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Research Article
Inhibition of Processed Cheese-late Gas Using *Candida pelliculosa* Yeast

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Abstract

Background and Objective: Yeasts have potential antimicrobial activities against the growth of putrefaction bacteria. The late gas defect is a major cause of spoilage in processed cheese. It results in the production of gas, off-odours and the liquefaction of the cheese. Some clostridial species are considered cause of late gas defect in cheese. So, processed cheese-late gas inhibition using dried supernatant of *Candida pelliculosa* yeast compared with nisin was studied. **Methodology:** Five processed cheese treatments were prepared. The treatments were A (control 1) fortified with clostridial spores only, B (control 2) fortified with clostridial spores and nisin (1000 IU g^{-1}), while, C, D and E treatments fortified with clostridial spores and 1, 3 and 5 mg of dried supernatant of *Candida pelliculosa* yeast DSCPY per gram, respectively. The resulting processed cheese treatments were storage at 30°C for 3 months. The chemical, physical and microbiological analyses of the resultant cheeses were performed every month of storage. **Results:** The treatments of A (control 1), C (1 mg DSCPY g^{-1} cheese) and D (3 mg DSCPY g^{-1} cheese) spoiled by producing high quantity of gas from the 1st month of the storage period and cheese glass jars were opened. Hence, the chemical, physical and microbiological analyses of these treatments weren't performed. The chemical composition of fresh processed cheese treatments and those of with 5 mg DSCPY g^{-1} or nisin during storage period was not significantly affected. Changing physical properties (penetrometer reading, oil separation and melting index) of studied processed cheese treatments did not happen at zero time. The penetrometer reading of E treatment (5 mg DSOCY g^{-1}) was higher than those of with nisin B (control 2) during storage period. The oil separation index increased but melting index and penetrometer reading decreased gradually during storage period in treatment E (5 mg DSCPY g^{-1}) or those of with nisin. Also, color properties of studied cheese treatments were determined. The microbiological results showed that the most effective concentrate of DSCPY against clostridial spores was 5 mg g^{-1}. **Conclusion:** It be concluded that the addition of 5 mg g^{-1} of DSCPY during processed cheese spread manufacture prevented of late-blowing in cheese.

Key words: Yeast, processed cheese, late gas, nisin, clostridial spores

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Processed cheese is an important section of the cheese market. Its market is very progressing. It is a complicated system consists of protein, fat, mineral salts and other ingredients[1]. The basic raw materials for processed cheese production are natural cheese which is treated by heat and adding emulsifying salts. From a point of view of the melting temperatures used (and the pH-value of the product), the method of processed cheese production can be considered "pasteurization of cheese". During the melting process, most vegetative forms of microorganisms, including bacteria of the family Enterobacteriaceae are inactivated. The melting temperatures are not enough to kill the endospores, which survive the process but they are often weakened. From a microbiological point of view, the major contamination problem of processed cheese is caused by the genus *Clostridium* which may leads to subsequent spoilage due to the production of gas and off-odours and the liquefaction of the cheese[2]. *Clostridium* is classified as a Gram-positive and rod-shaped microorganism[3], it is an obligate anaerobe[4]. Spoilage *Clostridium* species are primary cause of late blowing in cheese[5], consequently, these bacteria can cause microbiological problems in processed cheeses. Although, *C. tyrobutyricum* is the major relevant microorganism, other clostridial species such as *C. sporogenes* and *C. butyricum* have also been shown to contribute to this defect[6]. Spoilage, which occurs mainly because of microbial growth, might be detected by gas production, abnormal odors/colors or pH variation and possibly microscopy examination[7].

The most common protection against late gas is nitrate addition to milk. Nitrate is reduced to nitrite that is able to interfere with spore germination[8]. However, nitrite can react with aromatic amino acids to form nitrosamine that can cause cancerogenic effect[9].

Bactofugation is another used treatment in cheese manufacturing. It removes bacterial cells and spores by centrifugation at 9000×g. Bactofugate undergoes heat treatment and then it is returned to the manufacturing process not to reduce yield[10]. As with bactofugation, microfiltration allows the heat treatment for decontaminating milk to be minimized, but microfiltration appears to be more efficient than bactofugation. High retention levels (>99.98%) noticed for spore-forming bacteria are likely due to binding of bacterial spores to a part of the cell wall, consequently resulting in obviously larger cell size[11].

Nisin is a polypeptide antibacterial substance or bacteriocin produced from the fermentation of a modified milk medium by specific strains of the lactic acid bacteria, *Lactococcus lactis*[12]. Nisin has the ability to inhibit the outgrowth of *Clostridium* spores during the production of processed cheeses[13].

The yeasts are eukaryotic microorganisms, which are most commonly defined as unicellular fungi, although unicellular growth occurs within several fungal taxonomic orders and many types of yeast can grow by forming pseudo-hyphae[14]. Antagonistic characteristics of yeast have been attributed mainly to: (1) Competition for nutrients, (2) pH changes in the medium as a result of growth-coupled ion exchange or organic acid production, (3) Tolerance to high concentrations of ethanol[15] and (4) The secretion and release of antimicrobial compounds, such as killer toxins or "Mycocins"[16,17].

This study was carried out to compare the inhibitory effect of nisin and dried supernatant of *Candida pelliculosa* yeast (DSCPY) against *Clostridium butyricum* and *C. tyroburyricum* in processed cheese.

MATERIALS AND METHODS

Materials: *Candida pelliculosa* yeast isolated from grape leaves was screened and identified to inhibit of processed cheese-late gas. It showed high antibacterial activity against most studied pathogenic bacteria in former study[18].

Clostridium butyricum ATCC 8260 and *C. tyroburyricum* ATCC 25755 were obtained from Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University.

Ras cheese (1 month old) was obtained from Arabic Food Industrial Co. (Domety), 6th October city, Egypt.

Matured cheddar cheese (8 months old) and Kasomel emulsifying salts K-2394 were obtained from International Dairy and Food Co. (Milky land), 10th Ramadan city, Egypt.

Low heat skim milk powder and butter were obtained from local market. Nisin powder was obtained from Zhejiang sliver elephant Bio-engineering Co., Ltd., China.

Preparation of dried supernatant of *Candida pelliculosa* yeast (DSOCY): *Candida pelliculosa* was grown in potato dextrose broth at 30°C for 3 days and then centrifuged at 10000 rpm for 15 min. The supernatant was collected and subsequently dried under vacuum at 30°C.

Preparation of Clostridia spore suspensions: Clostridia spore suspensions were prepared using method of Senyk *et al.*[19]. Briefly, the *Clostridium butyricum* and *C. tyroburyricum* were inoculated into sterile thioglycollate broth (previously steamed to 100°C for 10 min and cooled) and incubated for

Table 1: Chemical composition of ingredients used in processed cheese manufacture

	Ingredients			
Component (%)	Ras cheese	Cheddar cheese	Butter	Skim milk powder
Total solids	55.17	65.89	84.00	96.00
Fat	25.00	35.00	82.00	00.29
Total protein	22.25	25.40	00.80	37.13
Soluble nitrogen	0.68	1.31	00.20	00.80
Lactose	1.65	0.10	-	47.50
Ash	5.30	5.02	01.00	07.88

Table 2: Cheese treatments made in this study

Treatments	Clostridial spores (spores g^{-1})	Nisin (IU g^{-1})	DSCPY (mg g^{-1})
A (control 1)	1000	-	-
B (control 2)	1000	1000	-
C	1000	-	1
D	1000	-	3
E	1000	-	5

DSCPY: Dried supernatant of *Candida pelliculosa* yeast

18-24 h. *Clostridium butyricum* was incubated at 32°C, while *C. tyrobutyricum* was incubated at 37°C. After a second subculture in thioglycolate broth, the cultures were heated at 63°C for 40 min to destroy all the vegetative cells. The broths were immediately cooled in ice water and subsequently stored at 4°C. Spore concentrations were determined by plating stock suspensions on reinforced clostridial agar. Dilutions were made in sterile peptone-saline (0.1% peptone, 0.85% NaCl). Plates were incubated in an anaerobic incubator at 32 or 37°C for 48 h depending on the species being enumerated. Duplicate plates were used for all dilutions.

Pasteurized processed cheese spread manufacture: Pasteurized processed cheese spread was prepared according to Tanaka *et al.*[20]. Chemical analysis (%) of the ingredients used in processed cheese spread manufacture is shown in Table 1. Five treatments were prepared and illustrated in Table 2. Ras cheese (38.44%) and cheddar cheese (12.80 %) were ground and placed into the processing batch type kettle of 10 kg capacities, a pilot machine at the National Research Center. About 5.12, 10.26, 2.5 and 30.88% of skim milk powder, butter, emulsifying salts and water, respectively were added. Nisin (1000 IU g^{-1}) was added in case of control 2. The tested dried supernatant of *Candida pelliculosa* yeast at 1, 3 and 5 mg g^{-1} were added to C, D and E treatments, respectively. The temperature of the cheese melt was increased to 80°C and held for 2 min. Previously prepared spores were added to the melted cheese mixture. The cheese spread was maintained at 80°C for 2 min following inoculation to facilitate spore activation. Cheese spread treatments were filled into sterilized glass jar and also covered with aluminum foil and their covers, then rapidly cooled at 7±1°C. The resultant cheeses were stored at 30°C for 3 months. The chemical, physical and microbiological analyses were carried out on fresh processed cheese treatments and after 1, 2 and 3 months of storage.

Cheese analysis

Chemical analysis: The chemical composition of the processed cheese treatments was analyzed. Total fat content was measured according to the method of AACC[21]. Total protein, moisture and ash contents were analyzed as described by AOAC[22]. Carbohydrate content was determined according to the method of Nielsen[23]. For pH measurement, about 20 g of the cheese sample were soften by mixing them with the same amount of deionized water previously warmed at 40°C and the whole mixture was kept for 5 min at room temperature. The pH was measured using pH meter (HANNA 8417).

Physical properties: The processed cheese firmness was measured using a penetrometer as described by Gupta and Reuter[24]. Meltability of the processed cheese was measured as described by Savello *et al.*[25]. Oil separation was determined according to the method of Thomas[26]. Color parameters were determined using a Hunter Lab., colorimeter model b25 A-2 (Hunter Assoc., Lab., Inc., Va, USA) and the instruction of user manual. The instrument was first standardizing using a reference with white surface. As in the Hunter L, a and b scale described lightness black (0) to white (100), redness (+) to greenness (-) and yellowness (+) to blueness (-), respectively were measured.

Microbiological analysis: Clostridial spores were determined according to Senyk *et al.*[19]. Also, spoilage was detected by the presence of gas and/or odor in the packages.

Statistical analysis: Factorial design 2×2 was used to analyze the data and Duncan's test was used to make the multiple comparisons[27]. Significant differences were determined at $p<0.05$ level.

RESULTS AND DISCUSSION

Chemical composition of processed cheese spread: Chemical composition of processed cheese manufactured using dried supernatant of *Candida pelliculosa* yeast (DSCPY) at different concentrations (1, 3 and 5 mg g^{-1}) or nisin (control) as anticlostridial agent are shown in Table 3. The chemical composition of fresh processed cheese was not significantly affected by adding (DSCPY) or nisin at zero time of storage period. Similar observations were found by Roberts *et al.*[28]

Table 3: Chemical composition of processed cheese manufactured using (DSCPY) or nisin (control) as anticlostridial agent

		Treatments				
Component (%)	Storage period (Month)	A (Control 1)	B (Control 2)	C	D	E
Total solids	Fresh	44.99[a]	44.90[a]	44.89[a]	44.88[a]	44.90[a]
	1	-	44.92[a]	-	-	44.92[a]
	2	-	44.93[a]	-	-	44.95[a]
	3	-	44.96[a]	-	-	44.98[a]
Fat/DM	Fresh	50.65[a]	50.76[a]	50.68[a]	50.70[a]	50.66[a]
	1	-	50.78[a]	-	-	50.70[a]
	2	-	50.80[a]	-	-	50.72[a]
	3	-	50.81[a]	-	-	50.78[a]
Total protein	Fresh	13.25[a]	13.30[a]	13.36[a]	13.34[a]	13.40[a]
	1	-	13.32[a]	-	-	13.41[a]
	2	-	13.38[a]	-	-	13.45[a]
	3	-	13.43[a]	-	-	13.52[a]
Soluble nitrogen	Fresh	1.60[a]	1.64[a]	1.65[a]	1.64[a]	1.66[a]
	1	-	1.65[a]	-	-	1.69[a]
	2	-	1.66[a]	-	-	1.70[a]
	3	-	1.69[a]	-	-	1.71[a]
Salt in moisture	Fresh	3.01[a]	3.00[a]	3.06[a]	3.04[a]	3.07[a]
	1	-	3.03[a]	-	-	3.08[a]
	2	-	3.05[a]	-	-	3.08[a]
	3	-	3.08[a]	-	-	3.09[a]
Ash	Fresh	5.05[a]	5.01[a]	5.06[a]	5.08[a]	5.08[a]
	1	-	5.03[a]	-	-	5.09[a]
	2	-	5.08[a]	-	-	5.11[a]
	3	-	5.11[a]	-	-	5.15[a]
pH	Fresh	5.72[a]	5.73[a]	5.75[a]	5.76[a]	5.78[a]
	1	-	5.71[a]	-	-	5.75[a]
	2	-	5.68[a]	-	-	5.74[a]
	3	-	5.62[a]	-	-	5.71[a]

A (control 1): With spores, no nisin, no DSCPY, B (control 2): With spores and nisin, no DSCPY, C: With spores and 1 mg g^{-1} DSCPY, D: With spores and 3 mg g^{-1} DSCPY, E: With spores and 5 mg g^{-1} DSCPY, DSCPY: Dried supernatant of *Candida pelliculosa* yeast, means with the same letter in the same raw are not significantly different

who reported that the control (without nisin) and nisin-containing cheese had similar levels of fat, protein and solids. The treatments of A (control 1), C (1 mg DSCPY g^{-1} cheese) and D (3 mg DSCPY g^{-1} cheese) spoiled by producing high quantity of gas from the 1st month until end of the storage period. Hence, the chemical composition of these treatments wasn't performed. No spoilage was observed for treatment E (5 mg g^{-1} DSCPY) during studied storage period. No significantly different between chemical composition of treatment E (5 mg g^{-1} DSCPY) and treatment with nisin was noticed during storage period.

Physical properties: Data presented in Table 4 showed penetrometer reading, oil separation and melting index of processed cheese manufactured using dried supernatant of *Candida pelliculosa* yeast (DSCPY) at different concentrations (1, 3 and 5 mg g^{-1}) or nisin (control) as anticlostridial agent.

The physical properties of control 1, C (1 mg DSCPY g^{-1} cheese) and D (3 mg DSCPY g^{-1} cheese) wasn't carried out due to high quantity gas production during the studied storage period. It could be seen from the results that the DSCPY or nisin addition did not affect significantly the studied physical properties at zero time. The penetrometer reading of processed cheese with 5 mg DSCPY g^{-1} was higher than those of with nisin during storage periods. The penetrometer reading (mm) of processed cheeses with 5 mg DSCPY g^{-1} or nisin decreased gradually with increasing storage period. The differences in the penetration values during storage could be related to the interaction between emulsifying salts and state of protein network[29]. The oil separation index increased but melting index decreased gradually during storage period in processed cheeses with 5 mg DSCPY g^{-1} or nisin.

The color properties of the studied processed cheese treatments are shown in Table 5. It could be seen from the results that , the cheese with nisin or 5 mg g^{-1} DSCPY had the highest lightness than other treatments at zero time of storage and the lightness decreased gradually in these treatments by progressing the storage period. The cheese with nisin showed the highest intensity of green (a-value) at the zero time of storage period. The a-value of cheese with nisin or 5 mg g^{-1} DSCPY increased during storage period. On the other hand, the highest yellow (b-value) was observed with 5 mg g^{-1} DSCPY cheese, but the cheese with nisin showed the lowest yellow. The yellow (b-value) increased with increasing storage period for cheese with 5 mg g^{-1} DSCPY or nisin.

Table 4: Physical properties of processed cheese manufactured using (DSCPY) or nisin (control) as anticlostridial agent

		Treatments				
Physical properties	Storage period (Month)	A (Control 1)	B (Control 2)	C	D	E
Penterometer reading (mm)	Fresh	172[a]	173.00[a]	175[a]	176[a]	176.00[a]
	1	-	171.00[b]	-	-	175.00[a]
	2	-	168.00[b]	-	-	172.00[a]
	3	-	165.00[b]	-	-	169.00[a]
Oil separation index	Fresh	25.62[a]	25.63[a]	25.66[a]	25.66[a]	25.63[a]
	1	-	26.33[b]	-	-	27.00[a]
	2	-	29.00[b]	-	-	31.00[a]
	3	-	32.66[b]	-	-	33.33[a]
Melting index (mm)	Fresh	154[a]	154.00[a]	155[a]	154[a]	155.00[a]
	1	-	141.00[b]	-	-	151.00[a]
	2	-	138.00[b]	-	-	148.00[a]
	3	-	131.00[b]	-	-	133.00[a]

A (control 1): With spores, no nisin, no DSCPY, B (control 2): With spores and nisin, no DSCPY, C: With spores and 1 mg g^{-1} DSCPY, D: With spores and 3 mg g^{-1} DSCPY, E: With spores and 5 mg g^{-1} DSCPY, DSCPY: Dried supernatant of *Candida pelliculosa* yeast, means with the same letter in the same raw are not significantly different

Table 5: Color parameters of processed cheese manufactured using (DSCPY) or nisin (control) as anticlostridial agent

		Treatmentsy				
Color parameter	Storage period (Month)	A (Control 1)	B (Control 2)	C	D	E
L	Fresh	88.60[d]	88.65[a]	88.64[ab]	88.62[c]	88.65[a]
	1	-	88.60[a]	-	-	88.59[a]
	2	-	88.51[a]	-	-	88.52[a]
	3	-	88.02[a]	-	-	88.00[b]
a	Fresh	-2.01[c]	-2.09[a]	-2.01[c]	-2.01[c]	-2.06[b]
	1	-	-2.20[b]	-	-	-2.22[a]
	2	-	-2.33[b]	-	-	-2.35[a]
	3	-	-2.41[b]	-	-	-2.44[a]
b	Fresh	22.44[d]	22.33[e]	22.50[c]	22.53[b]	22.59[a]
	1	-	22.85[a]	-	-	22.80[b]
	2	-	23.04[a]	-	-	23.01[b]
	3	-	23.49[a]	-	-	23.29[b]

L: Lightness (Black (0) to white (100)), a: Redness (+) to greenness (-), b: Yellowness (+) to blueness (-), A (control 1): With spores, no nisin, no DSCPY, B (control 2): With spores and nisin, no DSCPY, C: With spores and 1 mg g^{-1} DSCPY, D: With spores and 3 mg g^{-1} DSCPY, E: With spores and 5 mg g^{-1} DSCPY, DSCPY: Dried supernatant of *Candida pelliculosa* yeast, means with the same letter in the same raw are not significantly different

Table 6: Effect of DSCPY on clostridial spore numbers compared with nisin during storage at 30°C

	Clostridial spore numbers (spore g^{-1})			
	Storage period (Month)			
Treatments	Fresh	1	2	3
A (Control 1)	1000[a]	-	-	-
B (Control 2)	900[e]	250[b]	300[b]	355[b]
C	980[b]	-	-	-
D	965[c]	-	-	-
E	940[d]	320[a]	340[a]	360[a]

A (control 1): With spores, no nisin, no DSCPY, B (control 2): With spores and nisin, no DSCPY, C: With spores and 1 mg g^{-1} DSCPY, D: With spores and 3 mg g^{-1} DSCPY, E: With spores and 5 mg g^{-1} DSCPY, DSCPY: Dried supernatant of *Candida pelliculosa* yeast, means with the same letter in the same column are not significantly different

Microbiological analysis: Changes in clostridial spores of the cheese treatments during storage are shown in Table 6. It could be seen from the results that the clostridial spore numbers increased with progressing storage period up to 3 months at 30°C. The lowest number of clostridial spores was observed with nisin cheese treatment during the1st month of the storage period but then this number was little increased during the 2nd and 3rd months of the storage period. Addition of nisin at 100 or 500 mg kg^{-1} suppressed anaerobic spore counts in processed cheese[30]. Nisin activity may be reduced by interaction with milk proteins and fat[31]. The clostridial spore numbers of dried supernatant of *Candida pelliculosa* yeast (DSCPY) cheeses decreased with increasing DSCPY. The most effective concentrate of DSCPY against clostridial spores was 5 mg g^{-1}. And 5 mg g^{-1} of DSCPY showed the nearly same results of nisin.

Gas and/or odor observation: As might be expected, the non-anticlostridial agent cheese (Control 1) spoiled faster than other tested treatments. Clostridial growth produces major

Fig. 1: Effect of dried supernatant of *Candida pelliculosa* yeast (DSPCY) at different concentrations compared with nisin on gas production in processed cheese

texture defects and gas production of the cheeses interior and creates an acidic, fermented flavour as well as a vomit-like odour from butyric acid[32,33]. Cheese spread containing nisin {N} had longer shelf stability than those of containing lower concentrations {1 or 2} of DSCPY. Cheese spreads containing nisin had a significantly longer shelf-life (when inoculated with *C. sporogenes* PA 3679) compared to control spreads without nisin[34]. Also, the Cheese spread containing 5 mg g^{-1} of DSCPY {3} showed good shelf stability (Fig. 1).

CONCLUSION

It be concluded that the addition of 5 mg g^{-1} of DSCPY during processed cheese spread manufacture delayed of late-blowing in cheese manufacture.

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