

Effect of thiamine hydrochloride, pyridoxine hydrochloride and calcium-d-pantothenate on the patulin content of apple juice concentrate

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Thiamine hydrochloride, pyridoxine hydrochloride and calcium-d-pantothenate were applied apple juice concentrates (AJC) at various doses in order to reduce the patulin content. AJC samples containing high levels of patulin were stored at $22 \pm 2^\circ\text{C}$ and 4°C for 6 months after vitamins were added. Patulin was fully degraded at the end of a 6-month period in samples stored at $22 \pm 2^\circ\text{C}$, on the other hand, other quality parameters diminished significantly. Without any considerable reduction on other quality parameters, applications of 1000 and 2500

mg/kg calcium-d-pantothenate resulted in reduction of patulin of 73.6 and 94.3%, respectively, however, 42.1% of patulin reduction was observed in the control sample of AJC stored for 1 month at $22 \pm 2^\circ\text{C}$. Addition of thiamine hydrochloride (1000 mg/kg), pyridoxine hydrochloride (625 or 875 mg/kg) and calcium-d-pantothenate (1000 or 2500 mg/kg) into the samples and storage at 4°C for 6 months yielded 55.5 to 67.7% of patulin reduction which was only 35.8% for the control, while the other quality parameters were protected adequately.

1 Introduction

Patulin is a mycotoxin produced by several species of mould fungi, such as *Penicillium*, *Aspergillus* and *Byssoschlamys* [1]. It is regulated for apple juice in several European countries at a maximum permitted level of $50 \mu\text{g/L}$ [2]. There is evidence that some B vitamins are useful for reducing patulin [3, 4], however, degradation products of patulin have not been identified and further work is required to demonstrate that these do not have toxic properties [5].

2 Materials and methods

The clear apple juice concentrate (AJC) high in patulin was a commercial product obtained directly from a producer in Turkey in 1999. Samples were prepared as shown in Table 1. Vitamins were all food-grade purchased from Roche (Basel, Switzerland), mixed with AJCs in glass jars. Samples were kept at $22 \pm 2^\circ\text{C}$ and at 4°C . For analysis 15 g samples were taken from each jar at the end of the first, second and sixth month of storage. Patulin was determined by using HPLC [6] and assayed in duplicate to ensure statistical analysis. AJCs diluted to 11.6 brix with distilled water and turbidity was then measured by using a Hach Ratio XR Turbidimeter; colour and clarity of the samples were measured by using a spectrophotometer against the distilled water at 440 nm and 625 nm, respectively, and were expressed as transmittance unit. One-way analysis of variance and Dunnett's intervals for treatment mean minus control mean were done for evaluating the reduction in patulin content.

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Abbreviation: AJC, apple juice concentrate

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Table 1. Experimental design

No.	Sample		Storage temperature ($^\circ\text{C}$)	Amount of vitamin added (mg/kg AJC)
	Application	Abbrev.		
1	Thiamine	ThW1	22 ± 2	500
2	Thiamine	ThW2	22 ± 2	1000
3	Thiamine	ThW3	22 ± 2	2500
4	Thiamine	ThC1	4	500
5	Thiamine	ThC2	4	1000
6	Thiamine	ThC3	4	2500
7	Pyridoxine	PyW1	22 ± 2	125
8	Pyridoxine	PyW2	22 ± 2	250
9	Pyridoxine	PyW3	22 ± 2	625
10	Pyridoxine	PyW4	22 ± 2	875
11	Pyridoxine	PyC1	4	125
12	Pyridoxine	PyC2	4	250
13	Pyridoxine	PyC3	4	625
14	Pyridoxine	PyC4	4	875
15	Pantothenate	PaW1	22 ± 2	500
16	Pantothenate	PaW2	22 ± 2	1000
17	Pantothenate	PaW3	22 ± 2	2500
18	Pantothenate	PaC1	4	500
19	Pantothenate	PaC2	4	1000
20	Pantothenate	PaC3	4	2500
21	Nothing added	CtrlW	22 ± 2	–
22	Nothing added	CtrlC	4	–

3 Results and discussion

According to one way analysis of variance of patulin, p was found ≤ 0.05 . This result indicates that differences between averages were statistically significant. The Dunnett test was performed for comparison of series to the control samples. The samples were compared to control samples, CtrlW and CtrlC. Those which showed significant reductions in patulin content are indicated in Table 2. Patulin was fully degraded at the end of a 6 month period in samples stored at $22 \pm 2^\circ\text{C}$, on the other hand, other quality parameters, such as turbidity, colour and clarity diminished significantly. Without any considerable reduction on other quality parameters, applications of 1000 and 2500 mg/kg calcium-d-pantothenate resulted in reduction of patulin by 73.6 and 94.3%, respectively, however, 42.1% of patulin reduction was observed in the control sample of AJC

Table 2. Changes in patulin, clarity, colour and turbidity levels during 1, 2 and 6 months of storage^{a)}

Sample name	Changes as % of initial values ^{b)}											
	Patulin			Clarity			Colour			Turbidity		
	1	2	6	1	2	6	1	2	6	1	2	6
ThW1	55.0	25.0 ^{a)}	0	95.4	91.9	78.3	82.4	78.8	40.5	144	281	444
ThW2	26.9 ^{a)}	17.9 ^{a)}	0	88.3	88.2	43.5	71.5	73.2	15.8	2019	1219	6000
ThW3	16.9 ^{a)}	11.6 ^{a)}	0	71.4	67.0	53.2	44.2	39.5	21.4	7050	7750	7937
ThC1	94.7	88.4	63.2	95.6	93.4	96.5	90.3	83.4	90.3	125	131	119
ThC2	74.0 ^{a)}	68.9 ^{a)}	43.3 ^{a)}	95.3	92.4	95.6	89.6	82.7	90.0	156	231	144
ThC3	64.8 ^{a)}	61.9 ^{a)}	32.3 ^{a)}	87.7	83.2	81.5	74.5	65.9	59.0	2563	2819	3875
PyW1	48.4 ^{a)}	23.8 ^{a)}	0	94.0	85.4	85.2	80.4	67.9	43.2	169	581	594
PyW2	47.3 ^{a)}	25.2 ^{a)}	0	95.4	84.4	86.3	82.0	65.9	42.5	150	606	569
PyW3	48.0 ^{a)}	25.8 ^{a)}	0	92.2	87.1	83.5	80.1	68.9	41.2	219	550	469
PyW4	47.8 ^{a)}	25.5 ^{a)}	0	81.4	73.6	82.1	66.2	86.0	33.3	575	831	631
PyC1	70.0 ^{a)}	66.9 ^{a)}	58.8 ^{a)}	95.2	92.0	95.5	88.0	82.0	83.0	103	113	102
PyC2	76.6 ^{a)}	75.0 ^{a)}	60.9	96.8	93.5	97.3	93.3	87.6	86.0	106	109	103
PyC3	82.2 ^{a)}	46.4 ^{a)}	42.8 ^{a)}	97.2	94.5	96.8	93.9	88.0	86.7	110	110	102
PyC4	54.1 ^{a)}	52.2 ^{a)}	44.6 ^{a)}	97.3	94.7	97.3	96.5	88.3	88.6	89	109	83
PaW1	41.9 ^{a)}	27.2	0	93.8	85.1	86.3	81.4	63.3	44.5	181	281	232
PaW2	26.4 ^{a)}	17.2 ^{a)}	0	94.9	87.7	76.0	82.0	68.9	42.2	213	319	406
PaW3	5.7 ^{a)}	4.0 ^{a)}	0	94.2	86.1	81.5	82.4	65.9	40.5	156	500	506
PaC1	95.6	90.6	63.4	97.4	95.6	97.2	93.9	88.0	89.6	114	101	111
PaC2	69.6 ^{a)}	69.0 ^{a)}	43.4 ^{a)}	97.0	95.8	97.0	94.6	88.6	88.3	98	102	102
PaC3	72.3 ^{a)}	67.2 ^{a)}	43.8 ^{a)}	97.3	95.4	97.3	94.2	87.3	91.9	101	104	96
CtrlW	57.9	28.6	0	95.6	91.4	85.2	86.7	76.4	44.5	175	294	244
CtrlC	94.9	89.2	64.2	96.7	95.8	96.9	93.3	86.0	90.9	104	105	106

a) Numbers indicate significant reductions on patulin content according to Dunnett test

b) Initial values are 304 µg/kg, 96.4 TU, 30.35 TU and 1.60 NTU for patulin, clarity, colour and turbidity, respectively.

stored for 1 month at $22 \pm 2^\circ\text{C}$. Addition of thiamine hydrochloride (1000 mg/kg), pyridoxine hydrochloride (625 or 875 mg/kg) and calcium-d-pantothenate (1000 or 2500 mg/kg) into the samples and storage at 4°C for 6 months yielded 55.5 to 67.7% of patulin reduction which was only 35.8% for the control, while the other quality parameters were protected adequately. For practical applications, we suggest that the use of reduced amounts of vitamins mentioned above and increased storage periods at 4°C could be effective for reducing patulin content in AJCs.

4 References

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