Production of New Synbiotic Yoghurt with Local Probiotic Isolate and Oat and Study its Effect on Mice

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Abstract

Food companies put many expectations in food products made to meet the consumers' demand for a healthy life. In this meaning, 'Functional Foods' play special roles for primary health outcomes have been microbiological in normal limits. Oats is becoming more popular as part of a healthy diet; Oat attends to the human's energy and nutritional demands, as well as functional. This study aimed to: 1) develop probiotic yoghurt containing oat; 2) study the effect of selected prebiotic addition on rheological properties and syneresis of bio-yoghurt during refrigerated storage; 3) determine the effect of this symbiotic feeding on mice. Our results revealed that the formulae of yoghurt with 0.75% oat had presented excellent sensory evaluation, had satisfactory acceptance by the consumers and the cholesterol and bile acid levels in the serum of mice fed with yoghurt fermented by Lb. acidophilus P106 with 0.75% oats decreased significantly, while the cholesterol and bile acid content increased in mice feces. These effects may be due in part to the deconjugation of bile salts by strains of bacteria that produce the enzyme bile salt hydrolase (BSH).

Keywords: Functional food; Synbiotic; Prebiotic; Oats; Bio-yoghurt

Introduction

Yoghurts are prepared by fermentation of milk with bacterial cultures consisting of a mixture of Streptococcus subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus.

Ever since yoghurt was proposed as a health promoting food by Metchnikoff [1] in 1907, the oldest and still most widely used way to increase the numbers of advantageous bacteria in the intestinal tract has been the direct consumption of live bacteria. Such bacteria are called probiotics [2,3].

Most probiotics are bacteria similar to those naturally found in people’s guts, especially in breastfed infants who have natural protection against many diseases. The normal human digestive tract contains about 400 types of probiotic bacteria that reduce the growth of harmful bacteria and promote a healthy digestive system. The largest group of probiotic bacteria in the intestine is lactic acid bacteria, of which Lactobacillus. Probiotics are found to exert other health advantages such as improving lactose intolerance, increasing humoral immune responses, biotransformation of isoflavone phytoestrogen to improve post-menopausal symptoms, bioconversion of bioactive peptides for antihypertension, and reducing serum cholesterol level [4].

The term prebiotic has been used to describe a dietary component that stimulates the growth and/or activity of a selected group of microbes, thereby providing these organisms with a competitive advantage over other bacteria in the ecosystem [5]. Combination of both probiotics and prebiotics is known as Synbiotics. This combination can improve the survival of probiotic organism. Consuming a probiotic supplement that also includes the appropriate prebiotic has many beneficial effects. The combination of both probiotic and prebiotic has the ability to heal and regulate the intestinal flora, particularly after the destruction of microorganisms following antibiotic, chemotherapy, or radiation therapies. Without the beneficial organism digestion, absorption, and manufacture of nutrients cannot take place.

Oats are the seeds of the plant Avena sativa, a cereal grain that has been commonly consumed as whole grains and known to provide healthy nutrients to humans. Oats have been grown for thousands of years, mainly as an animal feed crop, but during the 19th century oats won acceptance as part of the human diet [6]. At that time oats were generally consumed as a foodstuff for both humans and livestock for millennia. The attenuation of postprandial plasma glucose and insulin levels and the control of cholesterol can be ascribed to the partly soluble and viscous dietary fibers found in oats [7]. As a dermatological agent colloidal extracts of oats have been various bioactive compounds, for example antioxidants such as vitamin E, anti-inflammatory, moisturizing, cleansing and even ultraviolet protective properties, phytic acid and phenolic compounds [8,9]. It has been shown that some phenolic compounds found in oats may have potential health beneficial properties [8-10].

Nutritionally oats are an excellent source of soluble fiber in the form of beta-glucans, alpha tocopherols, B vitamins, minerals, proteins, and plant fats. As a grain without gluten, oat flour and bran are used as an alternative food for persons suffering celiac disease [11-13]. The beneficial nutritional properties of oats have attracted attention from researchers and have resulted in the food industry wishing to use oats as a food ingredient more extensively than today and therefore more studies are needed in this area. The aims of this study were to develop a symbiotic yoghurt, combining the health benefits of a probiotic culture with the oat prebiotic beta-glucan.
through fermentation of oat substrate with lactic acid bacteria with research the influence of oats concentrations as prebiotic on the growth and viability of bacteria for bio-yoghurt production for development of a product enriched with oats, and settles the most beneficial probiotic-prebiotic combination, which fulfils the therapeutic requirement of presence 10^6 cfu/ml (g) for all storage period of the synbiotic products. Rheological properties of bio-yoghurt were also investigated. Finally the effect of selected oat concentration addition in yoghurt was studied on mice's health considering the fact that consumers today value health-promoting foods highly, as well as the convenience aspects.

Materials and Methods

Starter culture

A probiotic strain Lactobacillus acidophilus P106 was identify by Mahrous [14], was used in this study. The strain was isolated from breast-feeding infant (15 days old) and selected as probiotic in two repetitions. Plates were incubated anaerobically (GasPak System – Merck, Darmstadt, Germany) at 4-6°C. The commercial lyophilized starter culture (Goat Nutrition Ltd, Units B & C, Smarden Business Estate, Monks Hill, Smarden, Ashford, Kent, TN27 8QL, England.) containing a mix of Streptococcus salivarius ssp. thermophilus (ST) and Lactobacillus acidophilus (LB) strains was prepared for direct inoculation of milk for yoghurt production.

Oat substrate

Oats (Al-Nahrain CO. for food products, Egypt) ground into fine oat flour were used as a substrate in different concentrations 0.3, 0.5, 0.75 and 1.0% and mixed with tap water. The slurry was then used in the manufacturer of yoghurt.

Bio-yoghurt production

Tested probiotic strain was incubated in BBL anaerobic jar (Becton Dickinson Microbiology Systems, Sparks, MD) provided with disposable BBL gas generating pack (CO_2 system envelopes, Oxoid, Ltd., West Heidelberg, Victoria, Canada) for 48 h at 37°C, and then concentrated by centrifugation. The cell pellets were resuspended in 10% skim milk (SMP) at a concentration of 10^8-10^10 CFU mL\(^{-1}\), the number of viable cells (colony forming units per mL, CFU mL\(^{-1}\)) and used in the manufacturing of yoghurt with 0.0, 0.3, 0.5 0.75 and 1.0% (w/w) oats, according to Shah, 2000. After incubation yoghurts were stored in +4°C for three weeks. Every week each kind of fermented yoghurt was examined in order to check the growth of lactic acid bacteria for 21 days.

Microbiological analysis

Serial decimal dilutions in sterile peptone water (0.1%) were prepared from every yoghurt kind (1 g sample). Then 1 ml of aliquots was plated over selected culture media (BA-sorbitol agar) for Lactobacillus acidophilus P106. The method of count plate was used in two repetitions. Plates were incubated anaerobically (GasPak System – Oxoid) in 37°C for 48 h.

Determination of pH

The pH of yoghurt was measured using a digital pH-meter (model CP-401, Elmetron, Zabrze, Poland).

Rheological measurements

Apparent viscosity of the yoghurts was measured at 20°C using a texture analyzer (TA1000, Lab Pro (FTC TMS-Pro), USA). A two-bite penetration test was performed using the Texture Analyzer with the TA 17 probe (30 and 25 mm diameter) and operated at a crosshead speed of 1 mm s\(^{-1}\) and penetration distance of 10 mm. Hardness, cohesiveness, springiness, gumminess and chewiness were evaluated as previously in double described by Szczesniak [17,18].

Consumer panel

Ten volunteers participated on panel evaluated appearance, mouthfeel, flavor, and overall quality of yoghurt groups on a nine-point hedonic scale (1= dislike extremely to 9= like extremely). Panelists were served five samples at a time and asked to rinse their mouths between samples.

Animals and Conditions

Forty male ICR (CD-I) mice, approximately 4 week-old with the average initial body weight of (25.25 ± 4.50) g were obtained from Faculty of Science, Department of Zoology, Alexandria University, Alexandria, Egypt. All mice were examined for health status and acclimated to laboratory environment for 2 weeks prior to use. Temperature was maintained at 23 ± 2°C, and relative humidity at approximately 50%, with a 12 h: 12 h light: dark photoperiod. Animals were housed in stainless-steel cages and given standard diet and water ad libitum throughout the study.

Probiotic feeding

Mice were randomly assigned to treatment groups according to an approximately equal mean body weight to 4 treatment groups of 10 each. The treatments were: 1) group A, were fed by the normal yoghurt (as control); 2) group B (were fed by normal yoghurt plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal); 3) group C (were fed with combination with yoghurt starter and Lb. acidophilus P106 plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal) and 4) group D (were fed with combination with yoghurt starter and Lb. acidophilus P106 with 0.75% (w/w) oat plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal). The experiment was carried out for four weeks (5 days week-1, 20 days) by oral gavages; dose level 10^8-10^10 CFU mL\(^{-1}\). The administered volume of each dose was 1.0 mL kg\(^{-1}\) day\(^{-1}\), adjusted daily for recorded body weight changes during the treatment period. At the end of experiment, the mice were fasted for 12 hours before blood collection.

Animal observations and sampling

Health status of treated mice was monitored daily throughout the experimental period. The number of animals with diarrhea was recorded daily. Feces of each group of mice were collected daily on each of the last 5 days of the experimental period and lyophilized. After dosing (20 days), mice were anesthetized by using diethyl ether. Mice blood was obtained by cardiac puncture via aspiration through polyethylene tubing attached to a heparinized micro hemocrit capillary tube which had been flamed and pulled to a fine point.

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Fecal bile acid and cholesterol

1.0 g of crushed lyophilized feces were suspended in chloroform-methanol 1:1(v/v), sonicated for 5 min, and then extracted at 60°C for 60 h. The extract was evaporated and dissolved in methanol for measurement of total bile acids (TBA) with a commercial kit [19]. The total cholesterol of feces was determined as described by Rudel and Morris [20].

Serum cholesterol and triglyceride assays

Triglycerides and cholesterol were determined in blood serum of each specimen. Biochemical determinations were made using an automatic chemistry analyzer Dimension Max (DADE BEHRING) following the manufacturer’s prescriptions and using the reagents routinely applied in that type of apparatus.

Statistical Analysis

Data are presented as the mean ± standard deviation, and n represents the number of replicates from the different groups and the control.

Results and Discussion

The changes of pH and bacterial counts of yoghurts

Most probiotic foods at the markets worldwide are milk-based, and very few attempts are made for development of probiotic foods with the use of other fermentation substrates such as cereals. Human consumption of oats has been stimulated by an increasing need for high-soluble fiber in the daily diet of consumers [21]. Epidemiological evidence indicates that a higher intake of oats is associated with a reduced risk of coronary heart disease, the major cause of death in Western countries. It may also reduce serum cholesterol and glucose uptake, decreasing the insulin response in the blood and controlling weight through prolonged satiety and balanced gastrointestinal function [22].

The present work was to develop synbiotic yoghurt, combining the health benefits of a Lb. acidophilus P106 which has been demonstrated to assimilate cholesterol in vitro and in vivo [23], with the oat prebiotic. The pH values of yoghurt samples during refrigerated storage are shown in Table 1 for all samples analyzed the pH values decreased throughout the storage period. Similar tendencies for pH values were observed for commercial yoghurts containing probiotics during their storage [24]. The pH values for all yoghurt types ranged from 4.65 to 4.15 during the storage. Average pH value of yoghurt obtained with probiotic strain and 0.7% oat was found to be lower than the control and other samples addition exhibited the lowest pH values throughout the storage period. Akalin [25,26] also reported that pH values of yoghurt containing fructooligosaccharides was found to be lower than yoghurt without supplementation during refrigerated storage for up to 28 days.

The counts of probiotic strain increased during the storage period up to 14 days of storage after that it decreased. The results in Table 2 indicated that cell count changes at oat flour concentrations 0.3% and 0.5% were not significant, while, 0.7% was set as preferred in order to achieve higher b-glucan content in the yoghurt. However, the addition of 1% of oats did not show a clear change in the count.

Table 1: The changes in pH of yoghurts obtained with addition of 0.3, 0.5, 0.7 and 1.0% oats as prebiotic and Lb. acidophilus P106 as a probiotic during storage; Data are presented as mean ± SD

<table>
<thead>
<tr>
<th>pH</th>
<th>Storage days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 7 14 21</td>
</tr>
<tr>
<td>Yoghurt with 0.3% oats</td>
<td>4.65 ± 0.1</td>
</tr>
<tr>
<td>Yoghurt with 0.5% oats</td>
<td>4.64 ± 0.01</td>
</tr>
<tr>
<td>Yoghurt with 0.7% oats</td>
<td>4.55 ± 0.01</td>
</tr>
<tr>
<td>Yoghurt with 1.0% oats</td>
<td>4.50 ± 0.1</td>
</tr>
</tbody>
</table>


The oats addition to yoghurt caused an increase in the numbers of all bacteria in comparison to control yoghurt obtained without addition of probiotics. The initial viable cell concentrations in the five product variants were within 1.5x10^6-3.5x10^7 cfu/ml at the end of the fermentation process. The shelf life of the synbiotic oat yoghurt was estimated to 21 days under refrigerated storage. During this period, good viability of the probiotic culture was observed. The oats addition to yoghurt up to 0.7 caused an increase in the numbers of probiotic culture in comparison to control yoghurt. Oat at the concentration of 0.7% was useful for Lb. acidophilus P106 because the amount of these bacteria was stable for entire storage time. Vinderola et al. [27] observed that initial counts of probiotic bacteria ranged from 10^9 to 10^7 cfu/ml, while the final counts were lower than 10^4 cfu/ml. Bolin et al. [28] analyzed the viability of L. acidophilus strains in milk cultures in refrigeration conditions (7°C) and showed that the number of bacteria significantly decreased during storage time (35 days). The mean difference between initial count (7.97 log cfu/ml) and end count (6.21 log cfu/ml) was 1.76 log cfu/ml [28]. Dave and Shah [29] studied/investigated the viability of bacteria from commercial starter cultures during yoghurt manufacture and storage. The count of Lb. acidophilus was in the range of 1.8-3.8x10^7 cfu/g after incubation but decreased during storage and recommended level of 106 cells was maintained only for 20-25 days.

Rheological measurements and sensory analysis

Hardness; springiness; cohesiveness and rheological properties of yoghurt obtained with addition of 1% oat increased during 21 days of refrigerated storage in comparison to control yoghurt. It could be assumed that oats is the part of the structural network being formed during fermentation and structuring of yoghurt. In other concentrations of oats, no improvement in terms of rheological properties was observed for yoghurts (Table 3). Hardness and adhesiveness of yoghurts with the 1% oat addition increased during refrigerated storage for 21 days than other groups while yoghurt with 0.7% was set as preferred in order to achieve higher b-glucan content in the yoghurt. However, the addition of 1% of oats did not show a clear change in the count.
They observed that the apparent viscosity of examined yoghurts decreased during storage time. As reported by Tamime and Robinson [33], higher contents of solids in the yoghurt promote greater viscosity of final products.

The consumer panels did not detect significant differences in the appearance, mouth feel, flavor, or overall quality among yoghurts with all concentrations of oats as compared to the control yoghurt. Table 4 showed all yoghurts were above average on the scale and were liked by the panelists. The mean color; mouth feel; maintenance of shape; flavor; acidity; appearance and overall scores for yoghurt with 0.7% oats were 8.3, 8.5, 8.6, 8.5, 8.6, 8.7 and 8.8, respectively which were the highest scores in comparison to other groups and control. The overall acceptance of the control sample and sample with 0.3, 0.5 up to 0.7% oat was significantly higher than the sample with 1.0% oat. In general, increasing the percentage of blending oat flour, the sensory quality scores were not different from control until 0.7% oat, while adding 1% oat reduced the scores.

Animal observations

Probiotics are claimed to have beneficial effects on health. However, only few well-performed studies have looked at clearly defined health effects such as serum cholesterol concentrations. In this study we have shown the ability of probiotic strain *Lb. acidophilus* P106 with and without oat to assimilate serum cholesterol level and their effect on general health of mice. As previously indicated, because addition of 1% oat causes unwanted hardening and slow fermentation, we used 0.7% oats to feed mice.

Adverse Clinical Signs

No serious adverse effects were observed for control and group B, in particularly, weakness compared to the other treated groups. These signs appeared in group B on day 8 of treatment and particularly progressed throughout the treatment period in the same animals and disappeared in others treated groups. These results are in agreement with those reported previously [34]. Also, it has been reported previously [35] that probiotics have beneficial effects on the health of the host. Kikuchi and Yajima [36] showed that probiotic microorganisms’ strains are useful in the treatment of disturbed intestinal micro flora and diarrheal diseases.
Table 3: Apparent viscosity, instrumental texture parameters of yoghurts produced with addition of different prebiotics; Data are presented as mean ± SD

<table>
<thead>
<tr>
<th>Parameters/Treatment</th>
<th>Color</th>
<th>Mouth feel</th>
<th>Maintenance of shape</th>
<th>Flavor</th>
<th>Acidity</th>
<th>Appearance</th>
<th>Overall acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.9 ± 0.01</td>
<td>8.1 ± 0.10</td>
<td>8.3 ± 0.03</td>
<td>8.1 ± 0.03</td>
<td>8.2 ± 0.02</td>
<td>8.3 ± 0.02</td>
<td>8.4 ± 0.02</td>
</tr>
<tr>
<td>Yoghurt + 0.3% oat</td>
<td>8.0 ± 0.02</td>
<td>8.3 ± 0.01</td>
<td>8.4 ± 0.04</td>
<td>8.2 ± 0.02</td>
<td>8.3 ± 0.10</td>
<td>8.5 ± 0.02</td>
<td>8.5 ± 0.02</td>
</tr>
<tr>
<td>Yoghurt + 0.5% oat</td>
<td>8.1 ± 0.01</td>
<td>8.4 ± 0.03</td>
<td>8.5 ± 0.02</td>
<td>8.4 ± 0.04</td>
<td>8.5 ± 0.04</td>
<td>8.6 ± 0.02</td>
<td>8.6 ± 0.02</td>
</tr>
<tr>
<td>Yoghurt + 0.7% oat</td>
<td>8.3 ± 0.10</td>
<td>8.5 ± 0.02</td>
<td>8.6 ± 0.01</td>
<td>8.5 ± 0.03</td>
<td>8.6 ± 0.02</td>
<td>8.7 ± 0.02</td>
<td>8.8 ± 0.02</td>
</tr>
<tr>
<td>Yoghurt + 1% oat</td>
<td>8.2 ± 0.10</td>
<td>8.3 ± 0.02</td>
<td>8.7 ± 0.01</td>
<td>8.3 ± 0.03</td>
<td>8.5 ± 0.02</td>
<td>8.4 ± 0.02</td>
<td>8.1 ± 0.02</td>
</tr>
</tbody>
</table>

Table 4: Mean values for sensory evaluation parameters of yoghurts stored at 10°C; Mean values (Mean values from 10 panelists)

Bile acid and cholesterol concentration in feces
Lactic acid bacteria are normal components of the intestinal microflora in both humans and animals and have been associated with various health-promoting properties. One beneficial effect is a reduction in serum cholesterol levels. The effects of assigned diets on feces bile acid and cholesterol content of mice are illustrated in Table 5. The bile acid and cholesterol content of feces in mice given high-cholesterol diets (groups B, C and D) were higher than those in control mice (group A).

Table 5: Content of total bile acids (TBA µMOL/g) and content of total cholesterol (TC mg/g) of feeding mice with normal yoghurt and fermented milk with probiotic microorganisms and starter yoghurt; Data are presented as mean ± SD

<table>
<thead>
<tr>
<th>Mice groups</th>
<th>Content of TBA µmol/g</th>
<th>Content of TC mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.68 ± 0.1</td>
<td>11 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td>17.5 ± 0.2</td>
<td>38.2 ± 0.1</td>
</tr>
<tr>
<td>C</td>
<td>18.5 ± 0.1</td>
<td>46.8 ± 0.2</td>
</tr>
<tr>
<td>D</td>
<td>20.5 ± 0.1</td>
<td>49.9 ± 0.1</td>
</tr>
</tbody>
</table>

All probiotic strains were added at (10^8-10^10 CFU mL^-1). Group A, were fed by the normal yoghurt (as control); group B (were fed by normal yoghurt plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal); group C (were fed with combination with yoghurt starter and Lb. acidophilus P106 plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal) and group D (were fed with combination with yoghurt starter and Lb. acidophilus P106 with 0.7% (w/w) oat plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal).

Serum cholesterol level
The cholesterol levels were between 1.5–2.2 mmol/l and for the triglycerides 0.9–2.5 mmol/l. There was a lower levels of cholesterol and triglycerides in group D comparing with other groups (Table 6) that showed the prebiotic and probiotic effects. Serum cholesterol increased in the group B compared to the other treated groups especially groups C and D. The use of probiotic bacteria in reducing serum cholesterol levels has attracted much attention. However, the mechanism of this effect could not been explained definitely. There are two hypotheses trying to explain that mechanism. One of them is that bacteria may bind or incorporate cholesterol directly into the cell membrane. The other one is, bile salt hydrolysis enzymes deconjugate the bile salts which are more likely to be exerted resulting in increased cholesterol breakdown [40]. Various studies have shown that some lactobacilli could lower total cholesterol and low-density lipoprotein (LDL) cholesterol. High level of serum cholesterol has been associated with risks of coronary heart disease [41-44].
group C (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal) and group D (were fed with combination with yoghurt starter and *Lb. acidophilus* P106 with 0.7%(w/w) oat plus 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal)

<table>
<thead>
<tr>
<th>Mice groups</th>
<th>Cholesterol mmolL⁻¹</th>
<th>triglycerides mmolL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.5 ± 2.10</td>
<td>0.9 ± 1.10</td>
</tr>
<tr>
<td>B</td>
<td>2.2 ± 1.20</td>
<td>2.5 ± 2.10</td>
</tr>
<tr>
<td>C</td>
<td>1.9 ± 2.56</td>
<td>1.5 ± 3.10</td>
</tr>
<tr>
<td>D</td>
<td>1.6 ± 3.20</td>
<td>1.0 ± 2.10</td>
</tr>
</tbody>
</table>

Table 6: Cholesterol and triglycerides serum levels in mice after feeding with yoghurt and probiotic microorganisms; Data are presented as mean ± SD

Conclusion

Continuous development of new functional foods is the response of science and industry to the increased consumer awareness regarding health and the role of foods for improving quality of life. Oats is major sources of beta-glucan, recognized as the main functional component of cereal fibers. Numerous scientific studies proved the hypocholesterolaemic effect of this compound, and to an overall effect of reduced cardiovascular disease risk. In addition, beta-glucan is known as probiotic, stimulating the growth of some beneficial residential colon microorganisms.

The results obtained in this study showed that the addition of oat in different concentrations did not compromise the sensory characteristics of the product or its acceptance by the consumers. The cholesterol and bile acid levels in the serum of mice fed with yoghurt fermented by *Lb. acidophilus* P106 decreased significantly especial when oat add with 0.7% concentration, while the cholesterol and bile acid content increased in mice faeces. These effects may be due in part to the deconjugation of bile salts by strains of bacteria that produce the enzyme bile salt hydrolase (BSH). This would ensure *Lb. acidophilus* P106 beneficial health effect at regular consumption and enhance the organoleptic properties of plain yoghurt to improve consumer acceptability and to identify methods for improving the nutrient profile and possible therapeutic effectiveness of plain probiotic yoghurt.

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