Influence of freeze-shocked mesophilic lactic starter bacteria and adjunct lactobacilli on the rate of ripening Gouda cheese and flavor development

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Abstract: The objective of the present study was to determine the effects of *Lactobacillus acidophilus* on the sensory attributes, ripening time, and composition of Gouda cheese and to investigate the survival of *L. acidophilus* during ripening. Five types of Gouda cheeses, control cheese (Tc), made with with mesophilic lactic starter bacteria, Ta1, Ta2 , Tb1 and Tb2 cheeses made using modified mesophilic lactic starter bacteria by freeze–shocked at -10°C/-20°C for 24 , 96 hrs and probiotic Lactobacillus, as adjunct culture. Cheese samples were assessed for microbiological and compositional properties, proteolysis, and sensory evaluation at different ripening stages. The composition and the pH value were almost identical between control and experimental vats within a single trial cheese. Characterization of proteolysis of gel electrophoresis of cheese samples at various stages of ripening showed that the extent of casein degradation varied between samples in all cheeses, α_{S1}-Casein was more extensively degraded than ß-casein. However, levels of soluble nitrogen (SN/TN) increased with ripening period for all cheeses, only moderate enhancement of proteolysis as in amino acid −N in all trials. The formation of non protein nitrogen (NPN/TN) was slightly increased compared to control at the end of ripening. Organoleptic evaluation showed that probiotic cheese had higher sensory evaluation than control cheese, without probiotic strain. The population of Lactobacillus survived to numbers > 10^7 cfu/g, which is necessary for positive effects on health. These results showed that the contribution of modified mesophilic lactic starter bacteria by freeze–shocked and probiotic strain as adjunct culture can be successfully used in production of Gouda cheese.
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1. Introduction

Cheese is an excellent dietary source of high-quality protein, vitamins and minerals such as absorbable dietary calcium. Gouda Dutch type semi hard cheese is a traditional, creamery, hard cheese. It is round with very smooth, yellow, waxed rind. The flavor is sweet and fruity. As time passes, the taste intensifies and becomes more complex. Mature Gouda (18 months plus) is coated in black wax which provides a stark contrast to the deep yellow interior. Gouda is considered to be one of the world's great cheeses. A great deal of research in Cheddar cheese technology is devoted towards the addition of adjunct cultures which may accelerate ripening times or to improve flavor (Wilkinson, 1993).The use of attenuated starters was first proposed by Petterson and Sjöström (1975) to accelerate the ripening of Svecia, a Swedish semi-hard cheese; these authors attenuated cells by heat treatment. Besides heat treatment, other methods to achieve attenuation have been studied including freezing and thawing, freeze or spray drying, lysozyme treatment, use of solvents, and natural and induced genetic modification. Freezing cells at sub-optimal conditions reduces the viability of lactic acid bacteria. Stressed cells do not contribute significantly to acid production during cheese making, but may retain protease and peptidase activity (El-Tanboly 1991). Frozen cells may lyses to a greater extent than non-frozen cells, and thus release intercellular enzymes (Barteis *et al*., 1987). Dairy foods, including in particular, fermented milks and yogurt are among the best accepted food carriers for probiotic cultures. The aim of this study was to develop new probiotic foods, particularly, the production of high quality Gouda cheese containing high levels of probiotic bacteria. The dairy products

with probiotic bacteria recognition as functional foods that provide health benefits beyond basic nutrition and the emerging clinical evidence to their potential in preventing some diseases have notably enlarged their consumption and stimulated innovation and new product development (Boylston *et al.*, 2004; Ong *et al*., 2007). Although yogurt and fermented milks have received the most attention as carriers of probiotic bacteria, some cheese varieties such as Gouda, white and Cheddar cheeses (Gomes *et al.*, 1995; Kasmoglu *et al*., 2004; Ong *et al*., 2007). Cheeses have a number of advantages over fermented milks as a delivery system for viable probiotic microorganisms, because they generally have higher pH and buffering capacity, more solid consistency, and relatively higher fat content (Ong *et al*., 2007; Joutsjoki, 2009). These features give protection to probiotic bacteria during storage and passage through the gastrointestinal tract. To exert positive health effects, the microorganisms need to be viable, active, and sufficiently abundant, in concentrations of at least 10^6 cfu/g throughout the shelf life (Vinderola *et al*., 2000; Narvhus, 2009). The aims of this study was to influence of physically modified mesophilic lactic starter bacteria by freeze–shocked and probiotic strain of Lactobacillus, as adjunct on the rate of ripening Gouda cheese and flavor development.

2. Materials and Methods

Mesophilic lactic starter bacteria and adjunct lactobacilli conditions

The mixed strains of mesophilic lactic starter bacteria 022 and adjunct lactobacilli used for experiments were obtained from the Production Laboratory of Dairy Biopreparation in Olsztyn, Poland. Bacteria were inoculated at 2% (v/v) into sterile 10%(w/v) reconstituted non–fat milk (RNFM). It was sub-cultured at least twice for 18 hrs at 23°C before treatment. Overnight adjunct lactobacilli (37°C for 16 h) were obtained from (MRS) broth. Cells were harvested by centrifugation at 8,000 x g for 20 min at 4°C. The resultant pellet was washed twice with saline solution (0.9% NaCl in distilled water) and resuspended in 10% sterile skim.

Mesophilic lactic starter bacteria modification

Biomass cells of mixed mesophilic lactic starter bacteria 022 were physically modified by freeze–shocked at -10, -20°C for 24 and 96 hrs. thawed the following experimental moring at 40°C and added just prior to renneting.

Gouda cheese manufacturing

Cheeses were manufactured according to the standard procedure Fox *et al.,* (2004) from three trials, T_c (control) of milk with modified mesophilic lactic starter bacteria, T_{a1}(-10°C/24 hr), T_{a2} (-20°C/24 hr), T_{b1} (-10°C/96 hr) and T_{b2}(-20°C/96 hr) made using modified mesophilic lactic starter bacteria and probiotic Lactobacillus, as adjunct culture.

Microbiological analysis

Samples cheeses were tested for counts of mesophilic lactic starter bacteria, *L. acidophilus* and coliform bacteria using standard methods (Vanderzant & Splittoesser, 1992). Plate count agar was used for enumeration of mesophilic lactic starter bacteria. Plates were incubated aerobically at 30°C for 48 h. *L. acidophilus* was counted on acidified (pH 5.4) MRS agar and incubated anaerobically at 37°C for 3 days. For the count of coliform bacteria, violet red bile agar was used and incubated aerobically at 37°C for 48 h.

Chemical analysis of Gouda cheese

pH was measured by pH-meter 646 with glass electrodes, Ingold, Knick, Germany. Titratable acidity (°SH) was done with Soxhlet Hankel method as described by (IDF,1993). Moisture content and cheese fat content was determined according to (IDF, 1986). Secondary proteolysis was measured by nitrogen fraction in cheese. Total nitrogen content (TN) was determined according to method of Kjeidahl, soluble nitrogen at pH 4.6 (SN), Non protein nitrogen (NPN), Peptide-N and Amino acid-N (AAN) was estimated according to as described by (IDF, 1993).

Organoleptic assessment of Gouda cheese

The cheese were evaluated organoleptically by a team of experienced cheese graders. The cheese samples were characterized by appearance of body, texture and flavor during ripening period. Cheese samples were analyzed chemically, when fresh and after 3 and 6 weeks.

3. Results and Discussion

Gross chemical composition of Gouda cheese

The composition of Semi hard cheese was almost identical for control and experimental vats within modified mesophilic lactic starter bacteria and probiotic Lactobacillus, as adjunct culture. The composition was similar between trials with a moisture content ranging 40-41 % , fat 28-30 %, salt in moisture 6.9-8.7 %, protein 24.6-29.4 % and PH 5.4-5.8 at 6 weeks of ripening. However, the production schedules were not altered because of the added modified mesophilic starter and probiotic Lactobacillus, as adjunct culture as illustrated in Table (1). Similar results were described by Degheidi e*t al.,* (2007).

Microbiological analysis

Initial numbers of *L. acidophilus* inoculated into the milk were 10^5–10^6 cfu ml $^{-1}$, but they grew rapidly during the one week of ripening and reached to 10^7–10^8 cfu g^{-1} in trials cheeses, respectively. Rapid growth of *L. acidophilus* might be due to the fermentation of lactose by modified mesophilic starter bacteria. It is well known that lactobacilli grow best

under acidic conditions (Mäkeläinen, *et al*., 2009). The viable cell numbers of *L. acidophilus* began to decrease after two weeks of ripening, because of the decrease in moisture level, increase in salt content, and the low ripening temperature. Although *L. acidophilus* decreased until the end of the ripening period, it did not decrease below 10^7 and 10^6 cfu g^{-1} in trials cheeses, respectively. As indicated earlier, it is necessary to maintain the viability of *L. acidophilus* at $\geqslant 10^7$ cfu g^{-1} of cheese, to call the cheese probiotic (Jatila *et al.,* 2009). There were no differences between the trials cheeses for he number of modified mesophilic starter bacteria count during the ripening period. Also, survival and growth of modified mesophilic starter bacteria was similar to that of the *L. acidophilus* at different stages of ripening for trials and Tc cheeses. Similar results were described by Degheidi *et al*., (2007). Modified mesophilic starter bacteria showed a decline after the one week of ripening. This reduction might be due to the low growth ability of modified mesophilic starter bacteria under acidic conditions (Mundt, 1986). Coliform bacteria were not detected in any of the samples in the present study.

Proteolysis of Semi hard cheese during ripening

(A) primary proteolysis

Characterization of proteolysis of gel electrophoresis of cheese samples at various stages of ripening are shown in Fig.1. Polyacrymide gel electrophoresis (PAGE), as well as stacking gel electrophoresis (SGE), showed cheese made with modified mesophilic starter and probiotic Lactobacillus, as adjunct culture did not show any distinct proteolysis of α_{S1}-casein but slight proteolysis was evident as a very faint band in trials Tb1 and Tb2 after just salting of ripening. There was no evidence of proteolysis of ß-casein in 0-day old cheese for any trials. After 3-weeks of ripening, Ta1, Ta2, Tb1, Tb2 and Tc had a distinct proteolysis of α_{S1-1} peptide.

A ß-1 peptide appeared as very faint band in all trials. γ-casein were present in all cheeses after 6-weeks ripening. Major differences were observed in amount intact α_{S1}- and ß-casein. Ta1 and Ta2 showed extensive degradation of α_{S1-1} peptide and increased intensities of γ_2- Casein and ß-1 peptide bands. Trials and had smaller amount of α_{S1}- and ß-Casein present as shown in Fig. (1). These results are in agreement with those of Jensen and Ardö (2009). The forgoing results of Polyacrymide gel electrophoresis (PAGE) of cheese samples treated with modified mesophilic bacteria and probiotic culture at different stages of ripening indicate that the proteolysis of both α_{S1}-casein and ß-casein increased during ripening, ß-casein was more resistant to hydrolysis than α_{S1}-casein which rapidly degraded during ripening, there are also increasing amount of some low-mobility peptides were detected in the γ-casein regions of all cheese samples.

(B) Secondary proteolysis

Addition of modified mesophilic starter bacteria and probiotic Lactobacillus, as adjunct culture, increase soluble-N levels over those in the control in several trials. The rate of proteolysis in Ta2 and Tb1 was higher in the first stage of ripening than when ripening had progressed. On contrary, the rate of proteolysis in Tc , Tb1 and Tb2 was lower in the first stage of ripening (Fig. 2). The data indicated that the Non protein-N values generally increased slightly for Tb1 and Tb2 compared to the control (Tc) at the end of ripening period (Fig. 2). On the both previous cheeses trials contained approximately 6.85 to 8.04% of the Total-N contents at the end of ripening time. Furthermore, it was observed also that the levels of Non protein-N were 32.21 to 38.67% of soluble-N.

The accumulation of Peptide-N was increased slightly in modified mesophilic starter and probiotic Lactobacillus, as adjunct culture trials than control. Tb2 was greatest being 0.446 and 7.29% (relative to Total-N and soluble-N at the beginning of ripening of ripening time, increased to 2.495 and 14.075% after 6 weeks ripening, respectively.

It was found to increase rapidly in the course of ripening mainly due to the breakdown of protein and peptide. Enhancement of proteolysis was observed only in Tb1 and Tb2 approximately 4.664 and 26.315% increased to 6.601 and 33.920% at the end of ripening period (Fig. 2). Only slight enhancement of proteolysis in T1 and Ta2.

A comparison between the results and those by other investigators would reveal similar influences , Gagnaire *et al.,* (2009) who reported that a heat treated culture of *Lb. helveticus* could be used to increase proteolysis and enhancement of cheese flavour without introducing bitter taste in Swedish hard cheese . This might be due to the results of cell lyses and release of intracellular proteinase of modified starter into surrounding cheese matrix, high level and specificities (Gagnaire et al., 2009).

In view of the foregoing available evidence, it could be concluded that a combination of rennet, regular and modified mesophilic starter bacteria and probiotic Lactobacillus was successful in accelerating maturation of Gouda cheese. They were mainly responsible for accelerating casein breakdown and contribute to hydrolysis of medium sized peptides to amino acids nitrogen . it is also clear that maturation time for semi hard cheese can be halved by using modified starter and can improve flavour intensity and reduce bitterness (El-Tanboly *et al.,* 2010).

Table (1) The changes in chemical composition during ripening of Gouda cheese made from modified mesophilic lactic bacteria and probiotic culture during ripening

*Trials	Ripening period (weeks)	Composition (%)				**FDM (%)	***S/M (%)
		fat	protein	Moisture	salt		
TC	0	27.5	22.83	42.70	2.10	47.99	4.92
	3	27.5	24.09	39.04	2.39	45.11	6.12
	6	31.5	25.56	37.36	3.79	50.29	10.14
Ta1	0	26.5	19.89	44.53	1.95	47.77	4.38
	3	28.5	22.96	44.15	2.95	51.03	6.68
	6	30.8	24.58	40.11	3.24	51.43	8.08
Ta2	0	27.0	20.24	45.95	2.25	49.59	4.90
	3	28.3	23.91	44.31	2.42	50.82	5.46
	6	28.3	25.07	41.25	3.59	48.17	8.70
Tb1	0	26.0	24.31	41.46	2.36	44.41	5.69
	3	27.5	28.42	40.05	2.95	45.87	7.29
	6	28.0	29.41	39.86	3.39	46.56	8.50
Tb2	0	27.5	19.84	41.37	2.16	46.90	2.22
	3	27.5	27.44	40.80	2.48	46.45	6.08
	6	30.0	27.93	40.04	2.66	50.01	6.92

Ta1: -10°C/24 hr, Ta2: -20°C/24 hr, Tb1: -10°C/96 hr and Tb2: -20°C/96 hr **FDM (%): Fat dry matter
***S/M (%): Salt in moisture

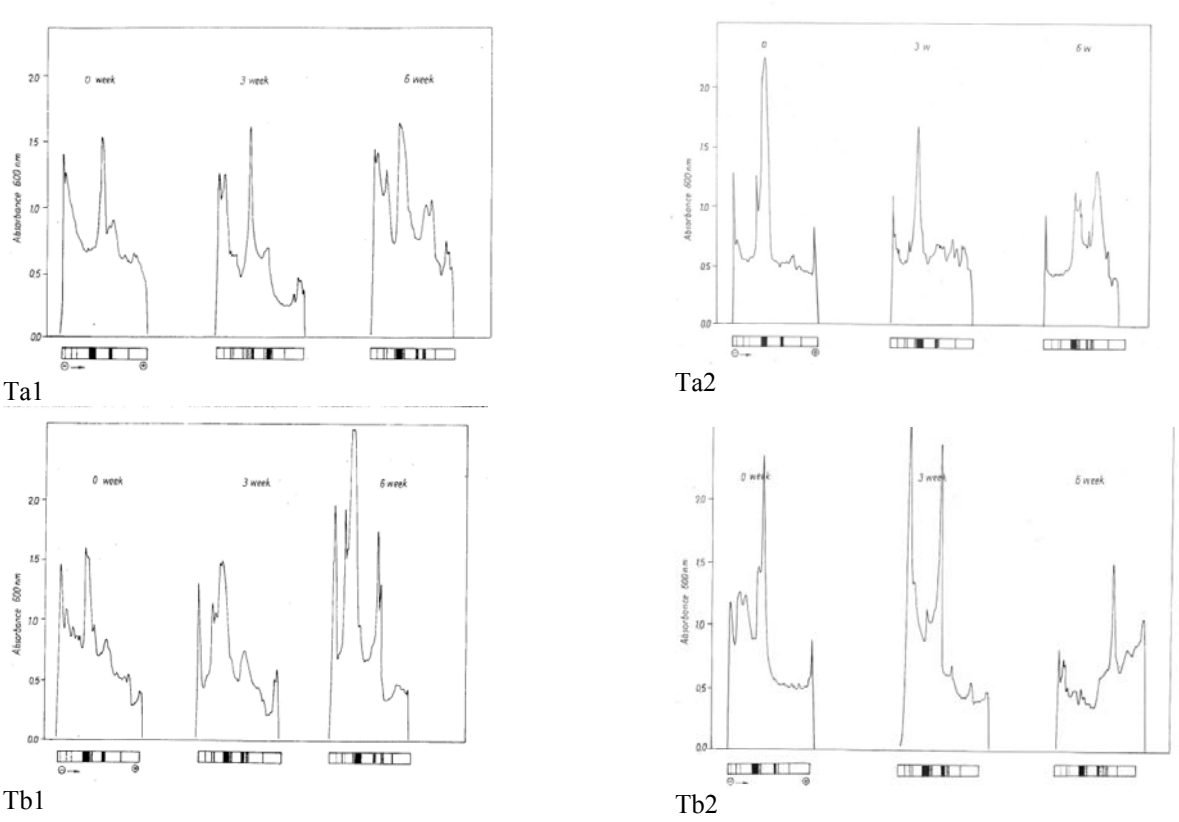
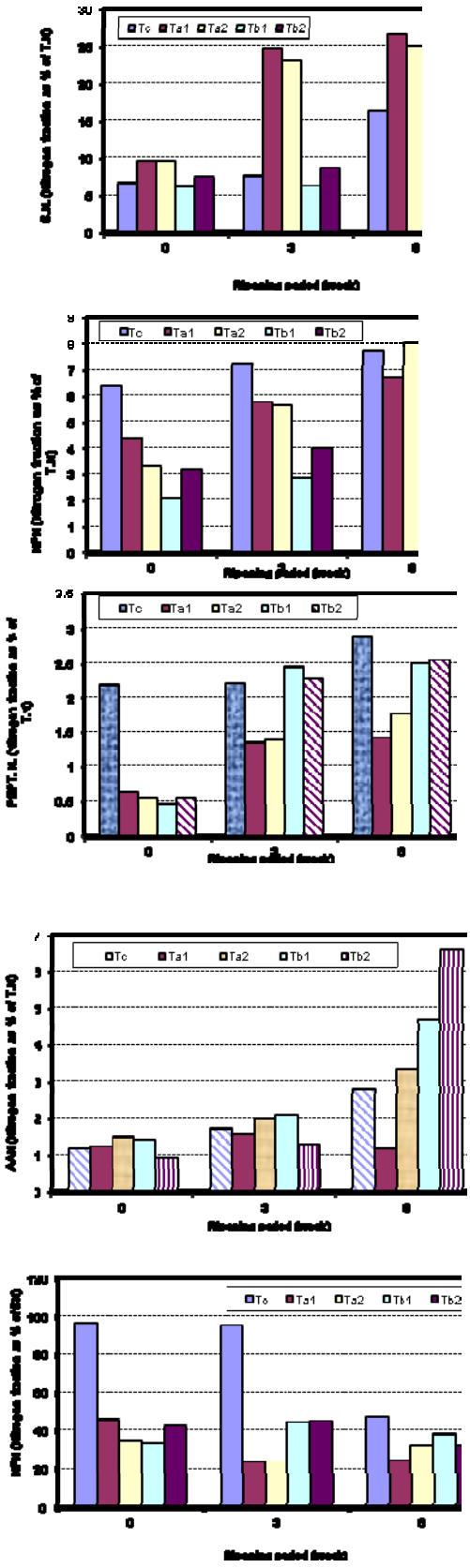


Fig. 1. Densitometric scans of PAGE during semi hard cheese ripening made with modified mesophilic bacteria and probiotic culture (Tc, Ta1, Ta2, Tb1 and Tb2).



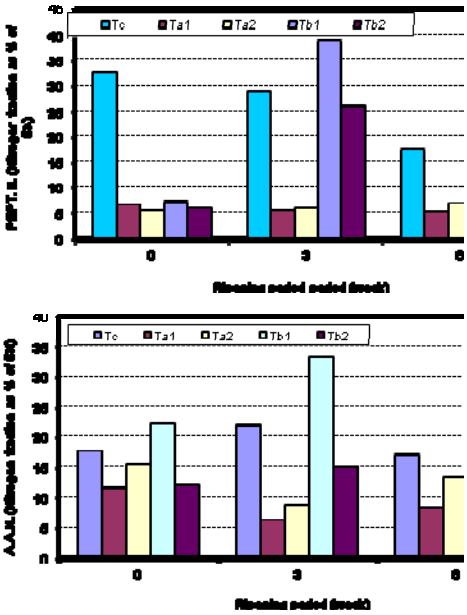


Fig. 2. The changes in nitrogenous compounds during ripening of Gouda cheese made from modified mesophilic bacteria and probiotic culture during ripening.

Organoleptic assessment of Gouda cheese

Cheeses made with modified mesophilic starter bacteria and probiotic Lactobacillus, as adjunct culture were found to have delicate pure taste, clean typical aroma , larger number of eyes and normal elastic consistency (Ta1, Ta2 and Tb2) Fig. 3. The increased eye formation was probably due to the higher number of citric acid fermenting bacteria (leuconostics or leuconostoc and *Str. diacetylactis* together also enclosed in the curd. Similar results have been reported (Møller *et al*., 2009). During the ripening of cheese, proteolysis is the most important pathway for flavour development. Short peptides and free amino acids are necessary for flavour development and are dependent on the extent of proteolysis. Gomes and Malcata (1998) reported that the high flavour scores of semi-hard goat cheese made with *B. lactis* and *L. acidophilus* strain Ki were associated with high levels of proteolysis. In addition, Flávia *et al.*, (2005) they reported that *L. paracasei* had a positive effect on the sensory Minas fresh cheese and its a great potential as a functional food.

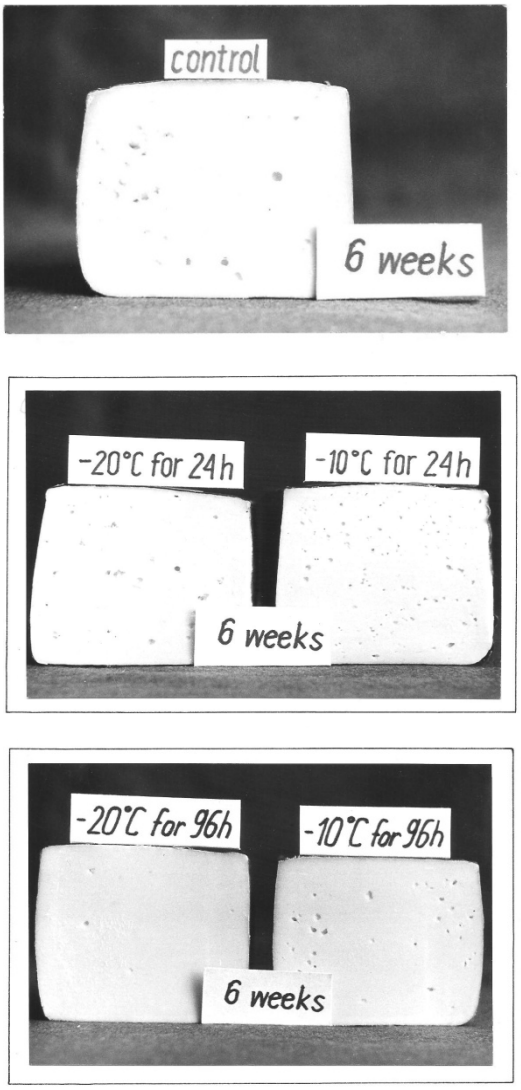


Fig. 3. Texture of 6 weeks old Gouda cheese made with modified mesophilic bacteria and probiotic culture.

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