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Effect of Water Activity on Growth of Certain Lactic Acid Bacteria

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ABSTRACT

The effect of reduction in water activity on growth and acid production by *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* was studied at water activity values of 0.998, 0.975, 0.967 and 0.959. Reduced water activity resulted in extended lag time of varied magnitude, varied growth rates, different cell population and acid production. The highest growth rates were observed at an a_w of 0.998 (control). *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* reached a maximum cell population of $80x10^6$ CFU/ml, $60x10^6$ CFU/ml and $60x10^6$ CFU/ml after 24th hour of incubation respectively.At water activity value of 0.975, *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* reached a maximum cell population of $40x10^5$ CFU/ml, $51x10^5$ CFU/ml and $51x10^5$ CFU/ml respectively after 24th hour of incubation , which indicate that *Lactococcus lactis* subsp *lactis* reached a maximum cell population which is lower than that observed for the other two strains. Which indicate that for each bacterial strain there is an optimum water activity at which the growth rate is faster comparing with the growth rate at lower or higher water activity. In addition the sensitivity to culture water activity varied between different bacterial strains. Also the obtained results indicated that the value of culture water activity had approximately simlar effect on rate of increase in developed titratable acidity as that found for the rate of increase in colony forming units.

Keywords: Lactic acid bacteria, colony count, water activity, Mrs Media, titratable acidity, *Lactococcus lactis* subsp *lactis Lactococcus lactis* subsp *cremoris Lactobacillus casei*

INTRODUCTION

Effects of some environmental parameters on growth kinetics of lactic acid bacteria might provide useful information concerning physiology and different reactions of the microorganism during growth under different conditions. Among these parameters water activity (a_w) seem to be of great importance, Tanigughi *et al*. (1987) and Bernard-Bibal *et al*. (1988).

Lactic acid bacteria now are used extensively as a starter cultures in the dairy industry, and therefore, optimization of growth conditions appears to be essential for successful industrial applications, Bernard-Bibal *et al* . (1988).

Water activity (a_w) is used to show a relation expressing the relative gas humidity as equilibrium with the system. The activity as ratio of two variables, represents a relative measure related to a standard which is distilled water. Pure water activity was conventionally fixed as equlling one (i.e., unit), water activity of any other solution is always lower than one, Rehacek *et al*, (1982).

Yoness (2002) demonstrated that reduced water activity resulted in extended lag phase by varied magnitude, varied growth rate, different maximum cell population, and rate of acid production of *Lactococcus lactis* subsp *lactis*. He found that the maximum growth rates at a_w 0.998 was at interval of time between 4th and 10th hr of incubation by a mean slope of 0.434 ± 0.003 log CFU/ hr and maximum cell population of $2.5x10^9$ CFU/ml.

This study emphasized the importance of water activity on growth of lactic acid bacteria and water activity can affect utilizing lactic acid bacteria in dairy manufacturing .

Therefore , the aim of the present study was to find water activity values which limit or completely inhibit the growth ability of lactic acid bacteria as well to develop acidity .

MATERIALS AND METHODS

Materials

1- Milk

Milk used in this study was obtained from Faculty of Agriculture herd, morning milking. As soon as milk was arrived to the laboratory it was skimmed by using Alfa-Laval separator operated at 16000 rpm.

Skim milk was distributed into 1 L conical flasks, each flask contained 750 ml skim milk, conical flasks containing skim milk were sterilized by using autoclave operated at 121°C/10 minutes (15 lph/inc^2).

2- Bacteria

Lactic acid bacteria used in this study were:

- *Lactococcus lactis* subsp *lactis* - *Lactococcus lactis* subsp *cremoris*
- *Lactobacillus casei*
- It was obtained from the Culture Collection of Botany Department, Faculty of Science, Assiut University. Pure culture of *Lactobacillus casei* was optained from professor doctor Ibrahim Abou El- Naga as agift .

All organisms were routinely maintained in sterile litmus milk fortified with 0.1% peptone and stored at 5–7°C.

For the preparation of the inocula, the procedure described by Hassan *et al.* (1989) was adopted. From each stored bacterial culture, a 1/10 dilutions were made in 500 ml conical flasks each one contained from 150–250 ml sterilized skim milk.

After overnight incubation at 34°C, the first non-coagulated flask in which the bacteria was expected to be in the exponential phase of growth was used for inoculating the experimental flasks.

Inoculation has been carried out by using certain volumes from the first non-coagulated flask in order to achieve about 10^5 CFU/ml when inoculated in the experimental flasks.

3- Growth media

MRS media was used in this study, plate counts were prepared on MRS agar, as described in Difco manual (1998).

4- Cultural conditions:

The obtained cultures was propagated under four different levels of water activity in the experimental flasks i.e.:0.998 (control, without addition of glycerol), 0.975, 0.967 and 0.959 in the experimental cultures.

Methods

To study the effect of water activity (a_w) on the bacterial growth rate, four of 1 L conical flask each of them contained 750 ml of sterilized skim milk were inoculated by the investigated bacteria. Inoculation had been carried out by volumes of the inoculum to give about 10^5 CFU/ml at zero time in each inoculated flask. Preparation of the inoculum had been carried out as previously described by Hassan and Richard (1990). One milliliter of the selected culture tube for inoculation of experimental milk was diluted to give dilution 0.1% w/v, this dilution was gently shaked for 30 sec., then this dilution (1/10) was used for inoculation of milk by calculated volumes to give about 10^5 CFU/ml in the experimental milk. The water activity (a_w) of the cultures was adjusted by using glycerol as previously described by Valik and Gorner (1995) to produce 4 different levels of water activity in the experimental flasks *i.e.*, 0.998 (control, without addition of glycerol), 0.975, 0.967 and 0.959 in the experimental cultures.

Conical flasks were incubated in water bath thermostatically controlled at 30°C.

Sampling had been carried out at zero time and each 2 hours intervals up to coagulation of culture in the control flask and up to 32 hours for other treatments.

Sampling

At the time of inoculation , zero time and each two hours intervals up to two hours after milk coagulation or up to 32 hours , a 15 ml aliquots of each culture was aseptically withdrawn in 25 ml sterilize conical flask.

1 ml of the aliquots was aseptically withdrawn in test tube containing 9 ml sterilized distilled water to give the first dilution 1/ 10 for the bacteriological analysis (10^{-1}) then it was shaked gently for 30 second and it was used for the preparation for other dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} MRS agar media was used in the present study. Inoculated petri dishes were incubated 34°C for 72 hours.

Colonies were determined by direct visual inspection.

Total viable counts were counted and was transferred into the corresponding log 10 values (log CFU/ml).

Acid development was followed by measuring of acidity of samples by using N/9 NaOH solution. The developed titratable acidity were calculated as previously described by Cogan (1978).

RESULTS AND DISCUSSION

The effect of reduced water activity on the growth rates and cells population of *Lactococcus lactis* subsp *lactis, Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* are shown in Tables 1, 2 and 3 and figures 1, 2 and 3.

Table 1. Effect of water activity (a_w) on the growth rate of *Lactococcus lactis* subsp *lactis* cultivated at 30°C in sterilized skim milk (CFU/ml).

Sampling time	a_w 0.998		a_w 0.975		a_w 0.967		a_w 0.959	
(hour)	CFU/ml	Log CFU/ml	CFU/ml	Log CFU/ ml	CFU/ ml	Log CFU/ ml	CFU/ ml	Log CFU/ ml
0	10×10^5	6.00	47×10^4	5.67	90×10^3	4.95	25×10^3	4.39
2	13×10^5	6.12	60×10^4	5.77	10×10^4	5.00	26×10^3	4.42
4	16×10^5	6.20	75×10^4	5.87	11×10^4	5.05	28×10^3	4.44
6	20×10^5	6.30	90×10^4	5.95	14×10^4	5.15	30×10^3	4.47
8	30×10^5	6.47	12×10^5	6.07	18×10^4	5.25	33×10^3	4.52
10	80×10^5	6.90	16×10^5	6.21	25×10^4	5.40	40×10^3	4.60
12	29×10^6	7.46	25×10^5	6.41	39×10^4	5.59	47×10^3	4.67
24	80×10^6	7.90*	40×10^5	6.60	57×10^4	5.75	59×10^3	4.77
26	90×10^6	7.95	51×10^5	6.70	72×10^4	5.85	67×10^3	4.81
28	98×10^6	7.99	64×10^5	6.80	11×10^5	6.05	77×10^3	4.88
30	10×10^7	8.00	78×10^5	6.89	13×10^5	6.11	81×10^3	4.90
32	93×10^6	7.96	98×10^5	6.99	15×10^5	6.17	92×10^3	4.96

***Coagulated sample.**

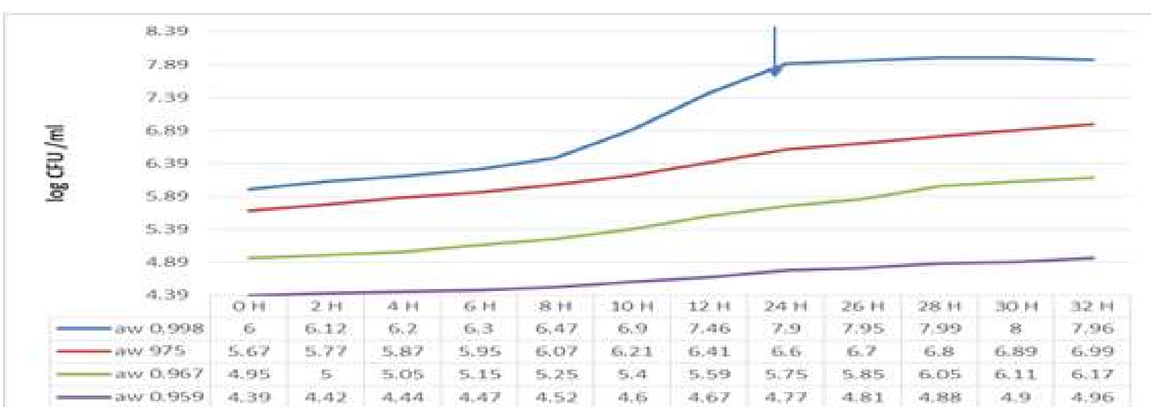


Fig .1. Effect of water activity (a_w) on growth rate of *Lactococcus lactis* subsp *lactis* cultivated at 30°C in sterilized skim milk (log CFU/ml).

↑ **Coagulated sample**

Table 2. Effect of water activity (a_w) on the growth rate of *Lactococcus lactis* subsp *cremoris* cultivated at 30°C in sterilized skim milk.

Sampling time	a_w 0.998		a_w 0.975		a_w 0.967		a_w 0.959	
(hour)	CFU/ml	Log CFU/ml	CFU/ml	Log CFU/ ml	CFU/ ml	Log CFU/ ml	CFU/ ml	Log CFU/ ml
0	23×10^{5}	6.37	30×10^{4}	5.47	30×10^{3}	4.47	30×10^{2}	3.47
2	40×10^{5}	6.60	50×10^{4}	5.69	50×10^{3}	4.69	40×10^{2}	3.60
4	50×10^{5}	6.69	72×10^{4}	5.85	64×10^{3}	4.80	50×10^{2}	3.69
6	70×10^{5}	6.84	90×10^{4}	5.95	80×10^{3}	4.90	60×10^{2}	3.77
8	10×10^{6}	7.00	10×10^{5}	6.00	90×10^{3}	4.95	70×10^{2}	3.84
10	15×10^{6}	7.17	12×10^{5}	6.07	11×10^{4}	5.04	80×10^{2}	3.90
12	30×10^{6}	7.47	20×10^{5}	6.30	16×10^{4}	5.22	97×10^{2}	3.98
24	60×10^{6}	7.77*	51×10^{5}	6.70	30×10^{4}	5.47	12×10^{3}	4.07
26	10×10^{7}	8.00	60×10^{5}	6.77	36×10^{4}	5.55	16×10^{3}	4.20
28	13×10^{7}	8.11	66×10^{5}	6.81	39×10^{4}	5.59	20×10^{3}	4.30
30	132×10^{6}	8.12	63×10^{5}	6.79	37×10^{4}	5.56	21×10^{3}	4.31
32	10×10^{7}	8.00	53×10^{5}	6.72	35×10^{4}	5.54	19×10^{3}	4.28

*Coagulated sample

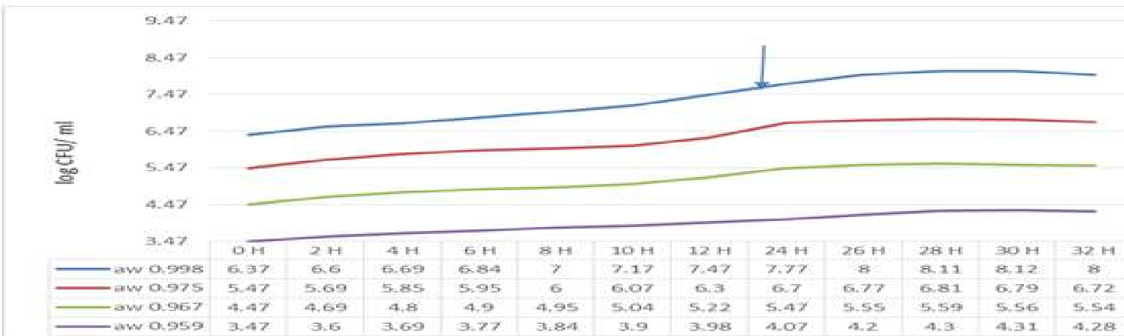


Fig .2. Effect of water activity (a_w) on growth rate (log CFU/ml) of *Lactococcus lactis* subsp *cremoris* cultivated at 30°C in sterilized skim milk.

↑ Coagulated sample

Table 3. Effect of water activity (a_w) on the growth rate of *Lactobacillus casei* cultivated at 30°C in sterilized skim milk (log CFU/ml).

Sampling time	a_w 0.998		a_w 0.975		a_w 0.967		a_w 0.959	
(hour)	CFU/ml	Log CFU/ml	CFU/ml	Log CFU/ ml	CFU/ ml	Log CFU/ ml	CFU/ ml	Log CFU/ ml
0	15×10^{5}	6.17	38×10^{4}	5.57	31×10^{3}	4.49	25×10^{2}	3.39
2	20×10^{5}	6.30	50×10^{4}	5.69	39×10^{3}	4.59	30×10^{2}	3.47
4	30×10^{5}	6.47	70×10^{4}	5.84	50×10^{3}	4.69	35×10^{2}	3.54
6	47×10^{5}	6.67	10×10^{5}	6.00	60×10^{3}	4.77	40×10^{2}	3.60
8	80×10^{5}	6.90	15×10^{5}	6.17	70×10^{3}	4.84	50×10^{2}	3.69
10	13×10^{6}	7.12	20×10^{5}	6.30	85×10^{3}	4.92	62×10^{2}	3.79
12	30×10^{6}	7.47	26×10^{5}	6.42	12×10^{4}	5.10	80×10^{2}	3.90
24	60×10^{6}	7.77	51×10^{5}	6.70	30×10^{4}	5.47	10×10^{3}	4.00
26	13×10^{7}	8.11	65×10^{5}	6.81	31×10^{4}	5.49	13×10^{3}	4.11
28	16×10^{7}	8.20*	76×10^{5}	6.88	35×10^{4}	5.54	16×10^{3}	4.20
30	15×10^{7}	8.19	86×10^{5}	6.93	39×10^{4}	5.59	18×10^{3}	4.25
32	150×10^{6}	8.17	95×10^{5}	6.97	43×10^{4}	5.63	20×10^{3}	4.30
34	145×10^{6}	8.16	10×10^{6}	7.01	44×10^{4}	5.64	22×10^{3}	4.34

*Coagulated sample

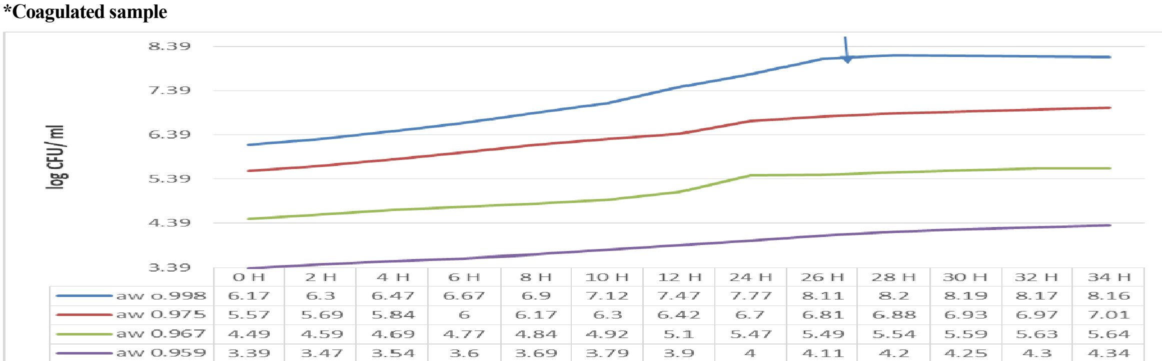


Fig. 3. Effect of water activity (a_w) on growth rate of *Lactobacillus casei* cultivated at 30°C in sterilized skim milk (log CFU/ml)

↑ Coagulated sample

The obtained results indicated that there was a significant changes in each of the three studied strains grown in sterilized skim milk in growth rates, lag phase, growth curves and maximum cell populations. The highest growth rates were observed at an a_w of 0.998 (control). *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* reached a maximum cell population of 80x10^6 CFU/ml, 60x10^6 CFU/ml and 60x10^6 CFU/ml after 24th hour of incubation respectively . As expected for *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* the maximum level of population was lower than *Lactococcus lactis* subsp *lactis*.

Growth suppression, in term of rates of growth and maximal cell population occurred as a_w levels were reduced from 0.998 through 0.975, 0.967 and 0.959.

It is clear from Tables 1, 2 and 3 that the effect of water activity on population levels increased as the water activity decreased. At water activity value of 0.975, *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* reached the maximum cell population of 40x10^5 CFU/ml, 51x10^5 CFU/ml and 51x10^5 CFU/ml after 24th hour of incubation respectively, which indicated that *Lactococcus lactis* subsp *lactis* reached a maximum cell population which is lower than that observed for the other two strains. As the incubation period increased, growth rates was decreased progressively reaching a minimum after 32 hour of incubation. The maximum population level was only 98x10^5 CFU/ml 53x10^5 CFU/ml and 95x10^5 CFU/ml, respectively after 32nd hour of incubation.

Although *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* were greatly affected when grow at water activity value of 0.975. The values for the same cultures grown at a_w of 0.967 were much more dramatic.

Results for colony forming units are shown in Tables 1, 2 and 3. *Lactococcus lactis* subsp *lactis* grow by a slower rate reached only a maximum cell population of 57x10^4 CFU/ml after 24th hour of incubation at 34°C.

The same decrease in colony forming units was observed with *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* reached a maximum cell population of 30x10^4 CFU/ml and 30x10^4 CFU/ml respectively after 24th hour of incubation.

When *Lactococcus lactis* subsp *lactis, Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* grown in sterilized skim milk at water activity of 0.959, the effect on the growth rates as measure by the colony forming units which reached maximum cell population of 59x10^3 CFU/ml, 12x10^3 CFU/ml and 10x10^3 CFU/ml respectively after 24th hour of incubation.

Although the three bacterial strains used in the present study were not particularly tolerant to the low water activity, it is interesting that *Lactococcus lactis* subsp *cremoris* was the most tolerant to reduce water activity. A similar results were reported by Raevuori and Genigeorgis (1975), Ismail (1990 b) and Hassan and Richard (1990).

Figures 4 , 5 and 6 present the results of developed titratable acidity for *Lactococcus lactis* subsp *lactis, Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei*, respectively.

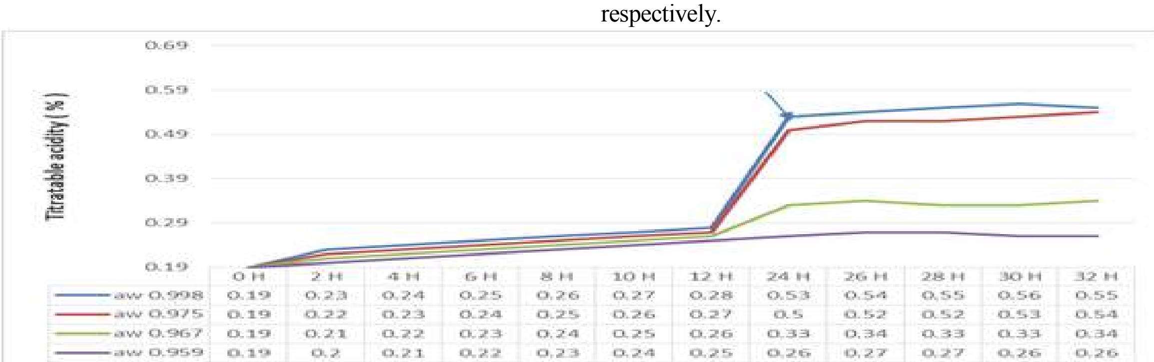


Fig. 4. Effect of water activity (a_w) on increasing rate in developed titratable acidity (%) during growth of *Lactococcus lactis* subsp *lactis* cultivated at 30°C in sterilized skim milk.

↑ Coagulated sample

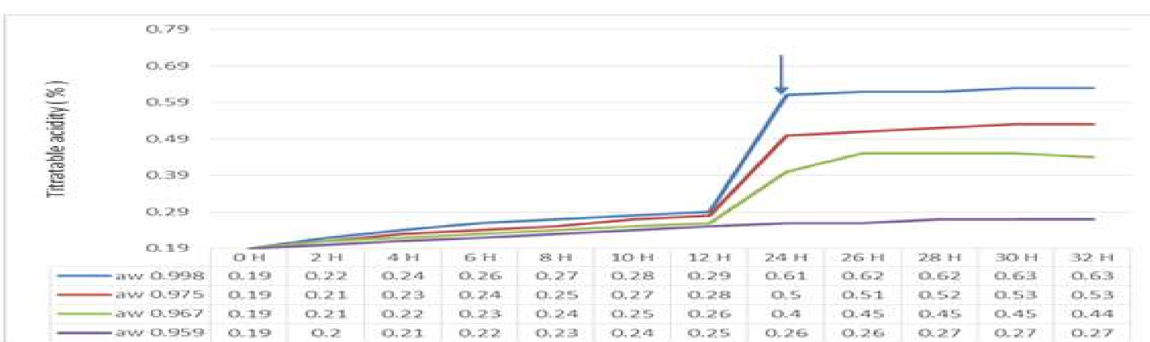
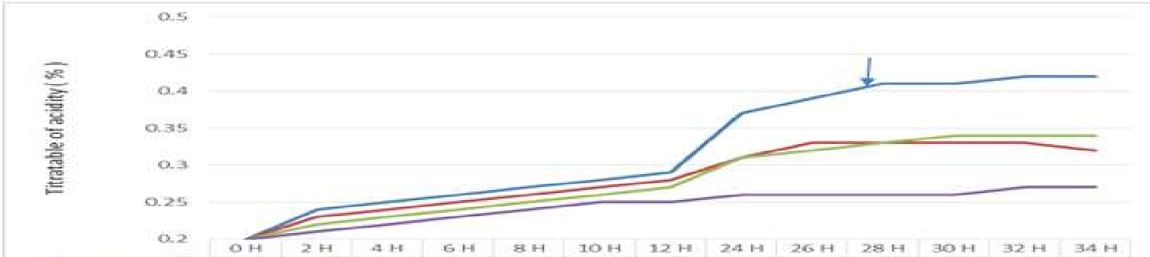


Fig .5. Effect of water activity (a_w) on increasing rate of developed titratable acidity (%) during growth of Lactococcus *lactis* subsp *cremoris* cultivated at 30°C in sterilized skim milk.

↑ Coagulated sample



	0 H	2 H	4 H	6 H	8 H	10 H	12 H	24 H	26 H	28 H	30 H	32 H	34 H
aw 0.998	0.2	0.24	0.25	0.26	0.27	0.28	0.29	0.37	0.39	0.41	0.41	0.42	0.42
aw 0.975	0.2	0.23	0.24	0.25	0.26	0.27	0.28	0.31	0.33	0.33	0.33	0.33	0.32
aw 0.967	0.2	0.22	0.23	0.24	0.25	0.26	0.27	0.31	0.32	0.33	0.34	0.34	0.34
aw 0.959	0.2	0.21	0.22	0.23	0.24	0.25	0.25	0.26	0.26	0.26	0.26	0.27	0.27

Fig. 6. Effect of water activity (a_w) on increasing rate in developed titratable acidity (%) during growth of *Lactobacillus casei* cultivated at 30°C in sterilized skim milk.

↑ **Coagulated sample**

In general reduction of the water activity causes a decrease of the rate of acid production.

All strains grow at water activity of 0.975 and 0.967 delayed acid productions, while at water activity 0.959 approximately no acidity was developed.

The effect was more intensity in the first few hours of incubation than in the final growth time.

This was especially evident in *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris* than *Lactobacillus casei* figures as shown in 4 , 5 and 6.

When *Lactococcus lactis* subsp *lactis* grow at a_w value of 0.998 (control), showed an exponential phase of acid production between 2nd and 24th hour of incubation reached a maximum of 0.53%.

Afterwards, the rate of acid production decreased between the 12th hour and 32th hour of incubation.

At a_w 0.975, this strain still gave an exponential phase of acid production, but with a considerable lower rate up to the 24th hour of the incubation reaching and developed titratable acidity of 0.50 %, on prolonging the incubation period , there was almost no increase in acid production.

On the other hand, with *Lactococcus lactis* subsp *lactis* the effect of water activity values of 0.967 and 0.959, was much more dramatic, acid production was completely stopped after 24th hour of incubation.

During exponential phase of *Lactococcus lactis* subsp *lactis* grow in sterilized skim milk at a_w 0.998, the increase in bacterial cell population was corresponded to the acid production, however at lower water activity growth did nertrtrtot correspond to acid production. Disagreement of growth with acid production have previously been reported by Turner and Thomas (1975) and Hassan and Richard (1990). This might be due to the decrease in water activity which restrained growth rate, but had less effect on lactose fermentation.

Reduction of the water activity from 0.998 through 0.975, 0.967 and 0.959 on the growth rates and acid production when *Lactococcus lactis* subsp *cremoris* and the *Lactobacillus casei* had approximately had the same effect obtained with *Lactococcus lactis* subsp *lactis* (Tables 2 and 3 and figures 2 , 3 ,5 and 6).

However, results indicated that *Lactobacillus casei* was less sensitive to decrease in water activity than *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris.* These differences suggest that same bacteria may not be found to grow at a_w below 0.959.

Although *Lactococcus lactis* subsp *lactis, Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* were able to grow at water activity value up to 0.959 the degree of reduction in growth rates and acid production varies with the degree of reduction in water activity.

CONCLUSION

These results indicated the importance of water activity as a variable which besides pH, temperature and nutritive substances of the medium play a fundamental role in growth and metabolism of a micro organism.

Having considered the obtained results showing the effects of reduced water activity on the growth rates and acid production of *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *cremoris* and *Lactobacillus casei* it is of interest to consider to what extent the findings may be generally applicable to other lactic acid bacteria which may be used in the dairy industry. The first general conclusion is that as water activity is reduced below an optimum level there is an increase in lag phase or latent period of growth, a decrease in the rate of growth and a decrease in the number of bacterial cells. In these respects the effects are very similar to those produced by reducing the incubation temperature below the optimum temperature.

On the other hand, it is likely that each bacterial strain has its own characteristics optimum water activity at which growth will occurs most rapidly.

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اثر درجة النشاط المائي علي نمو بكتريا حمض اللاكتيك

داليا جمال كامل ، نانيس حسنين جمعة ، دينا مصطفي عثمان و علي اسماعيل حسن

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تم دراسة تأثير الانخفاض في النشاط المائي علي النمو وإنتاج الحامض بواسطة *Lactococcus lactis* subsp *lactis* و *Lactococcus lactis* subsp *cremoris* و *Lactobacillus casei* عند تقييم نشاط مائي ٠.٩٩٨ ، ٠.٩٧٥ ، ٠.٩٦٧ و ٠.٩٥٩. وجد أن انخفاض النشاط المائي للوسط يعمل علي إطالة الطور التمهيدي للنمو وتسبب اختلافات كبيرة بالنسبة لمعدل النمو وكذلك العدد الكلي للخلايا وكذلك معدل إنتاج الحامض. وكان أكبر معدل نمو لوحظ عند نشاط مائي قدره ٠.٩٩٨ وقد كان أقصي عدد للخلايا بالنسبة للبكتريا *Lactococcus lactis* subsp *lactis* و *Lactococcus lactis* subsp *cremoris* و *Lactobacillus casei* ٨٠×١٠ ٦ مستعمرة/مل ، ٦٠×١٠ ٦ مستعمرة/مل و ٦٠×١٠ ٦ مستعمرة/مل بعد ٢٤ ساعة من التحضين علي التوالي. وعند نشاط مائي ٠.٩٧٥ كان أقصي معدل نمو للبكتريا *Lactococcus lactis* subsp *lactis* و *Lactococcus lactis* subsp *cremoris* و *Lactobacillus casei* ٤٠×١٠ ٥ مستعمرة/مل ، ٥١×١٠ ٥ مستعمرة/مل و ٥١×١٠ ٥ مستعمرة/مل علي التوالي بعد ٢٤ ساعة من التحضين. وتشير النتائج إلي أن *Lactococcus lactis* subsp *lactis* وصلت إلي أعلي معدل نمو لها وكان هذا المعدل أقل عن السلالتين الأخرتين. وان لكل سلالة بكتيرية لها درجة نشاط مائي مثلي حيث يكون النمو اسرع بالمقارنة بمعدل النمو علي اعلي او اقل نشاط مائي . وتشير النتائج المتحصل عليها الي ان قيمة النشاط المائي للمزرعة كان له نفس التاثير علي معدل الزيادة في الحموضة وأيضا علي معدل الزيادة في تكوين المستعمرات