Guaiacol Formation in Apple Juice, Effect of Selected Additives on *Alicyclobacillus* Growth

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Abstract: Disinfectant or card-board off-flavour formation was observed in the batch of pasteurised reconstituted apple juice. Aroma profile of products was analysed using SPME and GC/MS, guaiacol was detected in the apple juice sample as well as apple concentrate. Sporulating bacteria *Alicyclobacillus acidoterrestris,* which are able to growth in acidic conditions were isolated and identified as the cause of the off flavour formation. The possibilities of flavour formation inhibition using various additives were evaluated. The inhibitory effect of nisin, sulphur dioxide, benzoic acid and EDTA was followed. The best way of prevention is the sufficient washing and disinfection of apples prior the processing e.g. using chlorine dioxide. But when the treatment is insufficient and contaminated apples are to be processed the tested additives can prolong the lag phase and increase product stability. The inhibitory effect of the tested additives decreased in the order: benzoic acid, EDTA, nisin and sulphur dioxide.

Keywords: *Alicyclobacillus;* acid foods; apple juice; off flavour formation; inhibition

INTRODUCTION

About ten years ago the novel thermoacidophilic spore-forming bacteria were isolated from spoiled acidic beverages and were identified as *Alicyclobacillus acidoterrestris* [1]. *Alicyclobacillus* contains ω-alicyclic fatty acids as the major membrane fatty acid component in its cells, and is able to grow in a pH range of 2.5–6.0 and a temperature range of 25–60°C [1]. This microorganism growths slowly in the spoiled products with or without gentle gas formation and with production of offensive-smelling compounds such as guaiacol and 2,6-dibromophenol [2]. These microorganisms represent important problem in the processing of so called acid foods (with pH lower than 4.5), which are usually pasteurized only. The pasteurization with the temperatures below 100°C, which is used in the processing of acidic food, need not to destroy spores. The identification of sporoforming *Alicyclobacillus* capable of germinating and outgrowing under high acid conditions changed the traditional approaches to the heat treatment and there is a need for the related industries to develop a suitable method for inhibiting or controlling the germination and outgrowth of *A. acidoterrestris* in such products. The termoreinactivation parameters of the *Alicyclobacillus* spores are summarized in the Table 1.

Table 1. Termoreinactivation parameters of the *Alicyclobacillus* spores in apple juice

Strain	pH	SS °Brix	Temperature (°C)	D ± SD	Z	Reference
	3.5	nr	95	2.30 ± 0.03	12.2	[3]
Z (CRA 7182)			90	7.38 ± 0.85		
			80	54.3 ± 0.4		
	3.5	11.4	95	2.8 ± 0.7	7.7	[4]
VF			90	23 ± 7.5		
			85	56 ± 14		

The best way to prevent *Alicyclobacillus* growth in the apple juice is its elimination from apples before the processing, especially the treatment with the preparative containing active chlorine seems to be most promising (*Alicyclobacillus* spore numbers were reduced by > 4.8 log with 120 ppm free chlorine dioxide after only 1-min treatment) [5]. There is not enough results dealing with the inhibitory effects of food additives, Yamazaki *et al*. [6] described the inhibitory effect of nisin addition, which inhibits the growth of *Alicyclobacillus* and increases the thermoinactivation effect of pasteurization process. Since the problem is relatively young the inhibitory effect of other preservatives has not been tested yet and other data are not available.

The aim of the presented work was to analyze the cause of flavour defects of pasteurized apple juice and to evaluate the possible ways to prevent such undesirable changes.

EXPERIMENTAL

The spoiled samples of pasteurized apple juice aseptically packed in paper package (Tetra Pak) were obtained from the producer as well as the sample of apple concentrate used for the production of the complained products.

The aroma compounds were isolated using the SPME (Divinylbenzene/Carboxen/Polydimethylsiloxane (50/30 um) DVB/CAR/PDMS stable flex) Adsorption: 30°C 15 min and analyzed GC/MS.

GC/MS conditions:

column: DB 5 30 m × 0.25 mm × 0.25 μm (J & W Scientific, USA),

mobile phase: helium 0.7 ml/min, pressure 26 kPa

oven: 50°C (5 min), 7°C/min, 140°C, 30°C/min, 280°C (5 min),

injector: 240°C, splitless,

detector: 280°C, EI (70eV).

The compounds were identified by comparison of their spectra with the NBS library.

General microbial analyses were made, the presence of spores were observed, the isolated spores were identified at the Faculty of Science of the Masaryk University in Brno. The isolate was cultivated in PDA at 42°C. For the stability test the samples (commercial sterile apple juice 10 Brix with addition of nisin (50, 100, 150 IU/kg), SO_2 (25, 50, 75 mg/kg), EDTA (50, 10, 150 mg/kg), benzoic acid (15, 30, 45, 60 mg/kg) were inoculated to get the initial counts, and cultivated at 42°C.

RESULTS AND DISCUSSION

The complained samples had special disinfectant flavour, were significantly different comparing with the products of another batch. The sensory evaluation was done using the triangle test. The statistically significant differences were found with

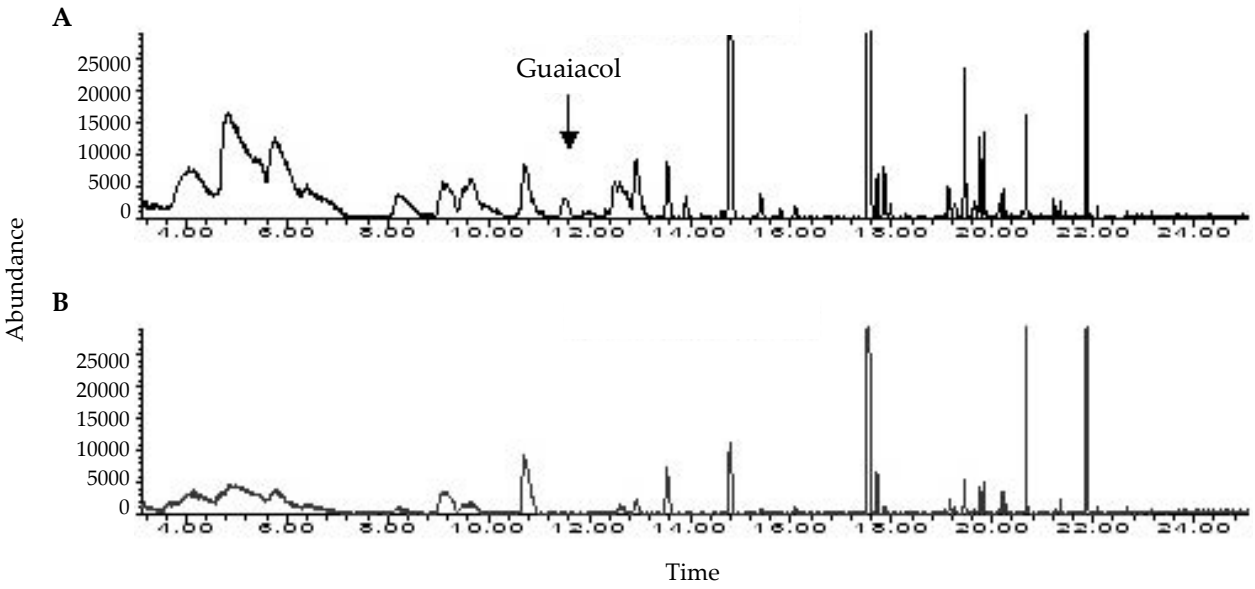

Figure 1. Volatile profiles of spoiled and good samples of pasteurized apple juice (A – spoiled juice, B – intact juice)

probability level 99% (N = 36, n = 20). The profile of volatiles of spoiled and good samples (Figure 1) differed in several peaks, in the spoiled samples guaiacol was determined. The other products degradation products described in the literature were not found.

The best prevention of spoilage with *Alicyclobacillus acidoterrestris* is the elimination of contaminating microflora prior the processing, but during the technological scale processing the risk of insufficient washing or cross contamination cannot be absolutely removed. Therefore it can be useful to have an opportunity to reduce the risk also by addition of inhibitory agents. Some generally used preservatives were tested, the results of inhibitory tests are in the Figures 2–5.

From the figures it is obvious that the addition of the all tested additives reduces the count of the *Alicyclobacillus* bacteria in the media, the decrease of the count after the inoculation is probably caused by the sensitivity of cells to the additive, probably also by sporulation of vegetative cells. The observed course of the curves is in agreement with the similar results obtained by Yamazaki *et al*. [6] for nisin.

CONCLUSION

Off flavour found in the batch of pasteurized aseptically packed apple juice was caused by *Alicyclobacteria acidoterrestris*, guaiacol formed by the contaminating microflora from ferulic acid in juice was detected in the spoiled samples.

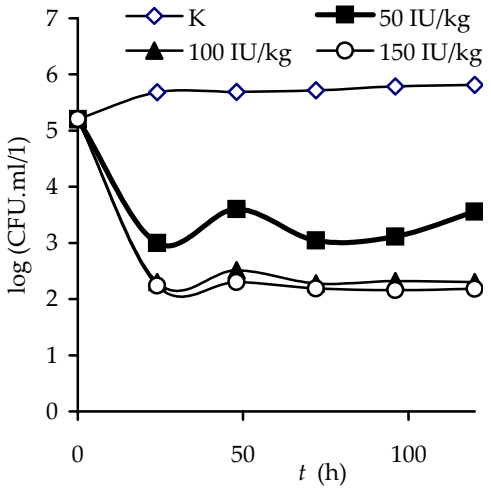

Figure 2. Effect of nisin on A*licyclobacillus* growth

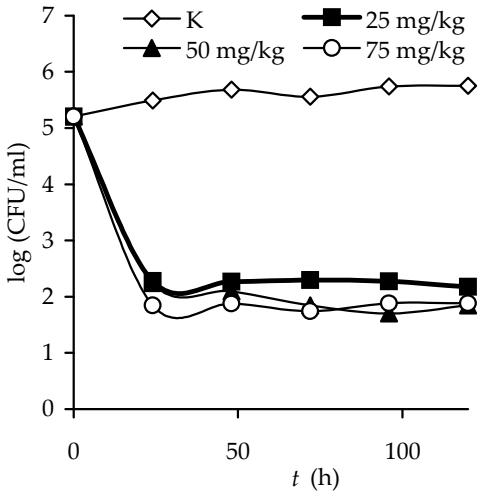

Figure 3. Effect of SO_2 on *Alicyclobacillus* growth

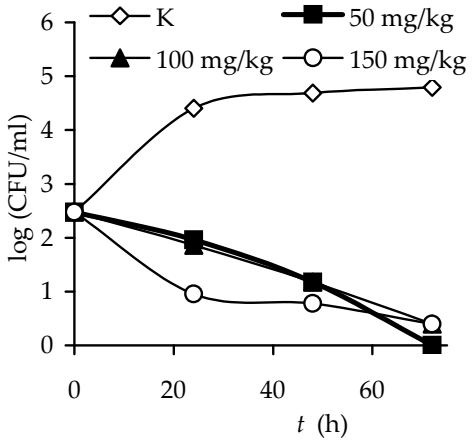

Figure 4. Effect of EDTA on A*licyclobacillus* growth

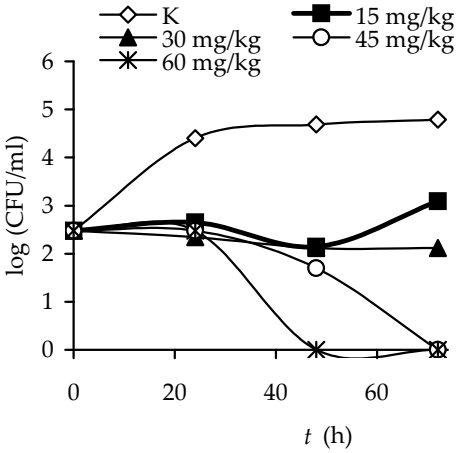

Figure 5. Effect of benzoic acid on *Alicyclobacillus* growth

The best way of prevention is the sufficient washing and disinfection of apples prior the processing e.g. using chlorine dioxide, but when the contaminated apples are processed the tested additives can prolong the lag phase and increase product stability. The inhibitory effect of the tested additives decreased in the order: benzoic acid, EDTA, nisin and sulphur dioxide.

The subsequent studies are needed, which will include the more systematic tests of other additives, also with the evaluation of the effect of additives on the termoresistance of *Alicyclobacillus* spores.

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