RESEARCH ARTICLE

The effect of yeast extract supplementation on activity of bulk starter in cheese manufacturing: A laboratory simulation

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ABSTRACT

Starter culture plays a crucial role in deciding ultimate flavor, aroma, texture and moisture content of natural cheese. Milk, the traditionally used bulk starter (BS) media for cheese starter growth, is found not to provide all the nutrients for starter culture growth. This study investigated the effect of yeast extract as a BS media supplement in increasing cheese BS activity. The BS system and cheese vats in a selected commercial cheese factory were simulated on laboratory scale. Conditions used in the BS system and cheese vats such as temperature, pH, neutralising agent, starter strains and inoculum sizes were used equivalent to those that are used in the selected cheese factory. Effect of two commercial yeast extracts (YE-A and YE-F) on activity of Lactococcus lactis sub sp. cremoris strains 49, 831, 1926 [ASCRC] in BS were evaluated. Starter growth during BS fermentation was analysed determining lactic acid (LA) production and pH reduction. Activity of cultures was evaluated by continuous pH monitoring in simulated cheese vats. YE supplementation increased LA production in the BS and reduced fermentation time by 20% compared to traditional BS media. 0.5% YE-A and 0.2% YE-F were chosen as optimum levels for simulated BS. YE supplemented BS cultures were more active compared to no YE added BS cultures, allowing inoculum levels required to inoculate cheese vats to be reduced by 40%.

Keywords: *Bulk starter (BS), cheese vat, fermentation, inoculum, Lactococci strains*

INTRODUCTION

Cheese is a product derived from milk, which is produced in a wide range of flavors, textures and forms by coagulation of the milk protein. There are more than 1000 varieties of cheese throughout the world, depending on the origin of milk, method of acidification, method of processing and ageing. Today cheese manufacturing utilises nearly one third of world's milk production (Farkye, 2004).

During cheese manufacture, starter culture plays an important role by acidification of cheese milk to the desired pH, which facilitates the curdling of milk. The consistent rate of lactic acid production by starter cultures in cheese vat is crucial to obtain the required final aroma, flavor and texture of cheese,

particularly for cheese varieties like cheddar (Powell *et al.,* 2011). Large scale cheese manufacture requires large volumes of suitable starter cultures to inoculate cheese vats. Therefore, most of the large-scale cheese factories in Australia, Italy and USA have bulk starter (BS) systems on-site at the cheese factory. In a such BS system, one or more starter culture strains, which are obtained from starter manufacturer, is grown in a BS vessel under controlled conditions prior to inoculate into cheese vats. The size of the BS vessel varies from 45 – 10,000 L depending on the size of the cheese factory (Limsowtin *et al.,* 1997; Fox and McSweeney, 2004). Modern BS units are generally fully enclosed stainless-steel vessels, equipped with strict Cleaning-In-Place (CIP), temperature control and pH control systems (Bylund, 1995; Powell *et al.,* 2011). pH control of the growth medium is a key factor in BS system, as lactic acid produced during the growth of starter culture lowers the pH of the medium, limiting the bacterial cell numbers obtained. Therefore, pH of the BS medium is controlled by manual or automated addition of alkali such as sodium hydroxide or potassium hydroxide throughout the fermentation period (Hoier *et al.*, 2010; Powell *et al.*, 2011). The purpose of the BS system is to produce a fresh inoculum of sufficient volume, cell number and acid producing activity to inoculate cheese vats, in order to achieve required rate of acid production in the cheese vat (Farkye, 2004; Fox *et al.*, 2017).

The type of media used for the propagation of starter cultures plays an important role in their growth and performance in BS system as well as on the subsequent activity in the cheese vat (Whitehead *et al.,* 1993; Hoier *et al.*, 2010). Principally, starter media should allow the starter species to produce high numbers of cells with high activity (Ibrahim and Daguri, 1996; Powell *et al.*, 2003; Fox and McSweeney, 2004). Fresh or reconstituted, skim milk or full-cream milk has been the most common starter media used in BS fermentations. In Australia, the use of Ultra High Temperature (UHT) treated fresh milk as the BS media is preferred by cheese manufacturers due to economic reasons (Limsowtin *et al.,* 1997).

Although, milk is the natural habitat for *Lactococci* species, milk cannot provide all the nutrients required for these organisms. The addition of different growth supplements such as yeast extract (YE) and peptones to the milk has been shown to enhance their growth rates (Benthin and Villadsen, 1996; Olsen and Sorhaug, 1998; Gaudreau *et al.*, 1999; Barrette *et al.,* 2001; Gaudreau *et al.*, 2002; Schepers *et al.*, 2002; Champagne *et al.,* 2003; Nancib *et al.,* 2005; Hoier *et al.*, 2010).

The objective of the study was to investigate the effect of yeast extract as a BS media supplement in increasing starter culture activity in BS.

MATERIALS AND METHODS

A particular cheese factory in Victoria, Australia was selected for the study and the factory BS system and cheese vats were simulated on a laboratory scale. The conditions used in the BS system and cheese vats such as temperature, pH control, neutralising agent, starter strains and inoculum sizes were used as equivalent to those that used in the selected cheese factory. Initial simulations

were carried out using 10% (w/v) reconstituted skim milk (RSM) while the final simulations used factory UHT treated BS milk and pasteurised cheese milk obtained from the selected cheese factory.

Yeast extracts

Two commercial yeast extracts used in the study; 60% Autolysate ® (YE- A) and Flavex powder type 0506-14 ® (YE-F) were obtained from Mauri Yeast Australia, Queensland, Australia and Halcyon Proteins, Victoria, Australia, respectively. According to the yeast extract manufacturer's specifications, different levels of yeast extracts, namely, 0.25, 0.50, 0.75 and 1.00% (W/V) of yeast extract-A and 0.05, 0.10, 0.20, and 0.30% (W/V) yeast extract-F were tested in the study.

Starter culture strains

The same starter culture strains, which used for the production of cheese in the selected cheese factory (*Lactococcus lactis* subsp. cremoris strains 49, 831, 1929 ASCRC) were obtained from Dairy Innovation Australia, Culture Division, Werribee, Victoria, Australia, were used for the study.

Bulk starter simulation

A factory BS unit was simulated using laboratory scale fermentation system developed at Culture Division, Dairy Innovation Australia (Figure 1). One Litre glass fermenter vessels containing the milk media were kept in a temperature-controlled water bath (Polystat CC1, CE hurber, Offenburg). Bulk starter growth was carried out at a controlled temperature of 27 °C. Three fermenter vessels were run simultaneously. The media were agitated continuously at a speed of 180 rpm using magnetic stirrer plates placed under the fermenters. The pH probes (L3015 Model GT-DJ-225-2m double junction, PH Scientific (Pvt.) Ltd., New South Wales, Australia) inserted to each fermenter vessel monitored the pH throughout the fermentation period. The pH data logging was carried out via the sensory logging software (Total Turnkey Solutions, Vic, Australia) while the pH of the media was maintained at 6.00 by automatic addition of 46% (v/v) NaOH connected to the fermenters via valves to control flow rate. The glass NaOH reservoirs were placed on load cells (Celtron LPS – 1.0 kg, Scale components, Queensland, Australia) to determine the mass of NaOH delivered over time. The 10% pasteurised (at 85 °C for 15 min) reconstituted skim milk (RSM) was used in initial trials, while UHT treated milk, which was obtained from the selected cheese factoies, was used in final trials.

The fermenter vessels containing appropriate media were inoculated with the given mix starter strains (*Lactococcus lactis* subsp. *cremoris* strains 49, 831, 1929 ^{ASCRC}) at a percentage of 0.006% and fermentation was run over night. Four fermentation experiments were conducted separately, following the same experimental conditions, in four different days.

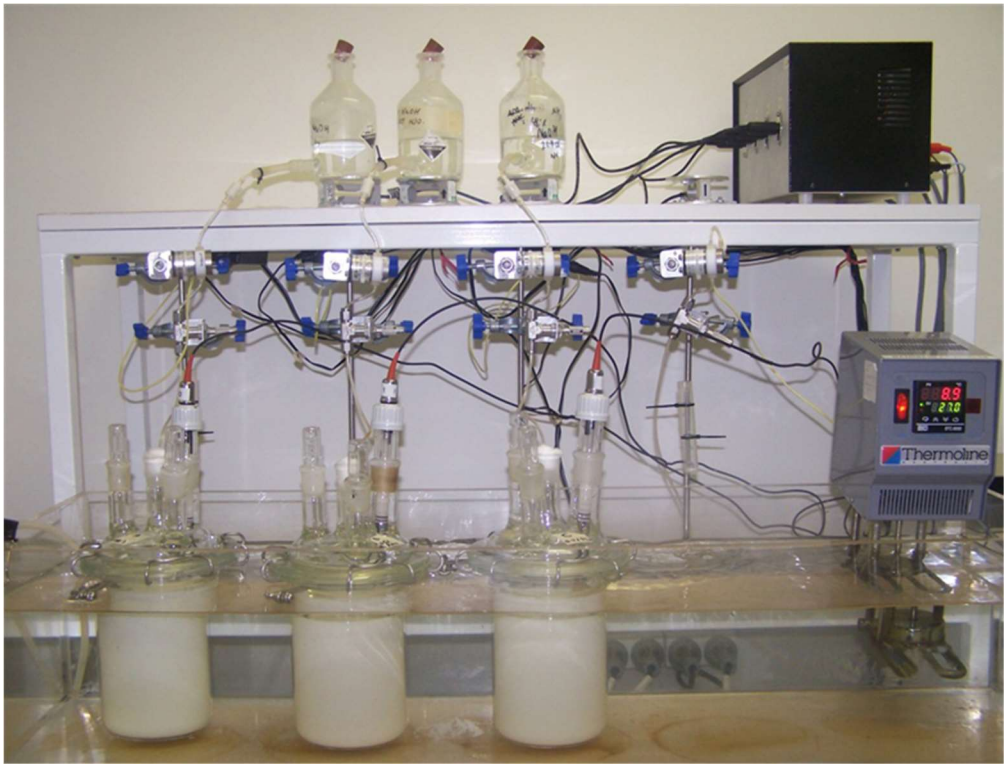


Figure 1: The lab scale fermentation system used for the simulation of factory BS unit.

Sampling

Bulk starter sampling was undertaken when the volume of 46% (w/v) NaOH was added to each of the fermenter vessel equaled to 0.4% of the fermenter volume, which is the harvesting time of BS in the selected cheese factory. A sample of 10 mL were taken from each fermenter vessel and immediately chilled to 4 °C and tested within 24 h.

Analysis of bulk starter growth

Measurement of pH and lactic acid production

The pH change during bulk starter growth was monitored using double junction glass pH electrodes. The rates of lactic acid production during BS growth were determined by calculating the amount of 46% (w/v) NaOH added to BS vessels over the fermentation period.

Cheese vat simulation

Factory cheese vats were simulated using glass test tubes containing 10 mL of 10% autoclaved RSM (at 121 °C for 15 min) held in a water bath at 32 °C for 5 h as described by Pearce (1969) and Rynne *et al.* (2007) with slight modifications.

The 10% autoclaved RSM was used in initial trials while factory milk, which was obtained from the selected cheese factory, was used in final trials.

Analysis of cheese vat performance

Continuous activity measurement

Autoclaved 10% RSM were inoculated with 2% (v/v) BS inoculum and incubated in a water bath in glass test tubes fitted with pH electrodes for 5 h at 32 °C. The pH of the samples was continuously monitored and data logging was carried out through the sensory logging software over the 5 h incubation period.

Activity Measurement at different inoculums levels

Autoclaved 10% RSM (10 mL) was inoculated with varying BS inoculums levels and incubated in a water bath at 32 °C for 5 h. The pH of the samples was measured at the end of the incubation period. Each inoculum level was triplicated.

Study I - The effect of yeast extract addition on bulk starter activity

Bulk starter media were prepared from 10% pasteurised RSM with added yeast extract levels of either 0.5% (w/v) YE–A or 0.05% (w/v) YE–F and BS growth was carried along with a control (level of each yeast extract was selected based on preliminary trials). Bulk starter simulations and cheese vat simulations were undertaken as described in below. Bulk starter simulation trials were carried out in triplicate for each yeast extract type.

Study II - Selection of the optimum level of yeast extract addition

Simulated BS growth was carried out in 10% pasteurised RSM medium at yeast extract addition levels of; 0.25, 0.50, 0.75 and 1.00% (w/v) for the YE–A and 0.05, 0.10, 0.20 and 0.30% (w/v) for the YE–F in order to determine the optimum level for each yeast extract type. The simulation trials were duplicated for each yeast extract type.

Study III- Yeast extract addition to factory UHT treated milk

UHT treated factory BS milk and pasteurised cheese milk was obtained from the selected cheese factory. Bulk starter media were prepared using factory UHT milk with yeast extract levels of 0.50% (w/v) YE–A and 0.20% (w/v) YE–F followed by pasteurising at 85 °C for 15 min. Bulk starter growth was carried out along with a control (pasteurised UHT milk minus yeast extract). The factory milk simulation was duplicated.

RESULTS AND DISCUSSION

Results

The effect of yeast extract on lactic acid production in simulated BS system

Supplementation of RSM medium with the yeast extracts increased the lactic acid production of the mixed starter culture (*Lc. lactis* sub sp. *cremoris* strains 49, 831 and 1929 [ASCRC]) and increased the subsequent rate of pH reduction in the simulated bulk starter system (Figures 2 and 3).

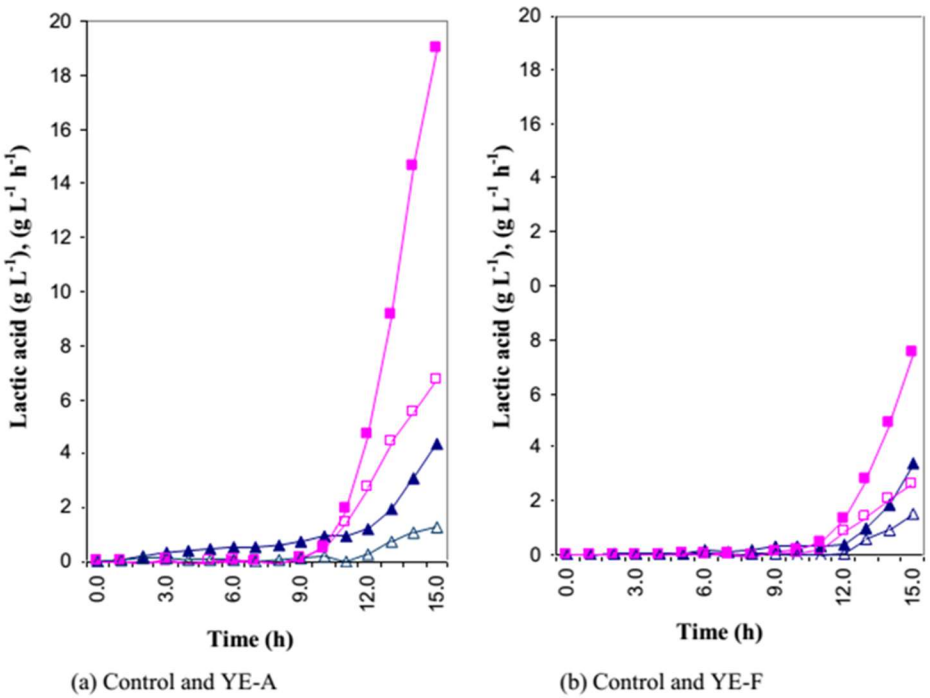
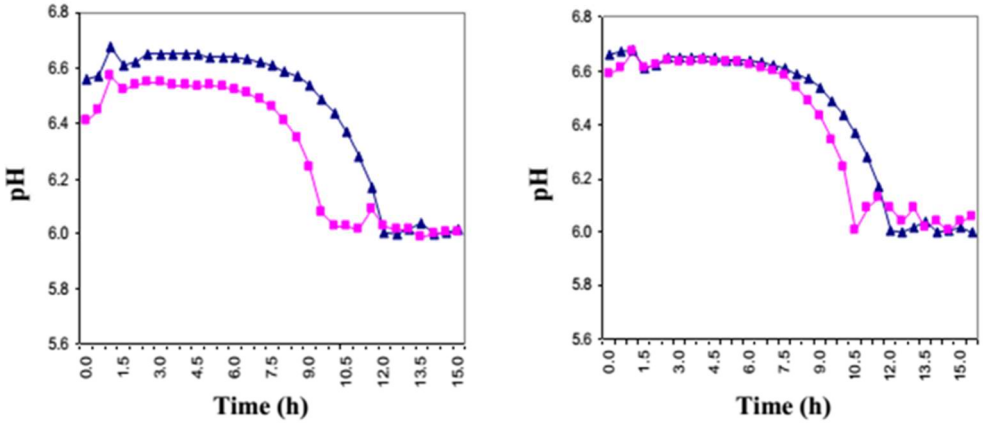


Figure 2: The effect of YE on lactic acid production by mixed culture (*Lc. lactis* sub sp. *cremoris* strain 49, 831 and 1926) in a simulated BS system, lactic acid concentration g L^{-1} (close symbols), rate of lactic acid production g L^{-1} h^{-1} (open symbols). (a) ▲: control (no YE); ■: 0.5% YE–A; Δ: control (no YE); □: 0.5% YE–A (b) ▲: control (no YE); ■: 0.05% YE– F; Δ: control (no YE); □: 0.05% YE–F.

As the lactic acid production is paralleled to the growth of *Lactococci* it appears that YE supplementation has increased the exponential growth phase of the culture to an upper limit (Figure 2). This result is in agreement with previous researchers Smith *et al.,* 1975, and Schepers *et al.,* 2002. The exponential growth was also extended by 60 – 120 min in YE supplemented media compared to the control.



(a) Control and YE-A (b) Control and YE-F

Figure 3: The effect of YE on pH reduction in the simulated BS system. The pH was maintained at 6.0 by addition of 46% (w/v) NaOH. (a)▲: control (no YE); ■: 0.5% YE–A (b) ▲: control (no YE); ■: 0.05% YE–F.

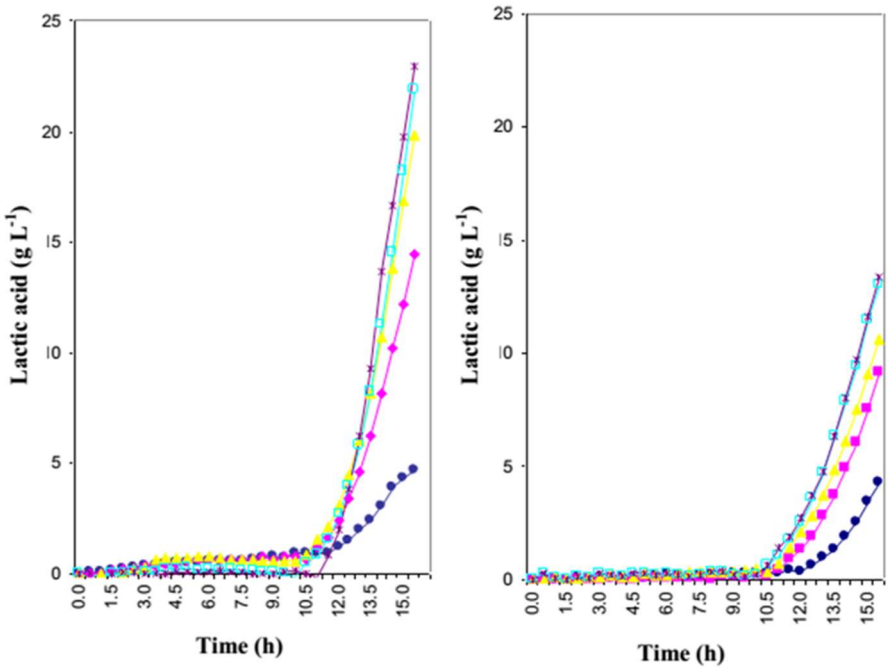
As shown in Table 1, during the whole BS fermentation period of 15.5 h, supplementation of the BS media with 0.5% YE–A, increased the final lactic acid concentration by 14.87 g L^{-1} whereas supplementation with 0.05% YE–F increased the lactic acid concentration by 5.03 g L^{-1} relative to the control (with no yeast extract), which resulted in lactic acid concentration of 4.13 g L^{-1}.

Table 1: Total lactic acid production in the different media.

Level of YE in 10% RSM medium	Lactic acid concentration of the media at 15.5 h (g L^{-1})
no YE	4.13 ± 0.71
0.05% YE-F	9.16 ± 0.97
0.5% YE-A	19.00 ± 1.21

Selection of the optimum level of yeast extract addition to the BS

As shown in Figure 4, during the 15 h fermentation period, lactic acid production increased with increasing yeast extract levels in both YE types. However, after a certain level of YE (0.5% level of YE–A and 0.2% level of YE-F) the increment effect tended to decrease (Table 2). It appears that, although, YE enhanced growth there was a point where the end products restricted growth. Therefore, the 0.5% of YE–A and 0.2% of YE–F were chosen as the optimum levels for addition to the simulated BS system.



(a) YE-A (b) YE-F

Figure 4: The lactic acid production of simulated bulk starter at different YE levels: (a) Different YE–A levels, ●: control (no YE); ■: 0.25% YE–A; ▲: 0.5% YE–A; □: 0.75% YE–A; ж: 1% YE–A. (b) Different YE–F levels, ●: control (no YE); ■: 0.05% YE–F; ▲: 0.1% YE–F; □: 0.2% YE–F; ж: 0.3% YE–F.

Table 2: Effect of yeast extract supplementation on fermentation time and lactic acid production.

Level of YE added to 10% RSM medium	Time taken to reach harvesting point (0.4% v/v 46% NaOH addition) (h)	Total lactic acid production (g L^{-1}) during 15.5 h fermentation period	Maximum rate of lactic acid production (g L^{-1} h^{-1})
0.25% YE–A	12.20	12.20 ± 1.30	4.069
0.50% YE–A	12.15	19.00 ± 1.10	6.502
0.75% YE–A	12.15	20.44 ± 1.13	6.969
1.00% YE–A	12.00	21.61 ± 2.57	7.108
0.05% YE–F	12.45	9.16 ± 0.98	2.374
0.10% YE–F	12.30	10.28 ± 0.98	2.689
0.20% YE–F	12.20	13.15 ± 1.98	3.262
0.30% YE–F	12.20	14.00 ± 1.52	3.136

The yeast extract supplemented bulk starters also reached the industrial harvesting point of (0.4% (v/v) additions of 46% (w/v) NaOH) 2.5 – 3 h before compared to the no yeast extract added bulk starters (Table 2). This revealed the fact that, YE addition leads to a reduction of the BS fermentation time.

The effect of YE addition to UHT treated milk in simulated BS

The UHT treated whole milk obtained from factory was supplemented with the selected optimal yeast extract levels (0.5% YE–A and 0.2% YE–F). This showed similarly enhanced lactic acid production as in YE supplemented RSM media (Figure 5).

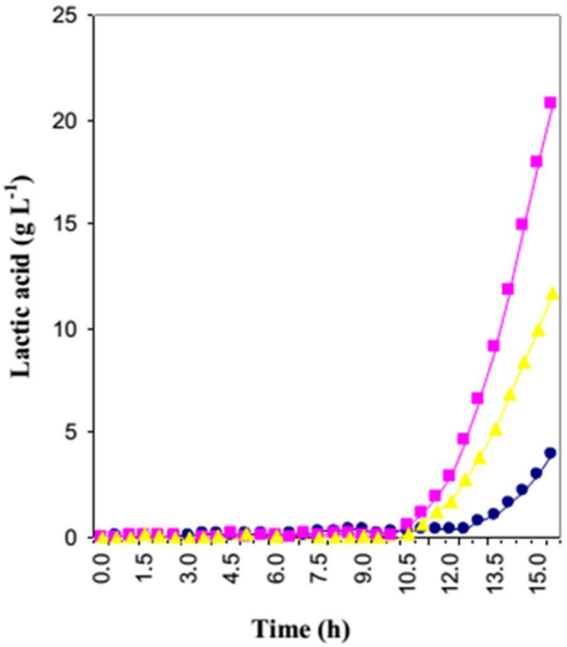
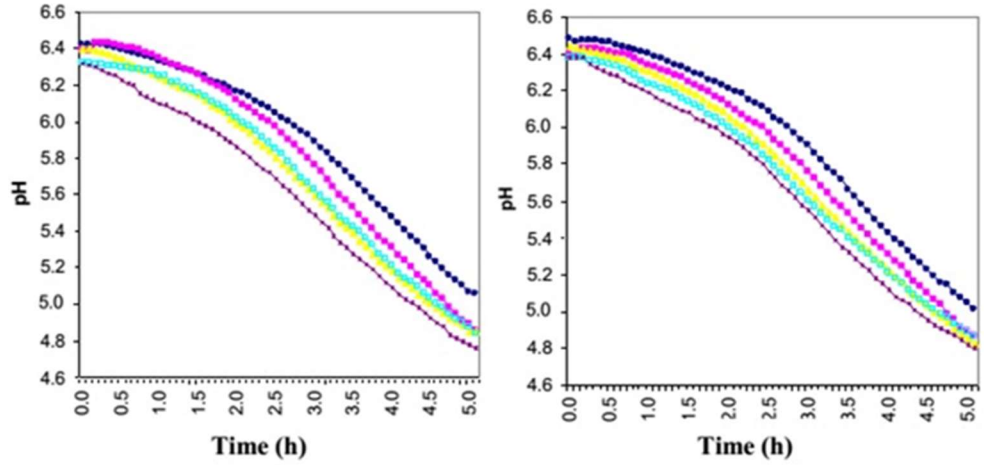


Figure 5: Lactic acid production in a simulated BS system with UHT treated whole milk medium at optimum YE levels. ●: control (no YE); ▲: 0.2% YE–F; ■: 0.5% YE–A.

The effect of YE on starter activity in the simulated cheese vat

Effect of yeast extract on continuous activity

As Figure 6 illustrates the BS cultures supplemented with yeast extract, reduced the pH of the cheese vat milk faster, compared to the bulk starters with no yeast extract supplementation. YE supplemented BS inoculated milk samples reached to pH of 5.4 (industrially preferred pH reduction in the cheese vat) in lesser time than the no YE supplemented BS culture samples indicating that YE supplementation of the BS media reduced the cheese making time in the cheese vat. Further activity improvements were observed with increasing yeast extract supplementation levels.



(a) YE-A (b) YE-F

Figure 6: The acid producing ability (activity) of different BS cultures in the simulated cheese va: (a) inoculated with BS cultures supplemented with different YE–A levels ●: control (no YE); ■: 0.25% YE–A; ▲: 0.5% YE–A; □: 0.75% YE–A; ж: 1% YE–A (b) inoculated with bulk starter cultures supplemented with different YE–F levels ●: control (no YE); ■: 0.05% YE–F; ▲: 0.1% YE–F; □: 0.2% YE–F; ж: 0.3% YE–F.

As shown in Figure 7, yeast extract added BS cultures showed considerable activity improvements in factory cheese milk (pasteurised whole milk). The cheese milk inoculated with YE supplemented BS cultures reached pH 5.4 faster than the no YE supplemented BS cultures, reducing the cheese making time approximately 30 – 45 min in the simulated cheese vat. The selected levels of the two yeast extracts gave similar activity results in simulated cheese vats.

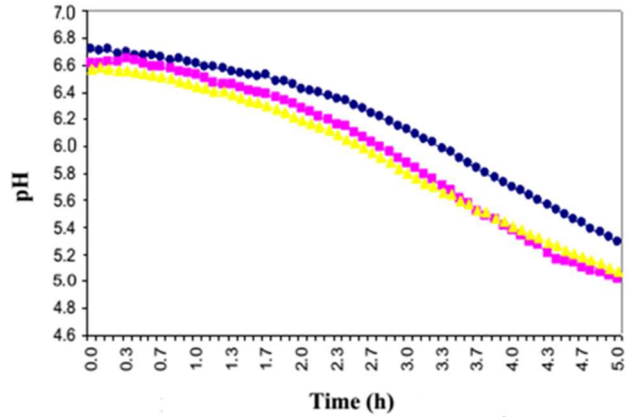


Figure 7: The acid producing ability (activity) of different bulk starter cultures in factory cheese milk. ●: control (no YE); ■: 0.5% YE-A; ▲: 0.2% YE-F

The effect of yeast extract on inoculum levels

It was revealed that the inoculum levels of YE added cultures required to reach pH of 5.4 in the simulated cheese vat of 0.3% (v/v) whereas inoculum level of no YE added BS culture was more than 0.5% (v/v). This indicates that YE supplementation of the BS media reduced the inoculum level required to inoculate the simulated cheese vat by approximately 40%. The selected levels of the two yeast extracts showed the same activity with different inoculum levels in the simulated cheese vat.

Discussion

The results showed that lactic acid production in the BS system was markedly increased by yeast extract supplementation of the BS media. Supplementation of the BS media by 0.5% YE-A increased the lactic acid production of the test *Lactococci* strains by 4 times while supplementation of 0.2% YE–F increased lactic acid production by 2 times compared to the no YE added BS. Similar results were obtained by Aeschlimann and Van Stockar (1990), Schepers *et al. (*2006) using *Lactobacillus helveticus*, which was grown in whey permeate media supplemented with yeast extract. The present study showed that yeast extract supplementation increased the volumetric production of lactic acid in the media as a result of cell biomass increase in the media.

Since lactic acid production of *Lactococci* strains in milk is parallel to the growth of *Lactococci* (Smith *et al.,* 1975; Ramachandran *et al.*, 2012) the growth was increased exponentially to a higher limit and then, it lasted for longer time in YE supplemented media, compared to the no YE added media. It is known that cultures of *Lactococci* species contain fast acid producing cells, which spontaneously produce slow acid producing variants. With the time these slow acid producing variants and fast acid producing parent cells come to an equilibrium in the growth medium, which tends to retard the acid producing activity of the culture (Smith *et al.*, 1975). Smith *et al.* (1975) showed that yeast extract has the ability to stimulate the acid producing activity of those slow acid producing variants so that their acid production resembled that of the fast acid producing parent cells. Therefore, cell biomass increment and the stimulation of slow acid producing variants to fast acid producers might have increased the lactic acid production in yeast extract supplemented bulk starters.

Yeast extract supplemented bulk starters showed higher rates of lactic acid production compared to the no YE added BS media. However, the rates of lactic acid production in both YE added and non YE added media tended to decrease relative to the lactic acid concentration in the media. This is apparently due to the end product inhibition as showed by Bibal *et al*. (1989), Loubiere *et al.* (1997), Rault *et al*. (2009) and Ramchandran *et al.* (2012).

Increasing YE levels beyond 0.5% of YE-A and 0.2% of YE–F did not result in any further substantial increment in lactic acid production. This result was in

agreement with Bibal *et al.* (1988) and Schepers *et al.* (2006) who carried out kinetic study of Lactic acid bacteria by enriching the medium with yeast extract. This is apparently because the end product restricted further growth of bacteria.

Yeast extract supplemented BS cultures showed higher activity when inoculated to simulated cheese vats and reduced the pH of milk faster than the normal bulk starter cultures. This indicates that YE supplemented BS cultures have higher activity compared to the no YE supplemented BS cultures.

CONCLUSIONS

The present study suggests the usefulness of yeast extract in improving BS activity in cheese manufacturing process. Further studies are required in finding the behavior of cell growth of *Lactococci* species in the YE added media and finding the constituents in yeast extract, which enhanced the growth of *Lactococci* species.

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REFERENCES

Aeschlimann, A. and Stockar, U.V. (1990). The effect of yeast extract supplementation on the production of lactic acid from whey permeate by *Lactobacillus helveticus*. Appl. Microbiol. Biotechnol. 32, 398–402.

Barrette, J., Champagne, C.P. and Goulet, J. (2001). Growth-promoting properties of yeast extracts produced at different pH values, with different autolysis promoters and bacterial populations. J. Chem. Technol. Biotechnol. 76, 203–209.

Benthin, S. and Villadsen, J. (1996). Amino acid utilization by *Lactococcus lactis* subsp. *cremoris* FD1 during growth on yeast extract or casein peptone. J. Appl. Bacteriol. 80, 65–72.

Bibal, B., Goma, G., Vayssier, Y. and Pareilleux, A. (1988). Influence of pH, lactose and lactic acid on the growth of *Streptococcus cremoris*: a kinetic study. Appl. Microbiol. Biotech. 28, 340–344.

Bibal, B., Vayssier, Y., Tournou, M. and Pareilleux, A. (1989). Enhanced inhibitory effect of lactic acid on growth kinetics of *Streptococcus cremoris* during nutritional medium limitations. Appl. Microbiol. Biotechnol. 30, 630–635.

Broome, M.C., Powell, I.B. and Limsowtin, G.K.Y. (2003). Starter cultures: specific properties. pp. 269–275. In: Roginski, H., Fuquay, J.W. and Fox, P.F. (Eds.), Encyclopedia of dairy sciences.: Elsevier, New York.

Bylund, G. (1995). Cultures and starter manufacture. pp. 233–318. In: Dairy processing handbook. Tetra pak processing systems AB.

Champagne, C.P., Gaudreau, H. and Conway, J. (2003). Effect of the production or use of mixtures of bakers' or brewers' yeast extracts on their ability to promote growth of Lactobacilli and Pediococci. J. Biotechnol. 6. 186–197.

Champagne, C.P., Gaudreau, H., Conway, J., Chartier, N. and Fonchy, E. (1999). Evaluation of yeast extracts as growth media supplements for lactococci and lactobacilli by using automated spectrophotometry. J. Gen. Appl. Microbiol. 45, 17–21.

Farkye, N.Y. (2004) Cheese Technology, Int. J. Dairy Technol. 57**,** 91–98.

Fox, P.F., Guinee, T.P., Cogan, T.M. and Mcsweeney, P.L.H. (2017). Starter cultures. pp. 121–183. In: Fundamentals of cheese science, 2nd ed. Springer, New York.

Fox, P.F. and McSweeney, L.H.P. (2004). Cheese: An Overview. pp. 1–17. In: Fox, P.F., McSweeney, L.H.P., Cogan, T.M. and Guinee, T.P. (Eds.), Cheese chemistry, physics and microbiology: General aspects. Elsevier Academic Press, London.

Gaudreau, H., Champagne, C.P., Conway, J. and Degre, R. (1999). Effect of ultrafiltration of yeast extracts on their ability to promote lactic acid bacteria growth. Can. J. Microbiol. 45**,** 891–897.

Gaudreau, H., Renard, N., Champagne, C.P. and Horn, D.V. (2002). The evaluation of mixtures of yeast and potato extracts in growth media for biomass production of lactic cultures. Can. J. Microbiol. 48, 626–634.

Hoier, E., Janzen, T., Rattary F., Sorensen,K., Borsting, M.W., Brockmann, E. and Johansen, E. (2010). The production, application and action of lactic cheese starter cultures. pp. 166–184. In: Law, B.A. and Tammie, A.Y. (Eds.), Technology of cheese making, John Wiley and Sons, West Sussex, UK.

Ibrahim, S.A. and Daguri, M.H. (1996). Bulk starter media for mesophilic starter cultures: A review. Dairy Food Environ. Sanit. 16**,** 823–828.

Limsowtin, G.K.Y., Bruinenberg, P.G. and Powell, I.B. (1997). A strategy for cheese starter culture management in Australia. J. Microbiol. Biotechnol. 7, 1–7.

Loubiere, P., Bousquet, M.C., Matos, J., Goma, G. and Lindley, N.D. (1997). Influence of end-products inhibition and nutrient limitations on the growth of *Lactococcus lactis* subsp. *lactis*. J. Appl. Microbiol. 82,95–100.

Nanacib, A., Nancib, N., Meziane- Cherif, D., Boubendir, A., Fick, M. and Boudrant, J. (2005). Joint effect of N sources and B vitamin supplementation of date juice on lactic acid production by *Lactobacillus casei* subsp. *rhamnosus.* Bioresour. Technol. 96, 63–67.

Olsen, E.S. and Sorhaug, T. (1998). Comparative studies of the growth of *Lactobacillus plantarum* in whey supplimented with autolysate from brewery yeast biomass or commercial yeast extract. Milchwissenschaft. 53, 367–370.

Parente, E. and Cogan, T.M. (2004). Starter cultures: General aspects. pp. 123–145. In: Fox, P.F., McSweeney, L.H.P., Cogan, T.M. and Guinee, T.P. (Eds.), Cheese chemistry, physics and microbiology: General aspects. Elsevier Academic Press, London.

Pearce, L. E. (1969). Activity tests for cheese starter cultures. New Zeal. J. Dairy Sci. Technol. 4, 246–247.

Powell, I.B., Broome, M.C. and Limsowtin, G.K.Y. (2011). Starter cultures: General aspects. pp. 552–558. In: Fuquay, J.W., Fox, P.F. and McSweeny, P.L.H. (Eds.), Encyclopaedia of dairy sciences. Academic Press, New York.

Ramchandran, L., Sanciolo, P., Vasiljevic, T., Broome, M., Powell, I. and Duke, M. (2012). Improving cell yield and lactic acid production of Lacto coccus *lactis* ssp. *cremoris* by a novel submerged membrane fermentation process, J. Membr. Sci. 179–187

Rault, A., Boux, M. and Béal, C. (2009.) Fermentation pH influences the physiological-state dynamics of *Lactobacillus bulgaricus* CFL1 during pH controlled culture, Appl. Environ. Microbiol. 75, 4374–4381.

Reiter, B. and Oram, J.D. (1962) Nutritional studies on cheese starters 1. Vitamin and amino acid requirements of single strain starters. J. Dairy Res. 29, 63–77.

Rynne, N. M., Beresford, T.P., Kelly, A.L., Tunick, M.H., Malin, E.L. and Guinee, T.P. (2007). Effect of exopolysaccharide-producing adjunct starter cultures on the manufacture, composition and yield of half-fat Cheddar cheese. Aust. J. Dairy Technol. 62, 12–18

Schepers, A.W., Thibault, J. and Lacroix, C. (2002). *Lactobacillus helveticus* growth and lactic acid production during pH-controlled batch cultures in whey permeate/yeast extract medium. Part1. multiple factor kinetic analysis. Enzyme Microb. Technol. 30, 176–186.

Schepers, A.W., Thibault, J. and Lacroix, C. (2006) Continuous lactic acid production in whey permeate/yeast extract medium with immobilized *Lactobacillus helveticus* in a two-stage process: Model and experiments. Enzyme Microb. Technol. 38, 324–337.

Smith, J.S., Hillier, A.J., Lees, G.J. and Jago, G.R. (1975). The nature of the stimulation of the growth of *Streptococcus lactis* by yeast extract. J. Dairy Res. 42, 123–138.

Whitehead, W.E., Ayres, J.W. and Sandine, W.E. (1993) A review of starter media for cheese making. J. Dairy Sci. 76, 2344–2353.