Effect of non-malted barley on low alcohol and non-alcoholic beer production

Ivan Petelkov¹, Vesela Shopska¹, Rositsa Denkova-Kostova¹,
Velislava Lyubenova², Kristina Ivanova³, Georgi Kostov¹

1 – University of Food Technologies, Plovdiv, Bulgaria

2 – Institute of Robotics, Bulgarian Academy of Sciences, Bulgaria

3 – Institute of Canning and Food Quality, Agricultural Academy, Bulgaria

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Corresponding author:

Georgi Kostov
E-mail:
george_kostov2@
abv.bg

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Abstract

Introduction. The aim of the present work was to substantiate for production of low and non-alcoholic beer by changing the wort composition by replacing part of the malt with unmalted barley.

Materials and methods. Industrial malt and non-malted barley were used. The alcohol fermentation was carried out using free and alginate-chitosan encapsulated top-fermenting yeast strain *Saccharomyces cerevisiae* S-33. The mashing regime included two pauses – 30 min at 50 °C and 60 min at 77 °C. The aldehydes were determined according to the bisulfite method, the ester concentration was determined by ester saponification with NaOH. Metabolites were determined after simple sample distillation of the beer.

Results and discussion. The wort extract decreased with the increase in the quantity of non-malted barley. In the selected mashing process, the fermentable sugars varied from 3.4 to 4.17% and accounted for about 50% of the laboratory wort extract. Use of 20% barley as adjunct led to a decrease in wort extract by 10% but it did not significantly affect viscosity. A major drawback of the use of non-malted barley is the increase in the laboratory wort filtration time. Filtration time was within 60–75 minutes when the adjunct was up to 20%.

It was selected to replace 20% of the malt with unmalted barley. Wort in semi-industrial conditions with the thus selected quantitative malt/barley ratio was produced (8.03% (w/w) extract and fermentable sugar content of 3.7%). The resulting wort was subjected to alcoholic fermentation at 10 °C for 7 days with free and immobilized cells, and for each variant the fermentation kinetics were determined.

The process with free cells was relatively slow and in the first 2–3 days up to 0.4% of alcohol was accumulated, which corresponds to a fermentation rate of 11%. Changes occurred in secondary metabolism – increased ester production, normal higher alcohol formation and weak carbonyl compounds synthesis. In the immobilized cells, the fermentation start was also delayed. The actual process started after the 4th day, the fermentation quickly caught up with the free cell fermentation. As a result, more alcohol (about 0.7% w/w) was accumulated in the laboratory beer, thus enabling its classification as low-alcohol beer, and, fewer metabolites accumulated in the beer, which, in combination with the low fermentation temperature, had a negative effect on the taste profile.

Conclusions. Technological regimes for production of low alcohol and non-alcoholic beer have been selected on the basis of analysis of wort sugars, as well as on a study of fermentation kinetics at low temperatures with free and immobilized yeast cells.

Introduction

The physical methods for non-alcoholic transformation of ready-made standard beer are mainly used [1, 2]. On the one hand, this is easier to do, even at an increased cost of purchasing additional equipment. The main disadvantage of the physical removal of alcohol are the drastic changes that occur in the organoleptic profile of the final product [3].

Biological methods – changes in fermentation and mashing regimes, the use of genetically modified microorganisms or transgenic barley, in turn, make it possible to reduce the alcohol content and preserve the organoleptic profile of beer [2, 3].

Two groups of methods out of these methods are most often used – changes in the mashing mode, which ensures the production of wort with reduced content of fermentable sugars or changes in the fermentation mode – limited fermentation or stopped fermentation. Data on these methods are relatively scarce, and recent research dates back to the last decade of the 20th century, which makes them out of date [1, 3].

In the context of brewing, adjuncts are alternative sources of fermentable extract and are used to replace a portion of the barley malt, which is usually more expensive. An obvious brewing adjunct is non-malted barley. It can replace up to 50% of the malt, but it has to be ground finely, the mashes have to be thin (ratio 1:4.5), and varieties with low gelatinization temperature are preferred. With the increase in the addition of barley, the β -glucan concentration rises arithmetically, and viscosity increases exponentially [5, 6].

Immobilized cells offer the possibility of scaling up the processes in a continuous mode, which ensures the production of beer with reduced alcohol content. Although some attempts have been made worldwide, detailed studies of the possibility of applying this type of systems in beer production have not been done so far [1, 4].

As already mentioned, the development of beer with reduced alcohol content is based on changes in classical technologies, which, however, is not well studied. The lack of data on the influence of the changes in the wort composition and the changes that occur in the fermentation process on the quality of the final product raises various questions. The data cited in the literature are insufficient [1].

The aim of the present work was to substantiate the production of low alcohol and non-alcoholic beer by changing the wort composition by replacing part of the malt with unmalted barley.

Materials and methods

Raw materials

Industrial malt (Weyermann®, Germany) and non-malted barley (Bulmalts, Bulgaria) were used. The malt and barley were coarsely ground with a Corona hand mill. The alcohol fermentation was carried out using top-fermenting yeast strain *Saccharomyces cerevisiae* S-33 (Fermentis, France).

Mashing methods

In the present study, 6 experimental variants of malt/non-malted barley (Table 1) were investigated. All mashing processes were conducted in a Lochner LB-8 modified laboratory mashing apparatus equipped with a programmable controller. 50 g of grist (according Table 1) were mixed with 250 cm³ of distilled water heated to a temperature of 45 °C. The sample temperature was raised to 50 °C and paused for 30 min. Then the temperature was increased

at a rate of 1.5 °C/1 min. After reaching a temperature of 70-72 °C, the samples were diluted with 150 cm³ of distilled water at the same temperature. Simultaneously, the temperature was raised to 77 °C, then there was a pause for 60 min. After the set time, the samples were removed from the mashing apparatus, cooled to 20 °C and filled with distilled water to sample weight of 500 g. The samples were filtered through Macherey-Nagel MN 614 ¼ Ø 320 mm filter paper [7].

Semi-industrial wort

The wort was produced in a 20 dm³ laboratory scale brewery (Braumeister, Germany). 4.5 kg of grist (80% malt and 20% barley) were mixed with water at a 1:5 ratio. The mashing was performed as previously described. Lautering and boiling were conducted in the same Braumeister. The boiling duration was approximately 1 h, and Nugget hop granules were added to the wort at the beginning of the process [8].

Table 1

Experimental variants						
Variant	1	2	3	4	5	6
Malt, %	100	95	90	80	70	60
Barley, %	0	5	10	20	30	40

Cell immobilization

The cells were immobilized in a 3 % calcium alginate gel. After autoclaving the alginate solution for 20 minutes at 120 °C, the solution was mixed with the cell suspension to obtain a cell concentration of 10⁷ cells.cm⁻³ of gel. This suspension was forced through a syringe needle by means of peristaltic pump and dropped into 2 % (w/v) CaCl₂ solution. The resulting beads were approximately 2 mm in diameter. The beads were left for 30 minutes in calcium solution and then number of beads were placed into 0.38 % (w/v) chitosan solution in 1% acetic acid (v/v). Alginate beads stayed in chitosan solution for 60 minutes. Afterwards, chitosan-alginate beads are washed with physiological solution (saline) to remove the excess of chitosan. Then the beads was transferred in in 0.05 M sodium citrate solution for 30 minutes for constructing microcapsules with liquid core. Afterwards, chitosan-alginate beads with liquid core were washed with physiological solution (saline) [8].

Wort fermentation

The fermentations were carried out in 500 cm³ plastic bottles equipped with an airlock system (in order to take samples daily and to reduce the impact of the changes in the sample volume). 400 cm³ of wort were placed into bottles and inoculated with a 10⁷ cells.cm⁻³ yeast suspension. The bottles were incubated at a constant fermentation temperature of 7 °C. For the immobilized cell variants, each bottle was inoculated with 5 g of microcapsules. The results were the arithmetic mean of two parallel fermentations [8, 9].

Analytical methods and procedures

Methods of the European Brewery Convention (EBC) [10]:

- Original, apparent and real wort extract – Methods 8.3 and 9.4;
- Final, real and apparent fermentation degree – Method 9.5;
- Alcohol concentration – Method 9.2.1;
- Vicinal diketones – Method 9.24.1.

Methods for metabolite determination [11]

- Determination of the acetaldehyde content of beer – the aldehyde concentration was determined according to the bisulfite method after simple sample distillation of the beer.
- Determination of the ester content of beer – the ester concentration was determined by ester saponification with NaOH after simple sample distillation of the beer.
- Determination of the higher alcohol content of beer – the higher alcohol concentration was determined according to the Komarovskiy-Felenberg method after simple sample distillation of the beer.

Biomass concentration [8]

The biomass quantity was determined by the calculation procedure based on Balling's equation.

Determination of fermentable sugars by HPLC [7]

Samples were diluted in a ratio of 1:1 with distilled water and injected directly into the apparatus under the following conditions: Apparatus – Waters 2695 (UK), Refractive index Detector 2414; Mobile Phase – 80% acetonitrile/20% water; column – Thermo ODS HYPERSIL, 250 x 4,6, flow – 1.5 ml/min; mode – isocratic [7].

The results of all standard and HPLC analysis were expressed as the mean $\pm$ the standard deviation of three replicates.

Kinetic evaluation

The kinetic evaluation was performed according to equations (1) to (5) [8].

$$\begin{aligned}
 \frac{dX}{d\tau} &= \mu X \\
 \frac{dP}{d\tau} &= qX \\
 \frac{dS}{d\tau} &= -\frac{1}{Y_{X/S}} \frac{dX}{d\tau} - \frac{1}{Y_{P/S}} \frac{dP}{d\tau} \\
 \frac{dE}{d\tau} &= Y_E \mu X \\
 \frac{dHA}{d\tau} &= Y_{HA} \mu X \\
 \frac{dA}{d\tau} &= Y_A \mu X - k_A XA \\
 \frac{dVDK}{d\tau} &= Y_{VDK} \mu X - k_{VDK} X VDK
 \end{aligned} \tag{1}$$

- Monod model with product inhibition

$$\mu = \mu_{\max} \frac{S}{K_{sx} + S + \frac{P^2}{K_{SPi}}}; \quad q = q_{p\max} \frac{S}{K_{sp} + S + \frac{P^2}{K_{SPi}}} \tag{2}$$

- Monod model with product and substrate inhibition

$$\mu = \mu_{\max} \left(1 - \frac{P}{P_M} \right) \frac{S}{K_{sx} + S + \frac{S^2}{K_{SXi}}}; \quad q = q_{p\max} \left(1 - \frac{P}{P_{MP}} \right) \frac{S}{K_{sp} + S + \frac{S^2}{K_{SPi}}} \quad (3)$$

- Mass transfer equations

$$\frac{dS_{im}}{d\tau} = K_{LS} (S - S_{im}) - \left(\frac{\mu X}{Y_{X/S}} \right) - \left(\frac{qX}{Y_{P/S}} \right) \quad (4)$$

$$\frac{dP_{im}}{d\tau} = -K_{LP} (P_{im} - P) + \left(\frac{qX}{Y_{P/S}} \right) \quad (5)$$

X – biomass concentration, g/dm³; P – ethanol concentration, g/dm³; S – real extract, g/dm³; Y_{P/S}, Y_{X/S} – yield coefficients; μ – specific growth rate, h⁻¹; q – specific ethanol accumulation rate, g/(g.h); E – ester concentration, mg/dm³; HA – higher alcohol concentration, mg/dm³; A – aldehyde concentration, mg/dm³; VDK – vicinal diketone concentration, mg/dm³; Y_{HA}, Y_E, Y_A, Y_{VDK} – yield coefficients of the corresponding metabolites, mg/(g.h); k_A, k_{VDK} – reduction coefficients for aldehydes and vicinal diketones, mg/(g.h); K_{SX}, K_{SP} – Monod constants, g/dm³; K_{SXi}, K_{SPi} – inhibition constants, g/dm³; P_M, P_{MP} – maximal ethanol concentration for full inhibition of the process, g/dm³; K_{LS}, K_{LP} – global mass transfer coefficients for the substrate and the ethanol, h⁻¹; P_{im} – ethanol concentration in the capsules, g/dm³.

Results and discussion

The results for the wort extract and fermentable sugars of the six experimental variants described in “Materials and methods” are presented in Table 2. The data show that the wort extract decreased with the increase in the quantity of non-malted barley. In the selected mashing process, the fermentable sugars varied from 3.4 to 4.17% and accounted for about 50% of the laboratory wort extract. Therefore, it can be suggested that the ethanol produced during fermentation would be approximately 2% ABV.

The results in Table 2 show that up to 10% of adjunct led to the increase in the quantity of fermentable sugars. This was due to the action of the malt enzyme systems on barley starch which resulted in its hydrolysis. An increase in glucose and maltose concentration only was observed, which indicated β-amylase activity. Although the pause at 63 °C was omitted, the β-amylase action could not be ignored since it occurred but at a far from optimal rate. The over 20% increase in the non-malted barley quantity led to the accumulation of lower amounts of fermentable sugars compared to the control sample.

The data are comparable with the data cited by other authors [13, 14]. On the one hand, the data show that removing the pause at 63 °C and increasing the pause temperature for the action of α-amylase leads to a decrease in the content of fermentable extract in the wort. This, combined with the replacement of part of the malt with unmalted barley, results in a wort which is suitable for the production of beer with a reduced alcohol content.

Table 2

Composition of laboratory wort

Parameter	Malt/non-malted barley					
	100/0	95/5	90/10	80/20	70/30	60/40
Laboratory wort extract, % w/w	7.84 ±0.25	7.53 ±0.22	7.45 ±0.23	7.13 ±0.21	6.72 ±0.20	6.44 ±0.24
Glucose, % w/w	0.47 ±0.03	0.54 ±0.05	0.23 ±0.03	0.38 ±0.04	0.33 ±0.03	0.17 ±0.02
Fructose, % w/w	0.07 ±0.01	0.05 ±0.001	0.03 ±0.001	0.04 ±0.001	0.05 ±0.001	0.03 ±0.001
Maltose, % w/w	3.23 ±0.11	3.58 ±0.12	3.61 ±0.10	3.24 ±0.12	3.09 ±0.11	3.20 ±0.11
Fermentable extract, % w/w	3.77	4.17	3.87	3.66	3.47	3.4
Fermentable extract/wort extract, %	48.09	55.38	51.94	51.33	51.63	52.80

A major drawback of the use of non-malted barley is the increase in the laboratory wort filtration time. The results showed that the filtration time was within 60-75 minutes when the adjunct was up to 20%. The increase in the barley amount led to an increase in the filtration time to a value between 90 and 120 minutes because of the higher wort viscosity caused by the unhydrolyzed β -glucans. The malt enzyme system was able to hydrolyze barley β -glucan only when the adjunct was up to 20%. When the barley amount rose over 20%, an enzyme preparation with glucanase activity had to be added, which would make production more expensive [15, 16, 17].

The determination of sugar distribution into the wort can be considered a new scientific element in the development of a production technology for low alcohol and non-alcoholic beer (Table 2). The existing mashing methods that have been patented so far [18, 19] are based on empirical knowledge of the wort original extract only, which does not allow good control of the alcoholic fermentation and the accumulation of esters and higher alcohols into the beer. The maintenance of balance between fermentable sugars in the wort lies at the heart of the production of beer having a balanced profile and high quality.

The replacement of 20% of the malt with non-malted barley was a variant selected on the basis of the data obtained for the production of low-alcohol beer.

Semi-industrial beer production with the selected variant was carried out, which included wort production in a Braumeister semi-industrial brewing system, Germany. After boiling, the resultant wort had an initial extract of 8.03 %(w/w) and fermentable sugar content of 3.7%. The wort was used for fermentation processes with free and immobilized top-fermenting yeast at 7 °C for 10 days.

The results are presented in Figure 1 and Figure 2. Identification of the parameters of kinetic models (1) to (3) was also carried out. The results are shown in Table 3, and the convergence of the models with the experimental data is presented in Figure 1 and Figure 2.

Figure 1. Dynamics of free cell alcohol fermentation

The free cell fermentation process started at a relatively slow rate. In the first 2-3 days, alcohol formation was relatively low, then culture activation began and the laboratory beer accumulated about 0.4% (w/w) of ethanol by the end of the 10th day, which gives a reason to classify the obtained beer as non-alcoholic beer. The extract consumption proceeded at a low rate and as a result, the actual fermentation rate reached 11% at the end of the fermentation. The biomass accumulation was also relatively low, which further reduced ethanol production (Figure 1a). Changes occurred in secondary metabolism. Increased ester production associated with the yeast strain used as well as with the striving of the cells to survive the stress factors was observed. The higher alcohol formation was normal. The basic amount of esters and higher alcohols were formed by the 4th – 5th day. The synthesis of carbonyl compounds was weak, and the aldehydes and the vicinal diketones in the beer were around the sensation threshold (Figure 2b). The results are comparable to those cited in [1, 20], although a more accurate comparison is difficult to make due to the strain specificity of the yeasts used. Another important feature is the differences in the analytical methods used, which also does not allow for more detailed comparisons.

In the immobilized cells, the fermentation start was also delayed. Once again, diffusion resistances affected the delay. At the low fermentation temperature, the actual process started after the 4th day, but since there was an increased volume of cells in the bottles, the fermentation quickly caught up with the free cell fermentation. As a result, more alcohol (about 0.7% w/w) was accumulated in the laboratory beer, thus enabling its classification as low-alcohol beer. The substrate uptake was also more rapid as a result of which the actual fermentation rate reached 15%. The results for the secondary metabolites were more interesting. The data reported by Naydenova, 2014 [21] that the strain used was sensitive to immobilization were confirmed. As a result, fewer metabolites accumulated in the beer, which, in combination with the low fermentation temperature, had a negative effect on the taste profile (Figure 2). However, there were no significant deviations in the taste and flavor of the beer since the high unfermented extract content concealed some of the drawbacks. The accumulation and reduction of carbonyl compounds was also delayed, which did not affect the organoleptic profile of the beer (Figure 2).

Two kinetic models reflecting different aspects of the alcoholic fermentation process were used to describe the process kinetics (Table 3). In the product inhibition model, the free cells grew at a lower specific growth rate than the immobilized cells, whereas in the product and substrate inhibition model, the reverse trend was observed due to the influence of the substrate. The first model showed that the cells demonstrated substantial lack of affinity towards the substrate (K_{SX} ranged from 3122 to 3600), which also affected the lower average specific growth rates. In the second model, there was higher affinity to the substrate, as the K_{SX} values were approximately equal to the absolute value of the substrate. The specific rate of ethanol accumulation followed a similar trend. The free cells had higher specific process rates than the immobilized cells, while the trend was reverse in the second model. Both models showed that the main substrate amount was consumed for the accumulation of ethanol. Based on the derived values for the primary metabolic model errors, the model that reflected the product and substrate inhibition showed a better match to the experimental data (Table 3). This was due to two main reasons. Firstly, it showed that the substrate had a significant effect on the fermentation process kinetics. The low concentration of fermentable sugars in combination with the low fermentation temperature resulted in greater cell dependence on the sugars. In the case of a temperature-limited fermentation process, the impact of the product inhibition was enhanced and, in combination with limited fermentable sugars, resulted in limited alcoholic fermentation. Secondly, the second model reflected the already mentioned fact that cells of the strain used were very strongly influenced by the immobilization process.

Legend:
with symbols – experimental data for fermentation parameters;
with lines – model parameters

Figure 2. Dynamics of immobilized cell alcohol fermentation

Table 3

Kinetic characteristics of alcoholic fermentation

Monod model with product inhibition								
Primary metabolism	$\mu_{\max}$	K_{sx}	$q_{p\max}$	K_{sp}	$Y_{x/s}$	$Y_{p/s}$	K_{sxi}	K_{spi}
Free cells	0.0080	3600	0.0103	0.958	0.0660	0.6079	0.339	0.823
	Error=0.0038467							
Immobilized cells	0.0122	3122	4.29	97	0.0634	3.046	0.889	3.723
	Error=0.0035487							
Secondary metabolism	Y_A	k_A	Y_E	Y_{HA}	Y_{VDK}	K_{VDK}	K_{LS}	K_{LP}
Free cells	0.491	0.00115	1.38	0.383	0.0323	0.0346	-	-
	Error=2.140							
Immobilized cells	0.608	0.0044	0.909	0.256	0.0076	0.0109	0.0464	0.0016
	Error=1.354							
Efficiency coefficients	η_{μ}	η_q	η_A	η_{KA}	η_E	η_{HA}	η_{VDK}	η_{KVDK}
	1.386	416.50	1.238	2.933	0.658	0.668	0.235	0.315
Monod model with substrate and product inhibition								
Fermentation type	$\mu_{\max}$	K_{sx}	$q_{p\max}$	K_{sp}	$Y_{x/s}$	$Y_{p/s}$	K_{sxi}	K_{spi}
Free cells	0.132	43.48	1.549	40.78	0.0034	0.718	0.187	0.568
	Error=0.0399							
Immobilized cells	0.0323	49.92	0.497	13.52	0.0055	0.397	0.681	6.98
	Error=0.00831							
Secondary metabolism	Y_A	k_A	Y_E	Y_{HA}	Y_{VDK}	K_{VDK}	K_{LS}	K_{LP}
Free cells	0.413	0.00019	1.445	0.396	0.0061	0.0043	-	-
	Error=1.3582							
Immobilized cells	0.600	0.0046	0.878	0.249	0.0056	0.00819	0.0019	0.0012
	Error=2.140							
Efficiency coefficients	η_{μ}	η_q	η_A	η_{KA}	η_E	η_{HA}	η_{VDK}	η_{KVDK}
	0.245	0.321	1.453	24.21	0.604	0.629	0.918	1.904
Full inhibition	P_{iM}	P_{iMP}	$\eta = \frac{\text{Parameter for immobilized cells}}{\text{Parameter for free cells}}$					
Free cells	44.55	15.952						
Immobilized cells	64.57	475.00						

* symbols and parameters – according legend for equation (1) to (5)

The higher accuracy of the product and substrate inhibition model also reflected the higher accuracy of the parameters of the secondary metabolism. The kinetic parameters in Table 2 indicated that the immobilization process affected the secondary metabolism of cells to a certain extent, and this resulted in lower yield coefficients and reduction coefficients of the major metabolite groups in beer (except for aldehydes and the reduction of the vicinal diketones), which was also reflected in the local concentrations observed. As already commented, the results are close to those in other studies, but a qualitative comparison is difficult to make, because in addition to the specific strain, the fermentation conditions and the wort composition have an impact [1, 20].

The values of the summarized mass exchange coefficients K_{LS} and K_{LP} are of interest. In our previous studies [8, 9, 21], it was found that internal diffusional resistances have greater influence on the fermentation process than external diffusional resistances. In this regard, the K_{LS} and K_{LP} data show that they are in the same range, which means that the mass transfer in the system is more limited by the diffusion of the substrate and the product in the capsules and by the concentration difference than by the mass transfer through the stationary

layer on the surface of the capsule. The second model demonstrated that full inhibition of the cell growth occurred at relatively high ethanol concentrations (between 44.55 and 475.00 g.dm⁻³), which were too far from the scope of the conducted study.

No data have been found in the specialized literature on studies of the kinetics of the fermentation process for the production of beer with reduced alcohol content. The data presented show that the study is innovative and allows the fermentation regimes studied to be transferred from laboratory and semi-industrial conditions to industrial production. In addition, the data obtained on the kinetic parameters for the accumulation of the relevant metabolites make it possible to evaluate the influence of the fermentation conditions on the organoleptic profile of the beer. As a future study, the resulting regimes will be transferred continuously to reduce beer production time to 3–4 days.

Conclusion

1. A mashing method by replacing 20% of the barley malt with unmalted barley in order to reduce the amount of fermentable sugars was proposed. As a result of the substitution, the amount of fermentable extract in the wort was 51.33%, which guaranteed the production of low alcohol and non-alcoholic beer.
2. Increasing the unmalted barley above 20% further reduces the wort extract, but worsens the conditions for lautering of the malt mash.
3. Limited fermentation with free and immobilized cells of a top-fermenting yeast strain *Saccharomyces cerevisiae* S-33 was performed. It was found that beer with reduced ethanol content and with a relatively balanced organoleptic profile under the selected fermentation conditions was obtained. The immobilization process led to further limitation of ethanol synthesis due to the influence of diffusion resistances in the system.
4. Based on the kinetics of the fermentation process described by the product inhibition model and the product and substrate inhibition model, the effect of the low temperature and the immobilization process on the changes in the fermentation process and the production of beer with a reduced alcohol content was determined.
5. The original elements in this study are the determination of sugars in the wort and the analysis of the fermentation kinetics. A thorough analysis of these elements determines the scientific nature of the study.

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