



Biomass production from *Kluyveromyces spp.* by using cheese whey

Karzan T. Mahmood

Food Science Dept. Faculty Of Agricultural Science University Of Sulaimani , Kurdistan Region- Iraq

Email: Karzan73@yahoo.co.uk

Article info

Original: 8 Apr. 2015
 Revised: 18 May 2015
 Accepted: 31 May 2015
 Published online:
 20 Dec. 2015

Key Words:

Biomass, Yeast,
Kluyveromyces, Cheese
 whey.

Abstract

Cheese whey was used as a medium for producing yeast biomass from *Kluyveromyces lactis* DSM 70800 and *Kluyveromyces fragilis*. The best conditions for yeast biomass production were 35°C with agitation speed of 150 and 200 rpm for *Kluyveromyces fragilis* and *Kluyveromyces lactis* DSM 70800 respectively. Both strains can grow in a wide range of pH. The maximum biomass yield recorded when whey with pH 6.5 was used. The crud protein based on dry weight was relatively high and the crud protein % of *Kluyveromyces lactis* DSM 70800 (50.56%) was insignificantly higher than that was obtained for *Kluyveromyces fragilis* (49.53%) while the protein yield was insignificantly higher for *Kluyveromyces fragilis*. On the other hand, the essential and non-essential amino acids were present in a various amount, among these amino acids arginine was present in high level in both strains which has the good nutritive value especially for children.

1. Introduction

Yeasts have been the most important group of microorganisms used by human, and they are natural organisms that can be used as suitable biomass sources with multiple uses, such as in the food industry, to improve and increase the nutritive value of the foods. In animal feeding, it can be used as a protein supplements for ruminant and non-ruminant animals, and also for preventions caused by pathogen infections both due to the barrier effect and by enhancing the host protective mechanisms (Jouany, 2001 cited in Pop et al. 2004). Yeast biomass was also used for production and isolation of specific enzyme (Mahmood, 2010). The production of microbial biomass is the technical manufacture of the cell mass of microorganisms and it is cost gainful because it uses industrial by-products such as molasses and malt for the biosynthesis process (Anghel, 1991 cited in Pop et al. 2004). *Kluyveromyces spp.* defined as Generally Recognize As Safe (GRAS) microorganisms (Mahoney and wildder, 1989), and it can be used for cell biomass production by using cheese whey as a by-product of cheese manufacture which contains many of the nutrients from milk and can be almost completely utilized for different purposes (ethanol, yeast biomass, lactic acid) (Tratnik, and Mlijeko cited in Grba *et al.* 2002). Cheese whey can also be used for biomass production from *Kluyveromyces* at various rates according to the growth conditions. Therefore,

investigation of the optimization conditions for biomass production should be concerned in order to improve its productivity process, maximize the yield and reducing the production cost. The aims of this study were to investigate the optimal condition of temperature, agitation speed and whey pH for cell biomass production from two species of *Kluyveromyces* and also to measure the protein and amino acids content in produced biomass.

2. Materials and Methods

2.1. Cheese whey

Cheese whey was provided from food science dept. /faculty of agricultural science/ Sulaimani University. Whey proteins were removed by thermal treatments (95°C for 30 min)

2.3. Microorganisms

Stock cultures of *Kluyveromyces lactis* DSM 70800 (K. lactis) and *Kluyveromyces fragilis* (K. fragilis) were obtained from the microbiological resources center (MIRCEN-Egypt), and maintained at 4°C on potato dextrose agar slants, which were used to inoculate the seed culture medium (cheese whey) and incubated at 25°C and 100rpm agitation speed . The growing active yeast was employed to inoculate biomass production medium.

2.4. Biomass production

To obtain the yeast biomass, cheese whey was used in batch cultures and inoculated with 1% (v/v) of the seed culture. The cells (wet weight) were harvested by centrifugation at 4000 rpm (2400 ×g) for 10 min. (Szczodark, 2000). Obtained biomass was dried by mini-spray drier (BUCHI mini spray dryer B-290 Switzerland) for protein determination.

2.5. Protein and amino acid determination

Micro Kjeldahl method according to A.O.A.C, 2000 was used for cheese whey and biomass proteins determination. Amino acids for biomass protein were determined. Automatic amino acid analyzer (AAA 400 INGOS Ltd, lab-Faculty of Agriculture-Cairo University) was used for the determination. Protein yield from yeast biomass was calculated according the following formula (Murad *et al.* 1992).

Protein yield (g l⁻¹) = {crude protein % × biomass yield (g l⁻¹)} /100.

2.6. Fat determination

Gerber method (A.O.A.C, 2000) was used to determine the percentage of remaining milk fat in the whey.

2.7. Effect of agitation on biomass production

The effect of agitation on biomass production was determined by incubation of the yeasts at various shaking speeds from 50 to 200 rpm at the 25°C for 24h.

2.8. Effect of Temperature on biomass production

The effect of temperature on biomass production was determined by incubation of the yeasts at various temperature degrees ranging from 20 to 60°C for 24h. The optimum agitation rate was used (150 rpm).

2.9. Effect of pH on biomass production

The effect of cheese whey pH which was adjusted by using concentrated lactic acid (92-94%) on biomass production was determined by incubation the yeasts at various pH values ranging from 3.5 to 7.0 at the optimum temperature and agitation rate for 24h.

2.10. Statistical analysis

The data were statistically analyzed according to the method of analysis of variance as a general test. Factorial experiment with three replications was used by XLSTAT program ver. 7.5.2 and conducted using Complete Randomized Design (CRD). All possible comparisons among the means were carried out by using Least Significant Difference (LSD) test at significant level of 0.05 or 0.01 after they show their significance in the general test.

3. Results and discussion

3.1. Whey compositions

Table 1 shows the average of whey compositions that was used for biomass production, according to its average compositions, cheese whey contains approximately 93% water and 7% total solids which protein comprise only 0.24% of it.

3.2. Effect of agitation speed.

From figure 1, it can be seen that the biomass yield increased by the increasing of agitation speeds, and the maximum wet biomass was produced when 150 and 200 rpm were used for *Kluyveromyces fragilis* and *Kluyveromyces lactis* DSM 70800 respectively, above 200 rpm decreasing in biomass yield was observed.

significant differences ($p \leq 0.05$) for biomass production was observed between yeast strains, the maximum biomass (17.01 g l^{-1}) was produced by *Kluyveromyces fragilis* compared with (10.72 g l^{-1}) for *Kluyveromyces lactis* DSM 70800.

Fadel and Degheidi (1998) reported that the maximum dry biomass yield (27.8 gm l^{-1}) from *Kluyveromyces fragilis* KHF-98 was obtained at 230 rpm after 18 h incubation by using whey permeate with inoculum size of 5%. Moeini, et al. (2004) showed that the agitation speed of 180 rpm was enough to produce maximum dry biomass yield of 11.79 gm l^{-1} by *Kluyveromyces lactis*, whereas Rech and Ayub (2007) observed that the biomass concentration reached its maximum value of 19.5 gm l^{-1} at 600 rpm after 15 h of incubation during the growing of *Kluyveromyces marxianus* on cheese whey medium in the batch culture. On the other hand, maximum production of wet biomass (22.67 gm l^{-1}) was obtained at 200 rpm after 24 hours of incubation in the whey medium by Mahmood (2010).

3.3. Effect of temperature

Biomass production from both species was increased with increasing temperature and gave maximum wet biomass production of 20.43 and 15.87 gm l^{-1} for *Kluyveromyces fragilis* and *Kluyveromyces lactis* DSM 70800 at 35°C respectively, and a significant ($p \leq 0.05$) increase in biomass production between two yeast species was observed. The yeast biomass was rapidly decreased above 35°C for both two species (figure 2).

This result is closer to that was obtained by Fadel and Degheidi (1998) and Genari et al. (2003) for producing biomass from *Kluyveromyces fragilis* KHF-98 and *Kluyveromyces lactis* respectively. It has been reported that the maximum dry biomass from both *Kluyveromyces lactis* and *Kluyveromyces marxianus* was produced at 25°C (Moeini, et al. 2004). Whereas, Mahmood (2010) showed that the highest biomass production from *Kluyveromyces lactis* DSM 70800 was obtained at 30°C . Raimondi et al (2013) shows that the optimum temperature for *Kluyveromyces marxianus* L3 growth falls generally around 42°C for.

3.4. Effect of whey pH

Data presented in figure 3 shows that both strains can grow in a wide range of pH. The biomass yields were increased by increasing whey pH up to 6.5. The maximum wet biomass production was obtained at pH of 6.5. and a significant ($p \leq 0.05$) differences for biomass production (21.03 and 15.65 gm l^{-1} for from *Kluyveromyces fragilis* and *Kluyveromyces lactis* respectively) has been seen between these two strains.

These results are closer to that was finding for *Kluyveromyces fragilis* when grown continuously on whey permeate, the cell mass yield remained constant at pH 3.9-5.1 (Shay and wegner 1986). Zayed (1991) reported that the best pH for *Kluyveromyces fragilis* growth on reconstituted skim milk whey ranged from 4.0-6.0, whereas the maximum biomass yield for *Kluyveromyces Lactis* NRRL1137 grown on whey permeate was obtained at pH 6.0 (Murad, et al. 1992).

3.5. Crud protein and protein yield of yeasts.

Table 2 shows that the percentage of crud protein based on dry weight was relatively high, crud protein % of *Kluyveromyces lactis* DSM 70800 was insignificantly higher than that was obtained by *Kluyveromyces fragilis*, while the protein yield also insignificantly higher for *Kluyveromyces fragilis* compared with *Kluyveromyces lactis* DSM 70800. Murad *et al* (1992) illustrated that the maximum crude protein for *Kluyveromyces Lactis* NRRL1137 was obtained when inoculation of 1% was used with a minimum protein yield, whereas the crude protein of 47.53 and 38.34% was obtained for *Kluyveromyces fragilis* KHF-98 and *Kluyveromyces marxianus* by Fadel and Degheidi (1998) and Somaye *et al* (2008) respectively. On the other hand higher protein content (53.3% DW) was obtained by Carrasco *et al* (1991) for *Kluyveromyces marxianus* va. *lactis* strain. The gained protein is satisfying the demand to use as a rich protein source in feeding.

3.6. Amino acid composition of yeasts.

Table 2 summarized the essential and non-essential amino acid compositions of both yeast strains. The essential amino acids were present in a various amount (Figure 4), among these amino acids arginine was present in a high level in both strains which has the good nutritive value especially for children. Non essential amino acids aspartic, glutamic and glycine were present in high levels. The same results were obtained for *Kluyveromyces Lactis* NRRL1137 grown on whey permeate by Murad *et al* (1992). On the other hand, phenylalanine from essentials and tyrosine from non-essentials are showed the lowest level of all detected amino acids for both strains. It is well known that the wheat and most cereals are very low in lysine and threonine, thus the concentration of these amino acids in *Kluyveromyces fragilis* indicates as the potential value of its biomass as a good supplement to cereal diets.

Table 1: cheese whey compositions

| Components | Percentage (%) |
|-------------|----------------|
| Water | 93± 0.5 |
| Total solid | 7± 0.5 |
| Fat | 0.1± 0.05 |
| Protein | 0.24± 0.04 |
| SNF | 6.9± 0.5 |

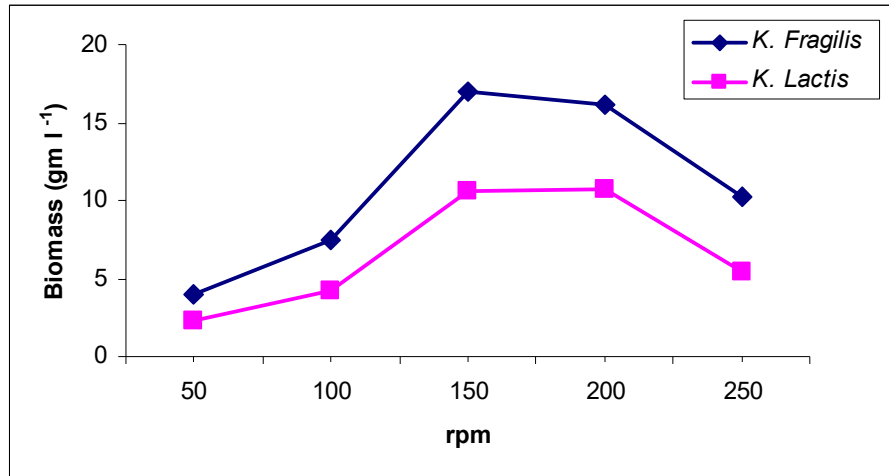


Figure1: Biomass production at different agitation speeds.

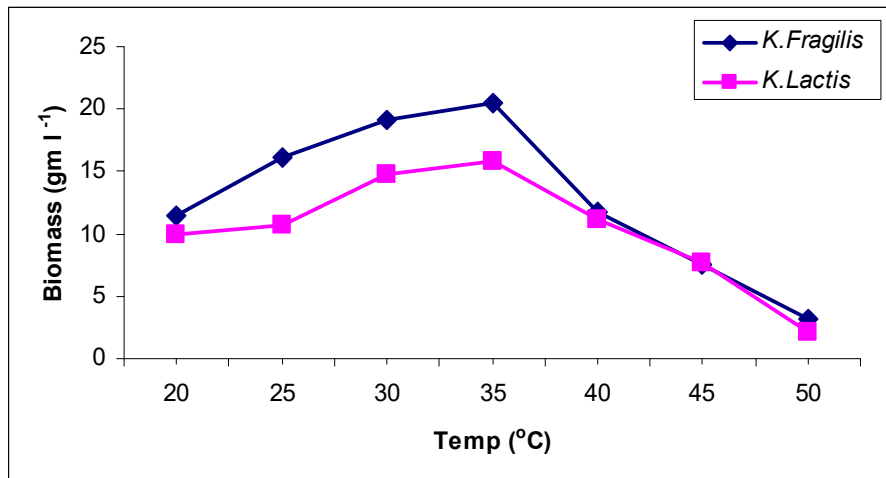


Figure2: Biomass production at different incubation temperature.

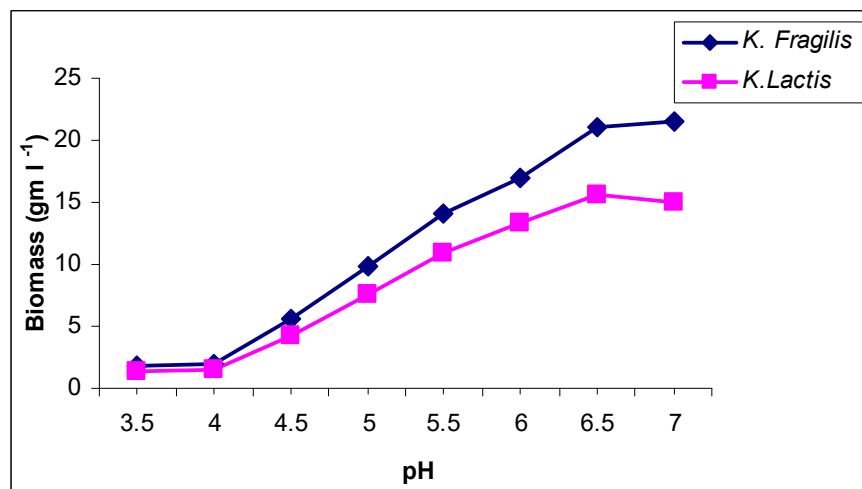


Figure3: Biomass production at different whey pH.

Table 2: Amino acid profile, %protein and protein yield of *Kluyveromyces lactis* DSM 70800 and *Kluyveromyces fragilis*.

| Yeast spp. | Biomass yield g l ⁻¹ | Crud protein % | Protein yield g l ⁻¹ | Amino acids | | | |
|---------------------------------------|------------------------------------|-------------------|------------------------------------|-----------------------|-------|---------------------------|------|
| | | | | Essential amino acids | % | Non-essential amino acids | % |
| <i>Kluyveromyces fragilis</i> | 5.46 | 49.53 | 2.70 | Val. | 3.12 | Asp. | 4.89 |
| | | | | Ile. | 2.31 | Ser. | 2.46 |
| | | | | Lys. | 2.37 | Glu. | 4.15 |
| | | | | (Arg.)* | 53.50 | Ala. | 3.67 |
| | | | | Leu. | 2.80 | Tyr. | 0.72 |
| | | | | Phe | 0.46 | Gly. | 3.83 |
| | | | | Thr. | 1.94 | His. | 2.35 |
| <i>Kluyveromyces lactis</i> DSM 70800 | 4.07 | 50.56 | 2.06 | Val. | 2.36 | Asp. | 3.51 |
| | | | | Ile. | 1.34 | Ser. | 1.85 |
| | | | | Lys. | 1.70 | Gly. | 2.68 |
| | | | | (Arg.)* | 61.24 | Tyr. | 0.46 |
| | | | | Leu. | 2.24 | Ala. | 2.79 |
| | | | | Phe. | 0.60 | Glu. | 3.23 |
| | | | | Thr. | 1.47 | His. | 2.15 |

*conditionally essential

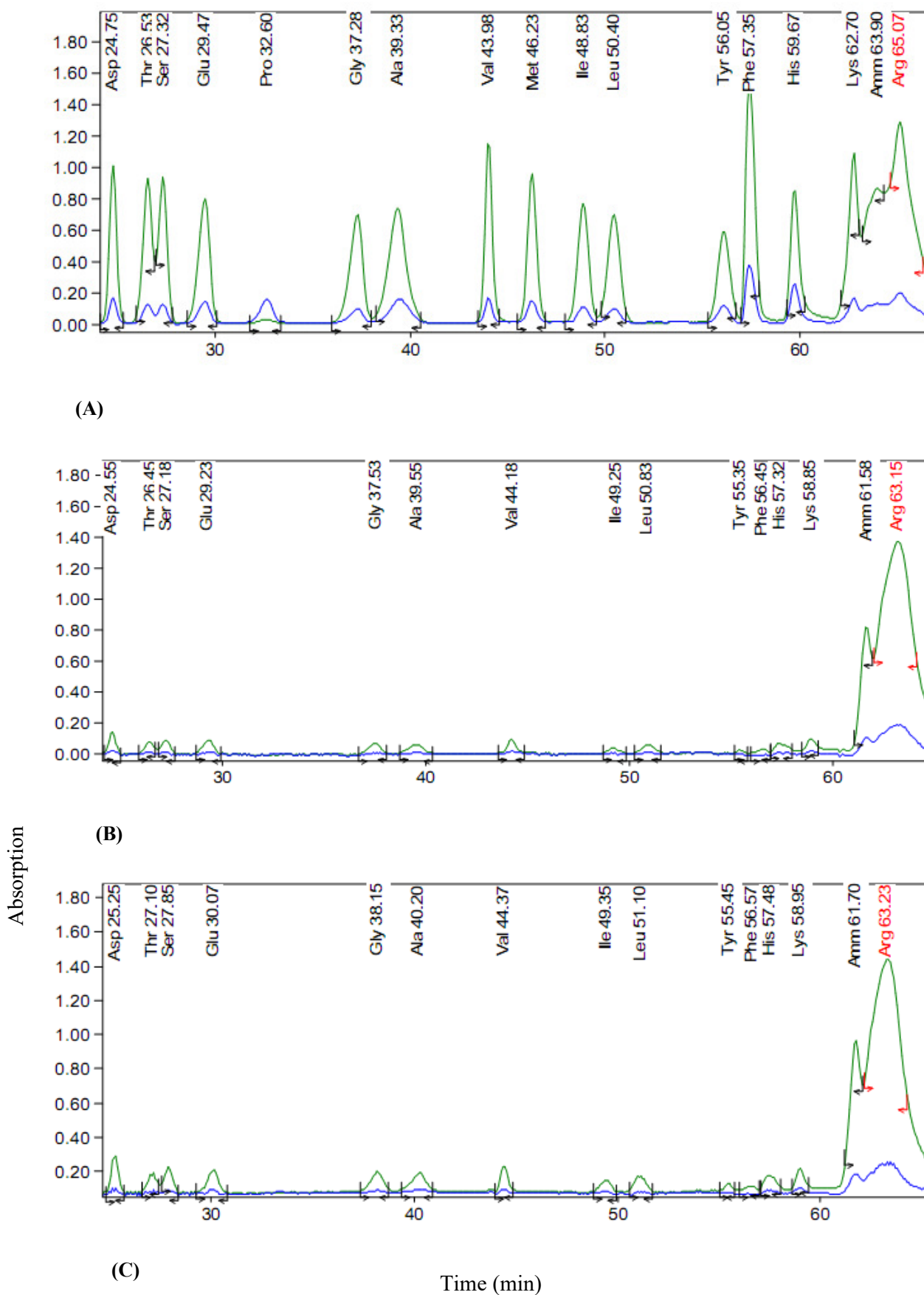


Figure 4: protein analysis for amino acid composition (A) standard (B) *K.Lactis* and (C) *K. Fragilis*.

References

- [1]. Carrasco de Mendoza M.S, Basílico J.C, Umansky G, and H.E. Scarinci. Biological value of the unicellular protein of *Kluyveromyces marxianus* var. *lactis*. Arch Latinoam Nutr. 41(1): pp 72-8. (1991).
- [2]. Fadel, M. and M.A. Degheidi. Utilization of whey permeate for yeast production. Egyptian Journal of Dairy Science. 26: pp 351-362. (1998).
- [3]. Genari, A. N., F. V. Passos and F. M. L. Passos. Configuration of a bioreactor for milk lactose hydrolysis. Journal of Dairy Science. 86: pp 2783-2789. (2003).
- [4]. Grba, S., V. Stehlik-Tomas, D. Stanzer, N. Vahèiæ, and A. Škrilin. Selection of Yeast Strain *Kluyveromyces marxianus* for Alcohol and Biomass Production on Whey. Chem. Biochem. Eng. Q. 16. 1. pp. 13–16 (2002).
- [5]. Mahmood, Karzan T. Isolation, Characterization and Immobilization of β -galactosidase from the Yeast *Kluyveromyces lactis* DSM 70800 and its Application in Some Dairy Products. Ph.D. thesis, Food science dept. Faculty of agricultural science, Sulaimani university (2010).
- [6]. Mahoney, R.R., and Wilder, T. Stabilization of lactase (*Escherichia Coli*) by milk components and related compounds. Journal of food science. Vol. 54. Issue 4. pp 899-901. (1989).
- [7]. Moeini, H., I. Nahvi and M. Tavassoli Improvement of SCP production and BOD removal of whey with mixed yeast culture. Electronic Journal of Biotechnology. 7, 3: pp 252-258. (2004).
- [8]. Murad, H.A. Abd-el-Ghani, S. and El-shenawy, K. Bioconversion of whey permeate into *Kluyveromyces lactis* biomass. Egyptian Journal of Dairy Science. 20: pp 261-271. (1992).
- [9]. Popa, A., Mihaela Tapai, and R.C. Duca. Survey on yeast strains biomass production capacity. Physical and chemical characterisation of the biomass. Archiva Zootechnica vol. 7: pp 81-85. (2004).
- [10]. Raimondi, S., Elena Z., Alberto A., Claudio P., Daniela U. and R., Maddalena, Thermal adaptability of *kluyveromyces marxianus* in recombinant protein production. Microbial Cell Factories. 12 (34). (2013).
- [11]. Rech, R. and M. A. Z. Ayub. Simplified feeding strategies for feed-batch cultivation of *Kluyveromyces marxianus* in cheese whey. Process Biochemistry 42: pp 873-877,(2007).
- [12]. Shay, L.K. and G.H. Wegner, Nonpolluting conversion of whey permeate to food yeast protein. Journal of Dairy Science., 69: pp 676-683, (1986).
- [13]. Somaye, F., Moosavi-Nasab M. and L., Nasiri, Single Cell Protein (SCP) production from UF cheese whey by *Kluyveromyces marxianus*. 18th national congress on food technology. Mashhad I.R. Iran. pp1-6, (2008).
- [14]. Szczodark, J. Hydrolysis of lactose in whey permeate by immobilized β -galactosidase from *Kluyveromyces fragilis*. Journal of Molecular Catalytalysis B: Enzymatic 10: pp 631-637. (2000).
- [15]. Zayed, G.Z. Utilization of whey from reconstituted skim milk from single cell production. Cultured Dairy Products Journal. 26: 22. (1991).

