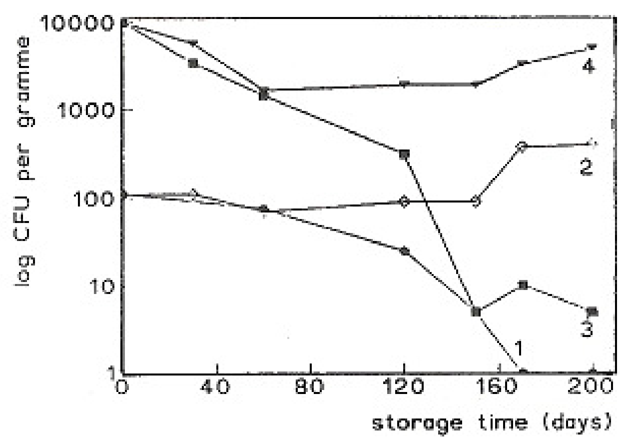


Fig. 6: Development of CFU during storage of 2nd remelt run-off syrup; 75% ds; pH 6; 20 °C

1 original with formaldehyde; 2 original without formaldehyde; 3 mixed with soil and formaldehyde; 4 mixed with soil without formaldehyde

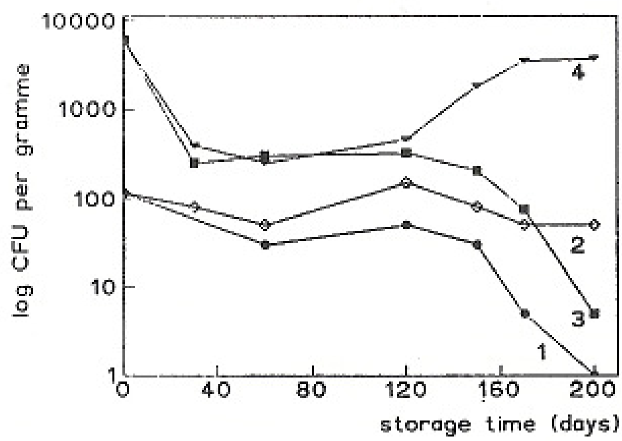


Fig. 7: Development of CFU during storage of 2nd remelt run-off syrup; 75% ds; pH 9; 20 °C

1 original with formaldehyde; 2 original without formaldehyde; 3 mixed with soil and formaldehyde; 4 mixed with soil without formaldehyde

120 days storage but only in samples without formaldehyde. The formation of reducing sugars is combined with the degradation of sucrose and we can assume the yield of reducing sugars to be about 50% of the sucrose destroyed [13]. It is thus possible to calculate the sucrose losses. In samples with formaldehyde treatment, this loss amounts to 2.9 to 3.5 g/100 g, respectively and without formaldehyde to 3.2 to 6.0 g/100 g after 180 days' storage time.

### 3.2 Influence of initial microbial count

The run-offs investigated with 75% ds contained originally 225 CFU (colony forming units) per gramme. Out of 10 samples all showed bacteria development, but only 6 showed yeasts growth. Moulds or osmophilic microorganisms could not be tracked down in any of these samples. If run-offs are inoculated with sucrose containing substrates adapted to microorganisms, the initial microbial count is significant for samples without formaldehyde treatment, as can be seen in Figures 6 and 7. In this case the microbial count increases strongly after 120 days of storage. The pH value 6 provides microorganisms with beneficial growing conditions, more effectively than pH 9. With the periodic addition of disinfectant it was possible to measure a decrease of the microbial count. This content is insignificant after 180 days of storage, independent of the pH-value. Parallel to microbiological alteration the chemical/physical quality changes of the inoculated samples yielded only higher values if no treatment with formaldehyde was carried out.

### 3.3 Influence of pH-value

As referred to in literature [14], the positive effect of a pH value 9 is evident during storage of beet thick juice. Our work has confirmed these comments only without addition of formaldehyde. After regular spraying of samples with formaldehyde, the pH values 6 or 9 had no influence on the storage result.

### 3.4 Influence of formaldehyde

The formaldehyde has the most significant influence on the storage result. The microbiological activity could be effectively suppressed and 180 days' storage of run-offs could be guaranteed. This behaviour is independent of initial microbial count and pH values.

## 4 Conclusions

After investigating the run-offs only 2nd remelt run-off syrup was suitable for storage over a period of 180 days under the following conditions:

- minimum dry substance content at 75%
- formaldehyde addition in fortnightly intervals and in doses of 15 $cm^3/m^2$ run-off surface.

For these conditions a minimum pH value 6 is necessary. If the process has to be done without the addition of formaldehyde, it is recommended to add alkali to increase the pH value to 9. In this case the total microbial count may not exceed $10^3$ CFU/g syrup. As a precaution microbial count must be controlled at a maximum interval of 20 days after 120 days' storage time in order to avoid a violent growth of microorganisms.

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## Essais avec des égouts stockés provenant du raffinage de sucre de canne (Résumé)

On discute les résultats d'essais microbiologiques et physico-chimiques effectués sur des égouts stockés provenant de différentes sucreries en Allemagne de l'Est. Après avoir testé des égouts tels que le sirop d'affinage, le sirop de 1re refonte et de 2de refonte provenant du raffinage de sucre brut de canne, on a remarqué que seulement le sirop de 2de refonte se prêtait au stockage. Ce sirop a été stocké à des teneurs en M.S. de 70 %, 75 % et 80 %. La valeur du pH fut de 6 et de 9 et le stockage se fit dans les comptages microbiens, dans la teneur en M.S., en sucre et en sucres réducteurs. En même temps on mesura l'activité de l'eau $a_w$. La difference entre l'$a_w$ dans les sirops à 70 et à 75 % de M.S. est nettement plus importante que pour les sirops à 75 et à 80 % de M.S. pour lesquels cette différence est très faible. Les principales séries d'essais on été effectuées à 75 % de M.S. et à 20 °C. Les échantillons ont été stockés durant 180 jours à des valeurs de pH de 6 et de 9, avec et sans inoculation, avec et sans addition de formol. Les résultats indiquent que l'égout à 20 °C peut être conservé pendant 180 jours, si le brix atteint au moins 75 et si on ajoute chaque quinzaine du formol dans le rapport de 15 $cm^3$ par $m^2$ de surface. Dans ce cas une valeur de pH d'au moins 6 suffit. En absence de formol, on recommande un pH de 9 et une teneur maximale en germes (au moment du stockage) de $10^3$ par gramme d'égout. Au bout de 120 jours on devra contrôler la teneur en germes tous les 20 jours.



## Estudios de jarabes almacenados obtenidos de la refinación de azúcar de caña crudo (Resumen)

Se discuten los resultados de estudios microbiológicos y físico-químicos acerca del almacenamiento de jarabes de varias azucareras del este de Alemania. De los productos jarabe de afinación, jarabe B y C, obtenidos de la transformación de azúcar de caña crudo, solamente el jarabe C resultó ser almacenable. En ensayos previos este jarabe C con 70, 75 y 80 % de materia seca, de valores de pH de 6 y 9, fue almacenado a 5 y 20 °C y se estudiaron los transtornos en el contenido microbiano, el contenido de materia seca, sacarosa y sustancias reductoras. Al mismo tiempo se midió el valor $a_w$ de la actividad del agua y se observó que las diferencias de los valores $a_w$ en materias secas de 75 y 80 % son considerablemente menores que las de 70 y 75 % materia seca. Los ensayos principales se llevaron a cabo en 75 % materia seca y a 20 °C. Las muestras fueron almacenadas hasta 180 días a valores de pH de 6 y 9, con y sin inoculación así como con y sin tratamiento de formaldehido. Los resultados mostraron que el jarabe C es almacenable a 20 °C hasta 180 días, si el contenido de materia seca es de por lo menos 75 % y si se añade cada 14 días formaldehido en cantidades de 15 $cm^3/m^2$ superficie. En este caso, un valor de por lo menos 6 es suficiente. Sin tratamiento de formaldehido, se recomienda una alcalinización a pH = 9 y se requiere un contenido microbiano total del jarabe a almacenar de maximal $10^3$ pro gramo de jarabe. Después de 120 días de almacenaje se deberá controlar el contenido microbiano cada 20 días.



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