Role of *Debaryomyces hansenii* yeast in improving the microbial and sensory properties of Monterey cheese

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Abstract

Debaryomyces hansenii yeast was grown in Malt Extract Broth medium at 30 °C for 5 days until the total count reached 5.6 $\times 10^8$ colony forming units/ milliliter, Monterey cheese was made from cow's milk and adding of starter bacteria *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp.*cremoris* with *D.hansenii* yeast as adjunct starter in 2% for each treatment included M1 (100% starter bacteria), M2 (50% starter bacteria + 50% live *D.hansenii*) and M3 (50% starter bacteria + 50% dead *D.hansenii)*,the ripening of Monterey cheese was done at 15°C and (85% relative humidity) for 56 days. The highest count of starter bacteria was 9.7 $\times 10^8$ cfu/g in M1 treatment after 3 days of ripening, the lowest number of starter bacteria was 6.9 $\times 10^8$ cfu/g in M2 treatment. The highest count of *D.hansenii* yeast was 4.3 x10^8 cfu/g after 3 days of ripening in M2 treatment, while the lowest count was 2.6 $\times 10^8$ cfu/g in the same treatment after 56 days of ripening, no yeast was found in the treatments that had dead yeast cells, all treatments under study were decreased in the total count of coliform bacteria, *Staphylococcus aureus* and psychrotrophic bacteria after 14 days as they were within the standard specifications of these groups of microorganisms, Then reached to zero in the late time of ripening. M2 showed the best results of sensory evaluation in taste, flavor, textures, color and bitterness after 56 days of ripening.

Keywords: ***Debaryomyces hansenii*, Monterey cheese, microbial properties, sensory properties.**
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دور خميرة *Debaryomyces hansenii* في تحسين الخصائص الميكروبية والحسية في جبن المونتري

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الخلاصة

نُميت خميرة *Debaryomyces hansenii* في وسط Malt Extract Broth في درجة حرارة 30 مْ لمدة 5 أيام إلى ان بلغ العدد الكلي 5.6$\times 10^8$وحدة مكونة مستعمرة/ مليليتر، صُنّع جبن المونتري من الحليب البقري وبإضافة كل من بادئ البكتريا *Lactococcus lactis* subsp. *lactis* و *Lactococcus lactis* subsp. *cremoris* مع خميرة *D.hansenii* بادئاً ثانوياً وبنسبة 2% لكل معاملة وتضمنت M1 (100% بادئ البكتريا) ، M2 (50% بادئ البكتريا + 50% بادئ خميرة حية) ، M3 (50% بادئ البكتريا + 50% بادئ خميرة ميتة) ، ونضج جبن المونتري في درجة حرارة 15 مْ ورطوبة نسبية 85 % مدة 56 يوم. بلغ اعلى عدد كلي لبكتريا البادئ 9.7$\times 10^8$ و.م.م/غم في المعاملة M1 بعد 3 أيام من الإنضاج، أما أقل عدد لبكتريا البادئ فكانت في المعاملة M2 إذ بلغت 6.9$\times 10^8$و.م.م/غم. بلغ العدد الكلي لخميرة *D.hansenii* اعلى قيمة محققاً 4.3$\times 10^8$ و.م.م/ غم بعد 3 أيام من الإنضاج في المعاملة M2 أما اقل قيمة فقد بلغت 2.6$\times 10^8$ و.م.م/غم في المعاملة ذاتها بعد 56 يوم من الإنضاج، ولم يظهر وجود للخميرة في المعاملات التي أضيفت لها خلايا الخميرة الميتة. أظهرت جميع المعاملات قيد الدراسة انخفاضاً في العدد الكلي لبكتريا القولون، العنقوديات الذهبية والبكتريا المحبة للبرودة بعد 14 يوم إذ كانت ضمن المواصفة القياسية المحددة لهذه المجاميع من الأحياء قيد الدراسة ولكافة المعاملات ثم تصل إلى الصفر في بقية مدد الإنضاج. أظهرت المعاملة M2 أفضل النتائج في كل من الطعم والنكهة، النسجة والقوام، اللون والمرارة بعد 56 يوم من الإنضاج.

الكلمات المفتاحية: *Debaryomyces hansenii*، جبن المونتري، الخصائص الميكروبية، الخصائص الحسية

Introduction

Debaryomyces hansenii yeast is a mesophilic yeast, the best temperature for its growth is 30° C , It had a pH range 4-6 and water activity of 0.99. It was classified as saline-tolerant for its ability to grow in salt concentration ranges from 3-5%, as well as its ability to grow in aerobic and anaerobic conditions, but the growth in anaerobic is less than aerobic, it is heterotroph since it is free from chlorophyll. *D.hansenii* yeast had several antimicrobial properties, including nutrient competition, pH change, production of high concentrations of ethanol alcohol, destruction of bacterial toxins by yeast proteolytic enzymes and inhibition of intestinal cell binding (1). Mehlomakulu reported(2). The *D.hansenii* had enzymatic degradation of lipids (triglyceride to diglyceride, then to free fatty acids and glycerol which is necessary to develop cheese flavor), the main factors of lipid degradation in cheese include the lipase enzyme produced from the starter bacteria (lactic acid bacteria) and the adjunct starter (*D.hansenii* yeast) to improve the flavor of cheeses, the short-chain fatty acids contribute directly to the enhancement of flavor, but fatty acids can act as raw materials to produce a wide range of other flavor compounds such as esters and methyl ketones that give distinctive flavors to the cheeses. *Debaryomyces* are characterized as safe, non-pathogenic and salt tolerant properties, and can be found in low water activity such as sea water, meat, cheese, fruits and soil (3). *D.hansenii* yeast was used as important eukaryotic starter for its role in food manufacturing and production. It was flavor enhancer in food products varieties for its ability to produce flavor compounds and production of lipase enzyme, which developed flavor during meat process and fermentation (4). Cheeses are common foods in many countries because of their health benefits associated with their consumption. The health benefits of cheese include the natural, therapeutic foods properties, an anti-tumor food, shown to reduce diabetes as well as a rich source of dietary calcium, vitamins, phosphorus and high nutritional value protein as well as other ingredients (5, 6). Monterey cheese is made from cow's milk and is one of the semi-dry American cheeses that ripened from 1-6 months (7). Monterey cheese is a concentrated food made from liquid bovine milk. It was made from pasteurized milk to kill pathogenic microorganisms and reduces the number of other microorganisms, encouraged the starter growth, which allowing the flavor development (8). The aim of this study to add *D.hansenii* yeast as adjunct starter in manufacture of Monterey cheese and studying the microbiological and sensory effects during maturation.

Materials and Methods

Monterey cheese was made from cow's milk after pasteurization at 65° C for 30 minutes and then milk was cooled to 32°C, *Lactococcus lactis subsp lactis* and *Lactococcus lactis subsp. cremoris* were added, and *D.hansenii* yeast was added as adjunct starter in 2%, for each treatment, included M1 (100% starter bacteria), M2 (50% starter bacteria + 50% live yeast), and M3 (50% starter bacteria and 50% dead yeast), 0.1% of microbial rennet made from *Mucor miehei* was added ,wait until coagulation, curd was cut and cooked at 32°C for 30 minutes, whey separated then fill the cheese in molds and salt added in 2%, cheese molds pressed for 24 hour, paraffin wax was heated at 118°C for 5 seconds then covered cheese (9). Ripening was carried out at 15°C with 85% relative humidity for 56 days. The total count of starter bacteria was estimated using the M17 medium according to the method given in (10). The total count of *D.hansenii* yeast was estimated using the Rose Bengal Agar medium according to the method in (11). Coliform bacteria and psychrotrophic bacteria were estimated according to the method in (12). Total count of *Staphylococcus aureus* bacteria was tested according to the method in (13). Al-Dahhan(14) was followed In testing of sensory properties included (taste, flavor, tissue, texture, color and bitterness) of the Monterey cheese during the ripening periods.

Results and Discussion

Fig. (1) showed the starter bacteria count in Monterey cheese during ripening periods. The highest count of starter bacteria at 3 days of ripening was 9.7×10^8 cfu/ g for (M1) treatment, the lowest starter bacteria count was 6.9×10^8 cfu/ g belong to M3 treatment.

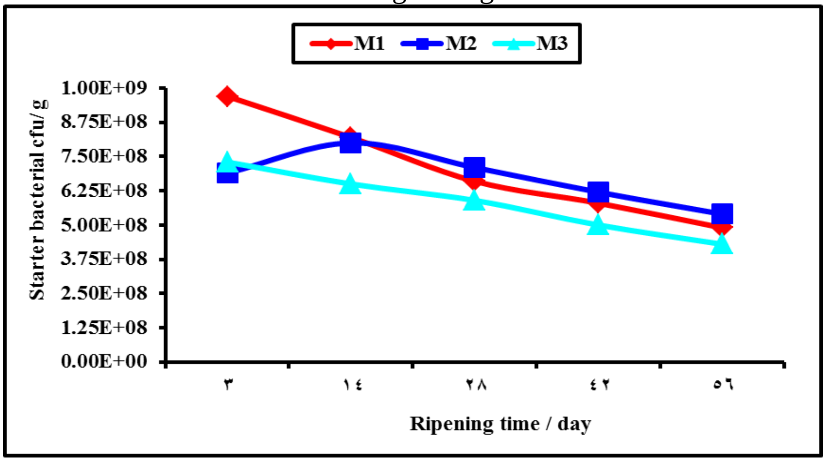

Fig. (1) Starter bacteria count in Monterey cheese treated with *D.hansenii* yeast

The results showed an increase in the starter bacteria count (8×10^8 cfu/ g) in the Monterey cheese for (M2) treatment after 14 days of ripening period. In contrast to the other treatments, the increasing may be due to the ability of yeast to consume lactic acid, which raised the pH, and provided better conditions to starter bacteria growth (2). M2, M3 treatments showed decreasing in starter bacteria count after 56 days of ripening reached (5.4×10^8, 4.3×10^8) cfu/ g respectively. Decreasing in starter bacteria counts may be attributed to nutrient consumption, low water activity and high salt concentration in cheese and may also decrease temperature 15° C at maturity, as the two types of *Lactococcus lactic ssp lactic* and *Lactococcus lactic ssp cremoris* 30° C, which reduces the growth of lactic acid bacteria (15). Fig. (2) showed the *D.hansenii* count for Monterey cheese on ripening periods, the highest *D.hansenii* count at 3 days was (4.3×10^8cfu/ g) for M2 treatment.

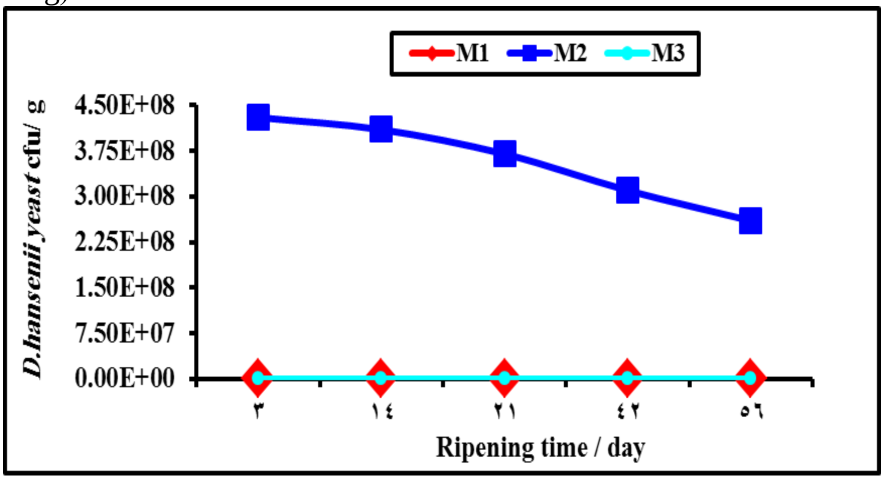

Fig. (2) *D.hansenii* count in Monterey cheese treated with *D.hansenii* yeast

D.hansenii yeast count were lowered in Monterey cheese during late ripening periods and was 2.6×10^8cfu/ g in the end of ripening time for M2 treatment. Decreasing in *D.hansenii* cell numbers may be due to low humidity of cheese (16), or because low temperature in cheese maturation which reduces the effectiveness of yeast (17). *D.hansenii* is mesophilic yeast and tolerant to high salt concentrations and low water activity (18). Fig. (3) showed coliform bacteria count in Monterey cheese during ripening periods. The higher count in 3 days was 8×10^2 cfu/ g for the treatment (M2), while the lower count had reached 3×10^2 cfu/ g for the treatment M1.

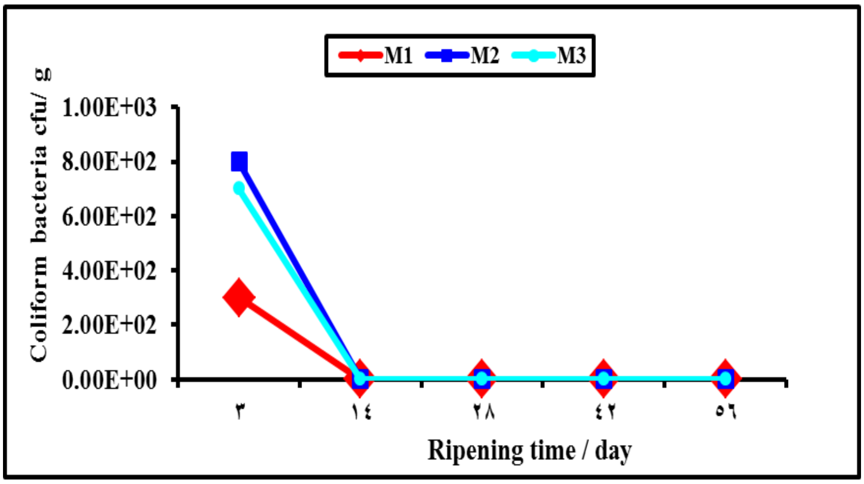

Fig. (3) Coliform bacteria count in Monterey cheese treated with *D.hansenii* yeast

The results showed decreasing of coliform bacteria count for all treatments in the Monterey cheese during the ripening period to zero after 14 days of ripening, this may be due to the ripening conditions and production of bacteriocin, lactic acid and acetic acid by the starter bacteria, as well as the production of lethal toxins from yeast in a pH ranging from 3-6, destroyed Pathogenic microorganisms such as coliform bacteria (19). Fig. (4) Illustrated *Staphylococcus aureus* count in the Monterey cheese during maturation. The highest count was 7×10^2 cfu/ g for M2 treatment, while the lowest count had reached (4×10^2 cfu/ g) for M1 treatment after 3 days of ripening.

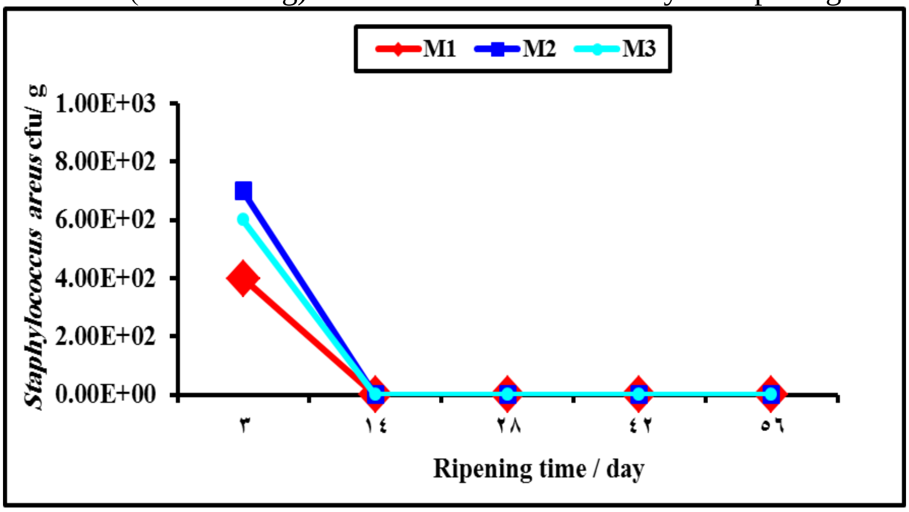

Fig. (4) *Staphylococcus aureus* count in Monterey cheese treated with *D.hansenii*

The results showed lowering in *Staphylococcus aureus* count for all treatments at early ripening periods to zero after 14 through the days of maturation. The reason for this may be yeast's ability to raise pH through the consumption of lactic acid, as well as production of inhibitory compounds from yeast or starter bacteria, and the consumption of nutrients necessary for growth, such as vitamins, amino acids, sugars and minerals (2). Fig.(5) showed psychrotrophic bacteria count during ripening periods. The higher count was 8×10^2 cfu/ g for M2 treatment, while the lowest had reached 5×10^2 cfu/g for M1 treatment after 3 days of maturation.

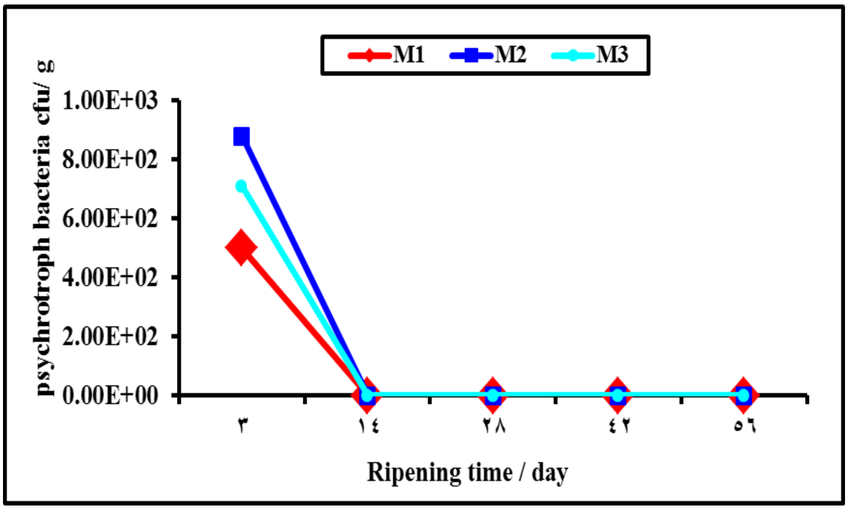

Fig. (5) Psychrotrophic bacteria in Monterey cheese treated with *D.hansen*ii yeast

The results indicated decreasing psychrotrophic bacteria count for all treatments in Monterey cheese to zero after 14 days of ripening, this may be due to the ability of starter bacteria and adjunct yeast to produce unsuitable organic acid to psychrotrophic bacteria growth on ripening periods, as well as the low water activity that prevents growth in maturation (20). The sensory evaluation results of cheese treatments showed in Table (1), the taste and flavor traits in M2 treated decreased during early double weeks of maturation compared with control (M1 just starter bacteria) reached 34/45, then increased at late ripening periods gotten 42/45 on 56 days of maturation when M1 treated was 35/45. The decrease in M2 treatment firstly may be due to the production of protein compounds with taste and flavor undesirable as a result of protein degradation by the starters either the height may be resulting of consumption of these compounds (2). The results showed highly values of sensory evaluation of different cheese samples at the end of the 56-day ripening periods and were directly proportional to the yeast concentration used. The M2 treatment had the highest value, indicated used of *D.hansenii* yeast had an influential role in improving taste and flavor of cheese products. Consumption of lactic acid by *D.hansenii* yeast and released ammonia during the decomposition of proteins and amino acids led to increased pH on the surface of cheese and would encouraged starter bacteria growth, which developed flavor and appearance (21). Mamo (22) reported that tissue and texture were affected by casein and fat decomposition as well as the physical changes caused by acidity changes and the distribution of salt particles in cheese during the maturation process, which protein degraded to simple compounds that give softness to the cheese tissue and texture. The tissue and texture traits decreased after 14-28 days of maturation, then increased at the end of the ripening periods as shown in Table 1, which reached 31/35, 27/35 for M2, M3 treatments respectively. The decline in scores may be due to the degradation of proteins and fats as a result of starter activity into simple compounds which would gave a softness (23). The raised of tissue and texture values may be the result of the consumption of *D.hansenii* yeast of simple compounds produced from the degradation of proteins and fats as a result of the activity of the starter used (2). The results showed that the increase in the degrees of sensory evaluation of the different cheese treatments at the end of the 56-day ripening period is directly proportional to the yeast state used. The M2 treatment has the highest value. Use of *D.hansenii* yeast may have an influential role in improvement of tissue and textures of cheeses, as the contribution of *D.hansenii* yeast with lactic acid bacteria to give the tissue and texture of cheese (24).

Table (1) Sensory evaluation in Monterey cheese treated with *D.hansenii* yeast

Maturation (Day)	Treatment	Taste & Flavor (45)	Tissue & Textures (35)	Color (10)	Bitterness (10)
3	M1	45	35	10	10
	M2	45	35	10	10
	M3	45	35	10	10
14	M1	37	28	10	10
	M2	36	27	10	8
	M3	38	27	10	9
28	M1	36	26	10	10
	M2	34	25	10	9
	M3	37	27	10	10
42	M1	35	27	8	10
	M2	41	32	10	10
	M3	38	28	9	10
56	M1	35	28	8	10
	M2	42	31	10	10
	M3	39	27	9	10

The color trait was not affected at early 4 weeks of ripening for all treatments as given Table (1), then it decreased M1 and M3 treatments comparing final weeks of maturation and achieved 8/10 and 9/10, respectively, comparing with M2 which as not affected. *D.hansenii* yeast may have an influential role in improving the color of the cheese; it contributes with the lactic acid bacteria to give a distinctive color of cheese (25). Bitterness trait values were decreased after 14 days of ripening for M2 and M3 treatments and achieved 8/10 and 9/10, respectively comparing with control treatment (M1), as shown in Table (1) bitterness was not affected with maturation, then increased all values of treatments at end of ripening periods recording 10/10. The decline in values at the beginning of the ripening period may be due to the role of starter in the degradation of proteins into high partial lipid peptides, which would give bitterness in taste (22). Raised values at the end of maturation may be due to the role of starter enzymes produced, which analyzed high molecular weights peptides and produced bitter less peptides and free amino acids (26).

References

1. Al-Asadi, E. M. M. (2016). Effect of *Debaryomyces hansenii* in fermented berger production. MSc. Thesis. Faculty of Agriculture, University of Baghdad.
2. Mehlomakulu, N. N. (2011). Yeasts as adjunct starter cultures in cheese making. Master Thesis, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein.
3. Reyes-Becerril, M.; Ascencio-Valle, F.; Meseger, J.; Tapia-Paniagna, S. T.; Morinigo, M. A. & Esteban, M. A. (2012). *Debaryomyces hansenii* L2-enriched diet enhances the immunity status, gene expression and intestine functionality in gilthead sea bream (*Sparu saurata* L.). J. Aquac. Res., 34:1107-1118.
4. Papagora, C.; Roukas, T. & Kotzekidou, P. (2013). Optimization of extracellular lipase production by *Debaryomyces hansenii* isolates from dry-salted olives using response surface methodology. J. Food and BioProducts Processing. 91(4): 413-420.
5. CDIC (Canadian Dairy Information Centre). (2014). Global consumption per capita of dairy products: Totalcheeseconsumption. http://www.dairyinfo.gc.ca/index_e.php?s1=dfffcil&s2=cons&s3=consglo&s4=tc-ft Accessed January 5, 2015.
6. Quigley, L.; O'sullivan, O.; Stanton, C.; Beresford, T. P.; Ross, R. P.; Fitzgerald, G. F. & Cotter, P. D. (2013). The complex microbiota of raw milk. FEMS Microbiol. Rev., 37(5): 664-698.

7. Al-Shati, Z. R. K. (2012). Use of ozone to prolong the mineral life of Monterey cheese. Master Thesis. Faculty of Agriculture, University of Baghdad. Iraq
8. Fox, P. F.; Guinee, T. P.; Cogan, T. M. & McSweeney, P. L. (2017). Chemistry of milk constituents. In Fundamentals of Cheese Science. Springer, US. PP. 71-104.
9. Al-Dahhan, A. H. S. (1983). Cheese industry and its types in the world. Dar Al Hekma Press, Mosul, Iraq.
10. Al-Hadithi, F. N. I. (2009). The use of unconventional cookers for the production of table cheese. Faculty of Agriculture, University of Baghdad. Iraq.
11. Corral, S.; Salvador, A.; Belloch, C. & Flores, M. (2014). Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii* yeast. Food Control., 45:1-7.
12. APHA (American Public Health Association), (1978). Standard Methods for the Examination of Dairy Products.14th ed. Marth. E.H. (ed.,). American Public Health Association, Washington. D.C.
13. Speak, M. (1984). Compendium of method for the microbiological examination for food. 2nd Ed. Washington, D.C. USA.
14. Al-Dahhan, A. H. (1977). A Study of Visible Characteristics of Cheese. Ph. D. Thesis, University Glasgow, Scotland.
15. Akpinar, A.; Yerlikaya, O.; Kinik, Ö.; Kahraman, C.; Korel, F. & Uysal, H. R. (2017). The volatile compounds, free fatty acid composition and microbiological properties of sepet cheese packaged with different modified atmosphere conditions. Kafkas Univ. Vet. Fak. Derg., 23(1): 123-129.
16. Andrade, M. J.; Thorsen, L.; Rodriguez, A.; Cordoba, J. J. & Jespersen, L. (2014). Inhibition of ochratoxigenic moulds by *Debaryomyces hansenii* strains for biopreservation of dry-cured meat products. Int. J. Food Microbiol., 170(17): 70-77.
17. Breuer, U. & Harms, H. (2006). *Debaryomyces hansenii* an extremeophilic yeast with biotechnological potential. Yeast., 23(6):415-437.
18. Banjara, N.; Suhr, M. J. & Hallen-Adams, H. E. (2015). Diversity of yeast and mold species from a variety of cheese types. Curr. Microbiol., 70(6): 792-800.
19. Çorbacı, C. & Uçar, F. B. (2017). Production and optimization of killer toxin in *Debaryomyces hansenii* strains. Braz. Arch. Biol. Technol., 60: e17160339.
20. Andrade, M. A.; Cordoba, J. J.; Casado, E. M.; Cordoba, M. G. & Rodriguez, M. (2010). Effect of selected strains of Debaryomyces hansenii on the volatile compound production of dry fermented sausage "salchichón". Meat Sci., 85(2): 256-264.
21. Gori, K.; Sorensen, L. M.; Petersen, A. M.; Jespersen, L. & Arneborg, N. (2012). *Debaryomyces hansenii* strains differ in their production of flavor compounds in a cheese-surface model. Microbiologyopen.,1(2):161-168.
22. Mamo, A. (2017). Cheddar cheese characterization and its biochemical change during ripening. Int. J. Adv. Sci. Res. Manag., 2(5): 53-59.
23. Sorensen, L. M.; Gori, K.; Petersen, M. A.; Jespersen, L. & Arneborg, N. (2011). Flavour compound production by *Yarrowia lipolytica, Saccharomyces cerevisiae and Debaryomyces hansenii* in a cheese-surface model. Int. Dairy J., 21(12): 970-978.
24. Irlinger, F. & Mounier, J. (2009). Microbial interactions in cheese: implications for cheese quality and safety. Curr. Opin Biotechnol., 20:142–148.
25. Banjara, N. (2014). Debaryomyces hansenii: A foodborne yeast that produces Anti-candida killer toxin. MSc. Thesis, The Graduate College at the University of Nebraska.
26. Al-Abbtan, A. A. (2007). Studying the proteolytic potential of Bifidobacterium spp. In different types of fermented milk and montropical cheese. Ph.D thesis. Faculty of Agriculture, University of Baghdad.