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A study on comparative analysis of gumminess attribute of Mozzarella cheese produced and developed by adjunct strain *Lactobacillus kefiranofaciens* ZW3

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Abstract

A novel exopolysaccharide producing strain *Lactobacillus kefiranofaciens* ZW3, isolated from kefir grain Tibet, was used for the first time as an adjunct strain to compare the gumminess attribute of Mozzarella cheese with market available starter cultures and acetic acid made cheese. The results demonstrated that gumminess was more in ZW3 made cheese compared to A.C, L.C and M.C made fresh Mozzarella cheese. Similarly, ripening of cheese at 4℃ also had a positive impact on gumminess of cheese. The gumminess in ZW3 made Mozzarella cheese was increased more during ripening for a period of 4 weeks. The fermentation time taken by *Lactobacillus kefiranofaciens* ZW3 was also calculated for the first time during cheese preparation and it took 16 h to reach pH 5.4.

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Introduction

Cheese making technology is critical and in many countries, it is a staple food in the human diet. Therefore, it is considered an ideal food due to its high nutrition, convenience, variety, availability, and good taste (Bogue *et al*., 1999). With its widespread production and utilization, the consumers' palate has become an important factor. Today, producing high quality cheeses, meeting all the consumer's expectations, is crucial in keeping the competition among cheese makers. One such consumer expectation is that the selected cheese must possess an excellent texture.

Texture and other sensorial attributes of cheese are important quality parameters of this food product and may be susceptible to undesirable changes due to production conditions (Santos *et al*., 2013). Texture is therefore an important quality attribute to meet the consumer's expectations. One of textural attributes is gumminess that imparts gum like characteristics to the final product especially Mozzarella cheese which is used more frequently in fast food industry.

Lactic acid bacteria (LAB) are Gram-positive microorganisms that not only produce lactic acid as a major end product of their metabolism but also produce rheologically important compounds like peptides and exopolys accharides. Similarly, the starter cultures also adopt specific fermentative and proteolytic activity that may have an impact on the functionality of Mozzarella cheese. For example *Lactobacillus helveticus* has been reported to give better functional properties, i.e. increase in melt, when used as starter culture with *Streptococcus thermophilus* (Oberg, 1991).

Some lactic acid bacteria (LAB) are able to synthesize extracellular polysaccharides also called exopolysaccharides (EPS). They are long-chain and high molecular weight polymers able to dissolve or disperse in water and enhance fermented product texture and viscosity (Welman and Maddox, 2003).The EPS polymers can be considered as natural biothickeners because they are produced in situ by the LAB-starters that have General Recognized As Safe (GRAS) status (Banik *et al*., 2000; Duboc and Mollet, 2001).

Our lab has isolated a novel strain of *Lactobacillus kefiranofaciens* ZW3 from kefir, a traditional dairy product that is known to provide many health benefits to humans, including antibacterial, immunologic and antitumor effects (Lin and Change, 2000).The complete genome sequences of *Lactobacillus kefiranofaciens* ZW3, representing the first genome of *Lactobacillus kefiranofaciens* ZW3 has already been reported (Wang *et al*., 2011).

The present study was designed to use a novel ropy EPS producing strain *Lactobacillus kefiranofaciens* ZW3 in Mozzarella cheese preparation in order to analyze the gumminess attribute which is one of the main quality parameters of this cheese especially when used in fast food industry. The main objective of this study was to enhance the quality parameter by making a comparative analysis of gumminess attributes of Mozzarella cheese produced by ZW3 with the Mozzarella cheese made by industrial used stains of *Lactobacillus burglarious* and *Streptococcus thermopiles* and direct acidified cheese by using acetic acid. The gumminess attributes of Mozzarella cheese were also studied during cheese ripening period of 4 weeks in order to analyze the impact of this novel strain ZW3 on this textural attribute. As this strain was used for the first time in cheese making process therefore the fermentation time of this strain was also determined in this study.

Materials and methods

Reagents

Milk coagulant enzyme rennet was obtained by Zhengzhou Wanjiu Chemical products Co. Ltd, Henan Province, China. Fresh cow milk was obtained from local dairy farm (Tianjin Tanggu dairy farm). Mozzarella cheese was prepared in stainless steel cheese maker purchased from Guangzhou WELLMAX Industry, China. Textural analyzer TA-XT-Plus was provided by the central Lab, Tianjin University of Science and Technology.

Strains

Mozzarella cheese was prepared by using different strains. A ropy and gum like nature of exopolysaccharide producing novel strain Lactobacillus *kefiranofaciens* ZW3 isolated from Tibet kefir grain, by our lab research group (Wang *et al*., 2008), was used as an adjunct strain along with *Lactobacillus delbruecki* ssp. *bulgaricus* CGMCC1. 2470 and *Streptococcus thermophilus* CICC6058, obtained from Chinese Academy of Sciences Institute of Microbiology, Beijing and stored in our lab at -80˚C.

Industrial grade starter culture MA14, *Lactococcuslactis* ssp. *cremoris* and *Lactococcuslactis* ssp. *lactis* was provided by Danisco company Beijing China, used as a market control (M.C) to prepare the mozzarella cheese. *Lactobacillus delbrueckii* ssp. *bulgaricus*CGMCC1.2470 and *Streptococcus thermopiles* CICC6058 were also used as a lab control (L.C) for mozzarella cheese manufacture. The strains were activated in 12% skim milk. The milk was first sterilized at 114˚C for 20min followed by inoculation of 200µL lab strains in 5mL of milk for 16h. 200µL of this first generation was further inoculated in 5mL of sterilized skim milk for 8h to get second generation. 1mL of second generation was further propagated in 100mL of sterilized skim milk for optimum duration of fermentation time to get the activated strains.

Mozzarella cheese preparation

Mozzarella cheese was prepared as described before (Scott, 1981) with little modifications. Fresh cow milk was procured from local dairy farm. The milk was standardized to 3% fat, pasteurized to 72˚C for 15 sec, followed by cooling to 30˚C. 2% adjunct strain of *Lactobacillus kefiranofaciens* ZW3 along with *Lactobacillus delbrueckii* ssp. *bulgaricus* CGMCC 1.2470 and *Streptococcus thermopiles* CICC6058, were inoculated to 1 L milk. The L.C batch used 1 L milk inoculated by 2% of *Lactobacillus delbrueckii* ssp. *bulgaricus* CGMCC1.2470 and *Streptococcus thermophiles* CICC6058 while M.C batch used 0.016g (as per recommendations of the company) of industrial grade starter culture MA 14, constituting *Lactococcuslactis* ssp. *cremoris* and *Lactococcuslactis* ssp. *Lactis*, in 1 L milk to prepare mozzarella cheese. After inoculation, the milk was stirred and incubated at 30˚C for an optimum fermentation time to reduce the pH level to 5.4. The direct acidification method implied the usage of 10% acetic acid solution to lower down the milk pH to 5.4. The rennet was added at concentration of 0.005-0.007g/L of milk and incubated at 30˚C.

The cutting of coagulated milk was done with the help of cutter into small cubes. The curd was kept at 30˚C for 20 to 25min to permit the whey separation, followed by cooking at 45˚C for 45min. The whey was drained, milled at pH 5.2 and the curd was salted (1.5% w/w) and held for 30min. The cheese was stretched in hot water (60˚C) at pH 5.1, shaped into round balls and the cheese was stored at 4˚C.

Texture study

Texture analyzer TA-XT-Plus was used to measure the textural characteristics of gumminess of Mozzarella cheese as determined previously Zisu and Shah, 2007). 2.00±0.06cm of mozzarella cheese cube with 10˚C sample temperature was placed vertically at the compression disk of the textural analyzer TA-XT-Plus at room temperature 19˚C.

The cylindrical probe of TA-XT-Plus continuously pressed the cheese samples for two times and the system (software) record the interacting force between the sample and the cylindrical probe. The TPA configuration parameters were as follows: pre-test, test and post test speed of 2 mm/sec, compression by distance of 16mm (a 40% compression was selected for TPA analysis in order to allow deformation to occur without breaking of the sample), a trigger system using force of 5g, and a waiting time between cycles of 5 seconds.

Statistical data

4 different batches of mozzarella cheese were prepared by using 4 different parameters. The parameters included the usage of experimental adjunct strain. In this study total 4 parameters were used with 3 repetitions.

The data for gumminess of the Mozzarella cheese were statistically analyzed using mean and standard deviation and the mean comparisons of parameters were performed by t-test, with the level of significance at 0.05.

Results and discussions

The gumminess attribute of Mozzarella cheese made by ZW3, L.C, M.C and acetic acid is depicted in Fig. 1. A significant difference (P>0.05) in gumminess was found between ZW3 and L.C, M.C and acetic acid produced mozzarella cheese. The Fig. 1 illustrates that gumminess of fresh Mozzarella cheese was improved by using the ZW 3 and it was 3.62 as compared to 2.34 in L.C, 2.23 in M.C while 0.73 in acetic acid produced Mozzarella cheese, had the lowest impact on gumminess.

LAB producing EPS have gained considerable attention in the fermented dairy industry because of their potential application as viscosities, texturizers, and emulsifying agents (Grobben *et al.*, 1996). One such EPS producing strain *Lactobaccilus kefiran* of aciens ZW3.

This strain is specific for its highly viscous and ropy formations of colonies which is evident by its EPS production. Previously the gas chromatography (GC) analysis of ZW3 EPS revealed that it was glucogalactan in nature; exopolymer showed similar flocculation stability like xanthan gum but better than guar gum (Wang *et al.,* 2008).

These specific characteristics of ropy formation and gum like nature of ZW3 EPS might be involved in improving the gumminess of Mozzarella cheese. This might also be due to the probable proteolytic pattern of the ZW 3, producing specific polypeptides and peptides responsible for the specific characteristics of gumminess. Previously it was found that gumminess.

Attribute was improved when using EPS-producing culture, whereas a great improvement was also recorded for the sensory properties including a much better flavor (El-Baz *et al.*, 2011). At same time the fat contents (data not shown) of the mozzarella cheese, produced by ZW 3 were maximum, which might also be the reason of increased gumminess.

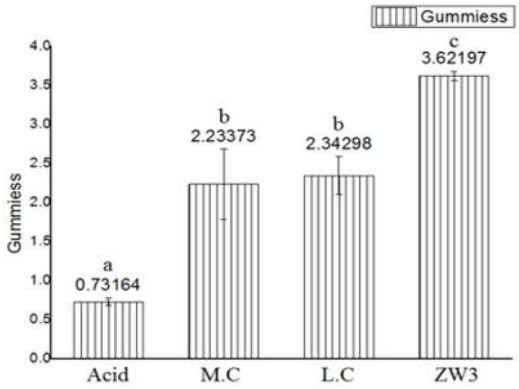


Fig. 1. A comparative analysis of gumminess (shown on y-axis) of fresh Mozzarella cheese prepared by ZW 3, M.C, L.C and Acetic acid (shown on x-axis). The values were measured by textural analyzer TA-XT-Plus. The data for gumminess was recorded by the system software. Results are expressed as mean ± standard error of means, n = 3 sets of data analyzed. Means between the treatments (ZW3, L.C. M.C and Acid) with like letters do not differ (P>0.05).

Similarly, ZW 3 also had a prominent impact on gumminess of Mozzarella cheese during ripening period of 4 weeks at 4°C as shown by the Fig. 2. There was a significant difference (P>0.05) found in the gumminess attribute between ZW3 and L.C, M.C and acetic acid produced cheese during ripening. A decreasing trend was found in gumminess of Mozzarella cheese during the process of ripening of 4 weeks however the gumminess was more in ZW3 made cheese as compared to market available cultures and acetic acid made cheese. These results suggest that the specific proteolytic pattern of ZW 3 during ripening might have also contributed in increased gumminess attributes of Mozzarella cheese.

The proteinases and peptidases that catalyze proteolysis in cheese during ripening originate from six primary sources, namely, the coagulant, the milk, starter LAB, NSLAB, secondary starters. The proteinases and peptidases of LAB have been the subject of active study over the past two decades and the extensive literature on this topic has been reviewed frequently (Christensen *et al.*, 1999; Grappin *et al.*,1985; Rank *et al.*, 1985; Fox 1989; Sousa *et al.*, 2001).

The polypeptides and peptides produced during proteolysis impart better functional properties and improve the textural parameters of the final product (Upadhyay *et al*., 2004). In this study the gumminess increased significantly compared to L.C, M.C and acetic acid made Mozzarella cheese during ripening period of 4 weeks indicating that ZW3 is unique in its proteolytic pattern. These results are in line with the findings of Jung *et al*., (2013) and Irudayaraj *et al.,* (1999) who found that the textural properties including gumminess increased significantly in cheese during the process of ripening.

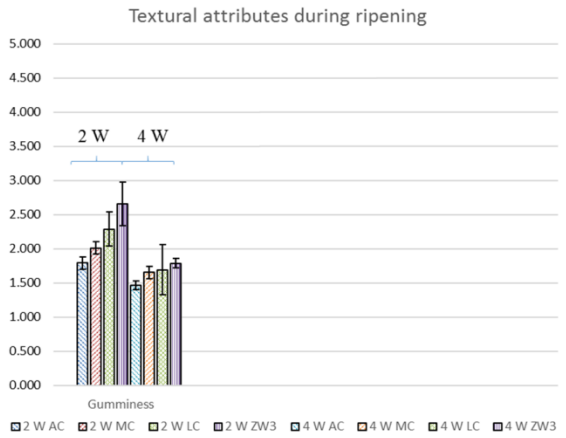
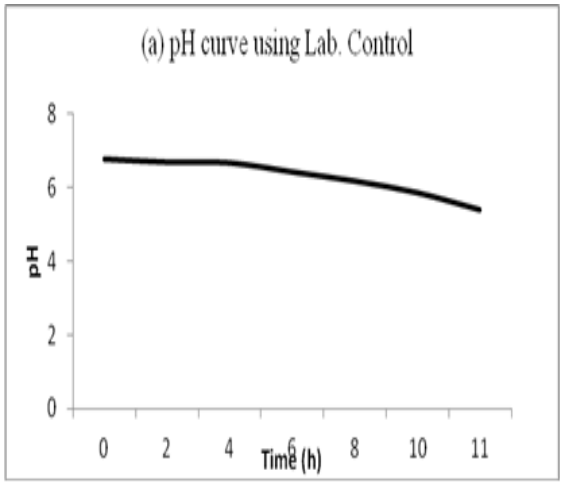


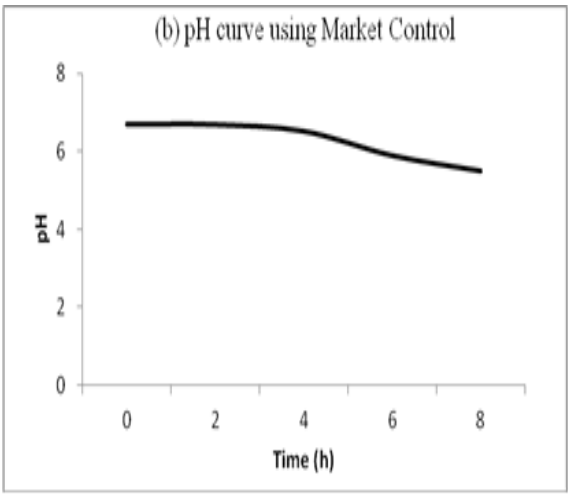
Fig. 2.A comparative analysis of gumminess of Mozzarella cheese prepared by ZW 3, M.C, L.C and AC (Acetic acid) during storage at 4˚C. 2 W indicates 2 weeks while 4 W indicates 4 weeks. Results are expressed as mean ± standard error of means, n = 3 sets of data analyzed.

The EPS production during ripening may also have contributed in increased gumminess of ZW 3 made cheese compared to controls. Diana *et al*., (2014) compared scanning electron microscopy micrographs of the different sections of the cheese and found that concentration of exopolysaccharide in the center was higher than in the outer sections, indicating that exopolysaccharide production continued during ripening and that the environment at the center of the cheese (moisture and/or oxygen concentration) favoure dexopolysaccharide production. They also found that these EPS had a significant impact on textural attributes during ripening. Similar findings were reported by Broadbent *et al*., (2001) and Low *et al*., (1998) who used EPS producing strains to improve the Mozzarella cheese texture.

In this study the fermentation time taken by *L. kefiranofaciens* ZW 3 along with *Lactobacillus delbrueckii* ssp. *bulgaricus* CGMCC1. 2470 and *Streptococcus thermophiles* CICC6058, *Lactobacillus delbrueckii* ssp. *Bulgaricus* CGMCC 1.2470 and *Streptococcus thermophiles* CICC6058 (Lab. control) and *Lactococcuslactis* ssp. *cremoris* and *Lactococcuslactis* ssp. *Lactis* (Market control), used in preparation of mozzarella cheese, to reach the pH level 5.4 is shown in Fig 3. Milk was inoculated by these three different inoculums and was kept at 30˚C and the pH was noted after every 2 hours.

The maximum time was taken by ZW 3 and it was 16 h while Lab control (L.C) and Market control (M.C) took 11 and 7 hours to reach pH 5.4 respectively. The EPS producing LAB has been unique in fermentation time point of view.





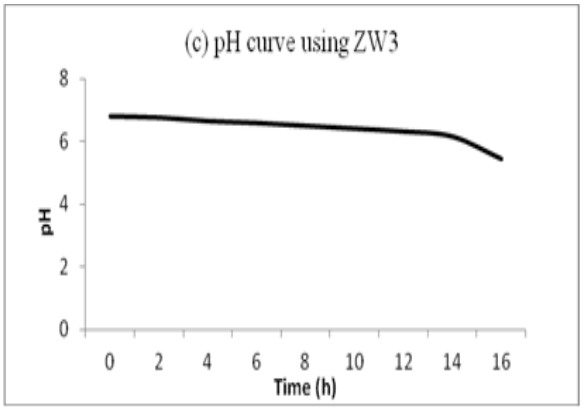


Fig. 3. Fermentation time taken by (a) Lab. control, (b) Market control and (c) ZW3. X-axis represents time (h) while y-axis represents pH.

Fermentation time is one of the critical environmental parameters affecting content, molecular mass, and sugar composition of EPS. EPS is a general term that refers to two types of secreted polysaccharides (Sutherland, 1972).

The first type of EPS is attached to the cell wall as a capsule (capsular polysaccharides or CPS), while the other is produced as loose unattached material (ropy EPS). Both ropy nature and high molecular mass EPS affect the fermentation time. Lin and Chang, (2005) evaluated the effect of fermentation time on EPS production by LAB strain and found that the *L. helveticus* BCRC 14030 took 60h to produce highest EPS yield. Further they investigated that the EPS produced by this strain was ropy in nature and their molecular mass was 26,500 kDa.

The strain used in this study *L. kefiranofaciens* ZW 3 also produced highest EPS yield amounting to 1215mg/l, ropy in nature (Wang *et al.*, 2008) and its molecular weight is 5.5×10^4Da (Zaheer *et al.*, 2013). These physicochemical properties of EPS produced by this strain resulted in high fermentation time. A similar result was reported previously who found that *Lactob accilluskefiran of aciens* K1, isolated from kefir grain, when used in preparation of fermented milk took 18h to reach pH 4.5 (Zisu and Shah, 2005). In another report the fermentation time of three EPS producing strains including *Enterococcus flavescens* DU-10, *Enterococcus faecium* DU-12 *and Lactobacillus amylovorus* DU-21, attaining pH 4.5 in 24, 15 and 18 hours respectively was calculated (Broadbent *et al.*, 2001). The high and low fermentation time depends upon the specific physiological processes of lactic acid fermentation capability of a specific strain to convert lactose into lactic acid.

Conclusion

Lactobacillus kefiranofaciens ZW 3 is a novel strain and is used for the first time in cheese making process. The EPS produced by this bacterium is ropy and gummy in nature. Therefore this gummy characteristic of ZW 3 EPS was explored in Mozzarella cheese which is very famous in fast food industry especially pizza pies for its elastic and gummy nature. In this study the gummy attribute of Mozzarella cheese was enhanced, as compared to industrially used starter culture, not only in fresh but also in ripened cheese indicating the specific proteolytic pattern and EPS physico-chemical properties possessed by this bacterium. Moreover being lactic acid bacteria, fermentation time of ZW 3 was also calculated for the first time in this study.

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Novelty statement

This is the first ever report on gumminess attribute Mozzarella cheese made by a lactic acid bacterium strain *Lactobacillus kefiranofaciens* ZW 3. This would lead to understand teguments characteristics of Mozzarella cheese made with an EPS producing strain. Also this study determined the evaluation of fermentation time taken by *L. kefiranofaciens* ZW 3 for the first time.

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