STUDY ABOUT THE INFLUENCE OF SOME FACTORS ABOUT THE LACTIC ACID FERMENTATION OF CARROT JUICES

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Key words: lactic acid fermentation, carrots, juice, temperature

Abstract: The influence of the storage time of vegetables – raw materials, the influence of fermentation temperature and the influence of the glucose addition about the lactic acid fermentation unfolding has been studied.

Fresh carrots and carrots stored five months were pressed to raw juice and heat-treated at 70 degrees C for 20 minutes in the aim of destroy the undesirable microorganisms.

Carrot juices were inoculated with lactic acid bacteria isolated from epiphytic microbiota at the concentration of $4 \times 10^5$ UFC/ml and fermented in a thermostat for 96 hours. During the fermentation the following analytical parameters were established: reducing sugars, total acidity, pH, amino acids content, nitrites content, ascorbic acid content.

During fermentation the pH of carrot juices decreases from 6.15 to 3.99, while the total acidity increases from 0.06 to 1.62% (as lactic acid). Less than 25% of the initial content of ascorbic acid rests in the juices after 96 hours of fermentation.

The amino acids content of juices, expressed as g N/100g, increase with a middle of 50% in 96 hours, due of the proteins decomposition. The pre-digestion of these compounds improve the nutritional quality of the lactofermented juices.

We found that the glucose supplement hasn’t a significant influence about the lactic acid accumulation. Also, at the end of the period of study, we found that the substratum metabolization was difficult in the sample with 1% glucose initial added.

The quality of the raw materials, especially concerning the sugars content and the total acidity at the beginning of the fermentation process is important because a rapidly increase of acidity minimizes the influence of spoilage bacteria.

To proceed from the results of the sensory analysis and the results of the chemical analysis we recommend stopping the fermentation of these juices after 72 hours.

INTRODUCTION

The lactic acid fermentation of vegetable products, applied as a preservation method for the production of finished and half-finished products, is considered as an important technology (Karovicova J. et. all, 1999). The fundamental reason for development and acceptance of fermented foods can be ascribed variably to preservation, improved nutritional properties, better flavour/aroma, upgrading of substrates to higher value products and improved health aspects (Kalantzopoulos G., 1997).

The vegetable juices processed by lactic acid fermentation introduce a change in the beverage assortment for their high nutritive value and high contents of vitamins and mineral compounds. For the juices processed by lactic acid fermentation, the content of lactic acid is important from the nutritive point of view. This acid shows disinfecting effects that are caused mainly by its acidity (Karovicova J., 2003). In a lot of countries consumption of the lactic acid fermented vegetable juices increases (Kopec K., 2000).
MATERIALS AND METHODS

Fresh carrots were purchased from the local free vegetable market from Dambovita County (Romania) at the end of July, respectively at the end of September and specifically conditioned by washing, scrubbing and non-edible pieces elimination. The juices were obtained using a home juice-maker. In the same conditions there are extracted juices from carrots stored from September to January.

In the aim to destroy the undesirable microorganisms, the juices were treated 20 minutes at 70 degrees Celsius. After rapid cooling at the thermostatation temperature, there are supplied with a brine inoculum with $10^5$ cells/ml lactic acid bacteria mixture isolated from epiphytic microbiota.

50ml juice from each experimental were distributed in sterile tubes and the anaerobiosis was created by covering the cotton stopper of the tube by a metal folia. The carbon dioxide formed during the fermentation process ensured anaerobic conditions over the surface of the juices. Each tube represented a single sample and the experiments were performed in double. The samples were noted as following:

A - carrots juice (from carrots purchased in July) fermented at 25-27 degrees Celsius

B - carrots juice (from carrots purchased in September) fermented at 25-27 degrees Celsius

C - carrots juice (from carrots stored until January) fermented at 30-32 degrees Celsius

D - carrots juice (from carrots stored until January) + 1% glucose, fermented at 30-32 degrees Celsius

The lactic acid fermentations were performed in a thermostat and the samples were daily examined through sensorial and chemical analysis, as following:

✓ reducing sugars, expressed in g/100g - by the Schoorl method, after a previous treatment with lead acetate,
✓ total acidity, expressed in g acid lactic/100g - by visual titration with a 0,1N solution of NaOH using phenolphthalein indicator
✓ pH - by electrometric method
✓ amino acids content, expressed as g glycocol/100g - by the Sörensen method
✓ nitrites content, expressed in mg NO$_2^-$/100g - by the Griess method
✓ ascorbic acid content, expressed in mg/100g - vitamin C extracted with HCl 2% was titrated with KIO$_3$ in KI and starch presence.

Analytical data were statistically processed and variation and dependence models were performed using the computer application Microsoft Excel.

RESULTS AND DISCUSSIONS

Comparing the initial reducing sugars of juices (figure 1) is obvious that this parameter has a significant value in the samples harvested at maturity, at the end of July (by 1,87 times higher than the juice extracted from carrots in September, respectively by 2,08 times higher than the samples obtained at the end of January).

The initial reducing sugars content seem to be very important regarding the rapidly increasing of the juices acidity. After 24 hours the lactic acid content in the samples A and B was by 6,72 respectively 10 times greatest comparatively with the moment of the fermentation starting. Consequently, the development of undesirable bacteria was prevented and the stability of lactic acid carrots juices was higher.
Table 1 shows the decreasing of reducing sugars (in percentages) during the 96th hours of fermentation process. The values were close by 30% after two days in the case of the samples A and B, respectively after three days in the case of the samples C and D. This aspect is correlated with the achieving or the exceeding of the total acidity by 1g/100g (figure 2). Although the carrot juice was supplemented with glucose (sample D), the substratum metabolism by lactic acid bacteria wasn’t so rapidly in the first 24 hours as in the case of samples A and B (with endogenous reducing sugars content).

<table>
<thead>
<tr>
<th>Sample</th>
<th>0-24h</th>
<th>0-48h</th>
<th>0-72h</th>
<th>0-96h</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.92</td>
<td>30.6</td>
<td>41.63</td>
<td>44.83</td>
</tr>
<tr>
<td>B</td>
<td>26.75</td>
<td>31.77</td>
<td>34.48</td>
<td>36.12</td>
</tr>
<tr>
<td>C</td>
<td>9.25</td>
<td>16.29</td>
<td>31.11</td>
<td>38.8</td>
</tr>
<tr>
<td>D</td>
<td>13.61</td>
<td>26.38</td>
<td>28.05</td>
<td>48.61</td>
</tr>
</tbody>
</table>

At the end of the analyzed period of time, the content of reducing sugars remained in the lactic acid fermented juices (table 1) was varied between 51.39 (sample D) and 63.88% (sample B). In values absolute, quantities about 1g glucose/100g metabolized in the samples B and C have been as result the lactic acid accumulation in quantity about 1g/100g (0.938g/100g in sample B, 1.003g/100g in sample C).

The glucose addition in the aim of substratum deficit compensation hasn’t been an evidently importance regarding the increasing of acidity than after 72 hours (table 2). Significant differences between the samples with and without glucose added have been occurring, from this point of view, after 96 hours, but the final acidities of the samples (1.219g/100g, respectively 1.624g/100g) can be named “excessive”, because the juices acquired a sour taste. On the other hand, the pH values of the samples were about “4” after 48 hours. In the aim of the final products preservation, the acquirement of this value in the mentioned period of time is advantageous.

Due of the small degree of lactic acid dissociation, the pH values don’t varied significantly in the last two days of analysis. Thus, if this parameter has been at the initial moment of fermentation the values 6.15 (sample C), respectively 6.2 (sample D), after 48 hours it became 4.11, respectively 4.08.
48 hours later, without to be straight proportional with the differences between the lactic acid content of the samples, this values were modified to 4.04, respectively 3.99. Because lactic acid has a low pK value, resulting a reduced pH value, its role consist in unlactic microorganisms control (by adulteration and pathogenic).

From intervals by 24 hours, the increasing of lactic acid content was significant in the first 24 hours in the case of carrot juices with a higher reducing sugar content, fermented at 25-27 degrees Celsius (6.72 times for sample A, respectively 10 for sample B). For the others, this indicator was varied between 2.07 (48-72 hours) and 1.03 (72-96 hours) in the sample without glucose added (C), respectively between 2.02 (48-72 hours) and 1.36 (48-72 hours) in the sample with glucose added (D).

Analyzing comparatively the juices obtained from carrots in September, respectively from carrots stored until January, it seems that the diminishing of the reducing sugars in the storage period of time can be compensated through the increasing of the fermentation temperature with about 5 degrees Celsius, in the aim of sufficient lactic acid accumulation in the carrot juices.

The amino acids content, expressed as g N/100g, was registered increases and decreases in time (figure 3). This evolution is due to the alternance in the amino acids consumption (as organic nitrogen source) by the lactic acid bacteria and the protein decomposition under the enzymatic equipment of microorganisms.

At the higher temperatures of fermentation, the mentioned indicator was registered successive increases (samples C and D) with a smaller reduction in the interval 48-72 hours. The differences from one interval of time to another weren’t very significant, because after 96 hours of fermentation the sample C had moreover by 0.0224g/100g, while the sample with glucose added (D) had only 0.0074g/100g.

<table>
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<tr>
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<th>0-72h</th>
<th>0-96h</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.72</td>
<td>11.02</td>
<td>12.94</td>
<td>13.66</td>
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<td>B</td>
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<td>16.63</td>
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<tr>
<td>C</td>
<td>1.8</td>
<td>2.63</td>
<td>5.47</td>
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</tr>
<tr>
<td>D</td>
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<td>2.8</td>
<td>5.67</td>
<td>7.73</td>
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</tbody>
</table>
In the first 24 hours of fermentation, the samples A and B, which were accumulated in this interval of time important quantities of lactic acid as result of lactic acid bacteria development, have been an opposite evolution. Thus, the nitrogen content increase about 4,4 times in the sample A, while the same indicator decrease about 1.14 times in the case of sample B.

If the initial content in amino acids of all the samples was closed (0.03-0.04 g/100g), after one week of lactic acid fermentation this parameter was about 10 times higher in the juice obtained from carrots at the end of July. The explanation can consist in the fact that the proteolysis process is intensified at the higher acidity of the environment. Consequently, the pre-digestion of proteins improve the nutritional quality of the lactofermented juices.

Figure 4 shows the evolution of the nitrites content of the carrot juices during fermentation process. In the first 24 hours, due of the nitrates reducing, it increase in all the samples. Differently from the others, this process continues still 24 hours in the samples A and B, with a higher NO$_3$ content at the time of analysis. The decreasing nitrites content after 96 hours of lactic acid fermentation represent 36.17% for the sample A, only 7.4% for the sample B (with a lower acidity), 84.44% in the case of the sample without glucose added (C), respectively 64.44% for the sample D. Calculating the coefficients of multiple correlation $R^2$, considering the nitrites content - variable dependant and the fermentation temperature, respectively the environment acidity - variables independents, we can concluded that it not exist a significant relation between them ($R^2 = 0.19872; R^2 < 0.2$.)

The ascorbic acid content of carrots stored during five months in autumn – winter period decrease drastically (until the null), reason for that the juices extracted at the end of January can’t be analyzed from this point of view.
The figure 5 shows the polynomial reducing of vitamin C content in the fermentation time in the case of juice obtained from carrots in September. This degradation can be caused by chemical oxidation (minor content of oxygen, the spread light). Enzymatic oxidation is excluded due of the thermic treatment of juices before fermentation. After 72 hours, the ascorbic acid content of the sample B represent only 22.74% from the initial content of the juice.

CONCLUSIONS

✓ The glucose addition at the juices with a lower reducing sugars content play an important role concerning the final acidity of the products and it haven’t a significant influence about the speed of lactic acid accumulation during 72 hours of fermentation
✓ Temperatures about 30-32 degrees Celsius seems to be favourable for the lactic acid process unfolding in the case of the raw material with lowest reducing sugars content; this temperatures are favourable for lactobacilli development
✓ As a rule the content in amino acids of all the analyzed samples increase during the lactic acid fermentation
✓ The lactic acid bacteria have an action by nitrites reducing considering the moment of obtaining the stabilized products
✓ The ascorbic acid content of carrots decrease during the five months of storage until the null; consequently, the lactic acid fermented juices are meagre in vitamin C
✓ The results of the sensory and chemical analysis (especially the taste and the lactic acid content) show that in all the experimental is indicate to interrupt the lactic acid fermentation of the carrot juices after 72 hours in the aim to obtain stable and agreeable products; in this case there are indicated soft methods for maintaining the nutritional properties of these beverages.

BIBLIOGRAPHY


Note. The researches were made in the frame of the CNCSIS Project nr. A 1086/2005, „Researches Concerning the Improvement of the Biotechnological Parameters of Obtaining Lactic-Fermented Juices from Vegetables”.

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