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A Novel Approach for Production of Highly Active Baker's Yeast from Fodder Yeast, a Byproduct from Ethanol Production Industry

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Abstract: To upgrade the fermentation activities, levening power and cells composition of the fodder yeast (the secondary by- product in distillation factories) successful studies were performed for ethanol production from *Saccharomyces cerevisiae* in order to acquire the standard properties of baker's yeast used in baking. The raising power activity (CO_2 evolved / g yeast cell/h) and improvement of protein content, viability and decrease of the ash content as to meet the specification of conventional *Saccharomyces cerevisiae* used in baking purposes. To achieve these objective the effects of duration time of ethanol production as well as ethanol concentration in fermentation mash were studied in order to identify suitable parameters necessary to avoid the adverse effects of ethanol on the viability and physiological state of yeast cells. Promising results have been attained for improving the raising power as well as remarkedly decreasing the ash contents of cells by application of five successive washing on the yeast cells after removal of the ethanol production fermentors. Furthermore, significant increases in yeast yield, total viable cells, total solid matter (TSM), protein content raising fermentative power, accompanied by marked reduction in ash content of cells were gained when the cells were resuspended in 1% (w/v) total sugars cane molasses after final washing and allowed to grow for 24 hrs. Comparative studies and chemical analysis on the revived resulting cells have shown that the new product exhibited typical baker's yeast properties with regard to levening power, protein and ash content of cells as well as the number of the active viable cells per gram of the product. The obtained results were discussed in the light of application feasibilities in production of baker's yeast in alcohol distillation factories.

Key words: Baker's yeast, fodder yeast, ethanol, by product

Introduction

Baker's yeast is considered as one of the largest microbial industries all over the world. It is hard to imagine the existence of a single country on the globe that does not either produce or at least import baker's yeast for breadmaking and or for the manufacture of various bakeries and of baking products. The magnitude of baker's yeast consumption in bread and other yeast-raised goods can be realized if we note that for every hundred Lb of flour about one Lb of yeast solids is required for adequate bread levening, (Oura *et al*., 1982). This approximation figure reflects the millions of tonnes of baker's yeast produced annually around the world for baking purposes.

The annual production of baker's yeast (in the fresh compresed form) has been increasing progressively in the last few years. According to a recent survey the annual production of the conpressed baker's yeast in Egypt amounted to about 25346 tonnes and its value reached 31,51,000 LE at that times since then the annual local consumption of bakers yeast has been on the residue to the population increase as well as the new application introduced for baker's yeast in new medical, agricultural and industrial fields. As a result a gap has been widening between the annual local production figures and those of actual consumption and this gap is now compensated for by the import of baker's yeast from foreign countries.

On the other hand several factories in Egypt are devoted for the production of ethanol alcohol for industrial purposes using molasses through alcoholic fermentation processes. These fermentations are carried out by ethanol-tolerant strains of *Saccharomyes cereviiae* i.e. belonging to the same yeast species employed in baker's yeast production. During ethanol production by fermentation substantial amount of yeast cells that are physiologically exhausted and alcohol injured and damaged are produced as a by product of the fermentatative processes. These large amounts of yeast cells are, then, further processed and dried at high temperature vons and sold as fodder yeast for animal feed. The annual production of fodder yeast byproduct exceeds 6000 tones annually and sold remarkably low prices as compared to baker's yeast.

In this studies scientific and experimental attempts were conducted to improve the physiological and fermentation capacities of the fresh (non-dried) fodder yeast byproduct as well as their biochemical composition. The objective was to simulate those properties of traditional baker's yeast to be used for baking and bread making. Such studies are hoped to add new local source of baker's yeast suitable for baking purposes and spare the need of importing baker's yeast to fill the gap between local production and actual requirements of the local market of this important product.

Materials and Methods

Molasses: The Egyptian sugar cane molasses used was the same employed for ethanol and fodder yeast production in Egyptian distillation factories.

Yeast cultures: Five strains of *Saccharomyces cerevisiae* applied for ethanol and fodder yeast production in Egyptian Distillation Factories were obtained through the combined project for development of ethanol production via fermentation approaches between National Research Center-Dokki-Egypt and Sugar and Integrated Industries Company Egypt.

Inoculum preparation: The yeast cultures were cultivated in 500ml conical flasks each contained 100ml of sterile medium composed of 10% (w/v) total sugar cane molasses 0.4% diammonium phosphate and 0.3% yeast extract. Then flasks were incubated in an incubator shaker for 24h at 34°C. The growing yeast was used as an inoculum to inoculate the experimental flasks. The medium is recommended in Egyptian distillation factories for ethanol and fodder yeast production

Screening for *S. cerevisiae* strains: The industrial medium for ethand and fodder yeast production at Hawamdia Distillation Factory which composed of 14 to 16% (w/v) total sugars cane molasses 0.05% urea and 0.05% calcium super

phosphate was used in the present studies. For selection more suitable yeast strain (s) on the basis of ethanol production, raising power ash content and viable cells.

Effect of ethanol concentration on fermentation activities and composition of yeast cells: Different concentrations of ethanol production were obtained by adjusting the molasses concentration namely 16, 14 and 12% (w/v) total sugar in the growth medium for the strains of yeasts under study

Effect of inoculum size: Three levels of the above inoculum i.e. 5,10 and 20% (v/v) were used to study their effect on the fermentation power, ethanol production and chemical composition of yeast strains.

Effect of aeration rate: Yeast cultures were placed on rotary shaker's adjusted at 50 rpm, 100 rpm and 200 rpm during the fermentation period to produce ethanol and fodder yeast the two selected yeast strains SH and A1 to study the effect of aeration rate 34°C.

Effect of successive washing: In the end of fermentation period the fermented mash was centrifuged to separate yeast. The yeast biomass was resuspended in flasks containing water and left to soak for 15 minutes, then centrifuged. The washing and soaking of the cells was carried out 5 times with repeated centrifugation.

Effect of feeding with diluted molasses syrup: After repeated washing and soaking the obtained biomass from the above step was resuspended in one fourth of the original volume of fermentation medium in water supplemented with different concentrations of molasses containing 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0 and 5.0% (W/V) total sugars. The yeast cultures were allowed to grow for 24h then harvested.

Effect of supplementation molasses and nitrogen source: Molasses feeding in the above step was replaced with molasses syrup of 1.0% (w/v) total sugar contained urea or urea and diammonium phosphate in ration 1:1 or 2:1. The yeast cultures were incubated for 24h before harvesting to obtain yeast yield.

Effect of incubation period: The yeast yield was introduced in a medium of 1% (w/v) total sugars molasses, 0.05% urea and 0.05% diammonium phosphate. The initial yeast cells concentration was 15 g /L. The culture was incubated under aeration (200 rpm) and the yield of yeast gained was taken at 4 hours intervals for analyses till 24 hours.

Effect of storage on the fermentative activity: The obtained yeast from the final steps was stored at 5-8°C. Samples were daily analyzed for fermentative activity up to 10 days.

Measurement of fermentation activities: The raising power for the obtained yeast cultures were determined using fermentograph (SJA) aparatus in Chemical Factories for baker's yeast production-Hawamida-Giza Egypt and defined as volume cm^3 Co_2 of evolved from five gram compressed yeast contain 28% total solid matter (T.S.M) under experiment conditions. Measurement were periodically performed within fermentation period of three hours.

Ethanol determination: The ethanol produced in the fermentation medium was determined using Ebulliometre apparatus which used as standard apparatus for determination of the ethanol concentration in distillation factories.

Chemical analysis of yeast cells: Total protein was determined by Micro Kjldahel method described in A. O. A. C. (1980). Total solid materials was determined by oven drying for samples at 105 °C until constant weight. Ash was calculated from ignition specific weight of yeast culture at 550 °C for 5 hours.

Determination total viable count: One gram fresh yeast (28% TSM) was transferred to flasks containing 100ml sterilized saline. The flasks well shaked. Different dilutions were performed till 10^{12}. One ml of each last five dilutions was transferred to 15 cm diameter petri-dishes contained 20 ml of YMPA medium. The plates were incubated for 24h at 30°C the dishes containing 50-150 colonies were taken for counting.

Results and Discussion

A comparative study on 5 industrial strains of *S. cerevisiae* used for commercial ethanol and fodder yeast production at Hawamdia Distillation Factories was carried out. The industrial strains were compared with respect to their potential ethanol productivity, viable counts per gram fresh weight, dough raising power as well as the biochemical composition of cells including total solid matter, protein percentage and their ash content. These parameters were measured under standard fermentation conditions using the same sugar cane molasses medium used in industrial scale production at Hawamdia Distillation Factories, Hawamdia, Giza, Egypt. Table 1 showed that all yeast strains had reasonable viable counts of more than 9.0×10^9 per gram fresh weight, total solids (T. S. M) was in the range of 26.3 – 27.6% depending on the yeast strain. On the other hand the ash contents were notably high ranging between 11.2 – 14.1%. These levels are higher than the level demands in baker's yeast (8.0%).

Furthermore, the yeast cells had exhibited low raising power especially in the first hour compared to the standard raising power for baker's yeast (460 cm^3 CO_2). This data reflects the adverse effect of anaerobic fermentation conditions on the raising power of the cells due to the harmful effects of ethanol produced in the fermentation medium.

The results also showed that strains SH and A1 gave the highest alcohol yields (8.3 and 8.0% (v/v) after 22 and 24 h fermentation respectively. Further more, cells of these two strains exhibited the highest fermentation power (373 and 350 cm^3 Co_2 respectively) in the first hours as compared to other strains tested. They were also higher in protein content and viability counts.

These all tested strains had high ash contents in their cells may be attributed to cultural anaerobic conditions in the presence of high concentration of sugars and alcohol formation on the multiplication rate of the cells. Under such a situation the cells tend to adsorb more minerals (Walter, 1953). Furthermore, cells membrane under these conditions can function as adsorbents for very small particles and metal ions (Krylova and Eroshim, 1990). Based on the obtained results yeast strain SH and A1 were selected for further studies.

Effect of ethanol concentration on fermentation activity, viability and chemical composition of the yeast cells: Three levels of sugar cane molasses were incorporated in the fermentation medium namely 16, 14 and 12%. The fermentation process are show in Table (2). The resulting alcohol concentration 6.1, 7, 1. and 8.3% (v/v) in case of

Table 1: Comparative properties of some industiral strains of *Saccharomyces cerevisiae* used in ethanol and fodder yeast production on cane molasses medium in the Egyptian Distillation Factories, Hawamdia, Giza, Egypt

Yeast strain	% produced alcohol (v/v)	Fermenta tion time (h)	Raising Power (cm^3CO_2/ha)**			Percent/cells dry weight				Total viable count x 10^9/g fresh yeast
			1st hour	2nd hour	3rd hour	Total	T. S. M.	Ash	Protein	
F111	7.8	26	340	730	875	1945	26.7	13.1	42.1	9.05
F4	7.9	24	325	740	875	1930	27.1	12.6	40.6	9.01
SH	8.3	25	375	725	920	1920	26.8	12.3	40.3	9.4
A1	8.1	24	350	725	850	1975	27.6	11.2	42.1	9.5
F25	7.9	25	300	625	800	1725	26.3	14.1	41.6	9.02

Table 2: Effect of resulting yield of ethanol (% v/v) in the fermentation mash on duration time, the fermentative activity, yeast cells components (T.S.M, protein and ash) and total viable counts of the two industrial yeast strains of *S. cerevisiae* SH and A1

Yeast strain	% produced alcohol (v/v)	Fermenta tion time (h)	Raising Power (cm^3CO_2/ha)**				Percent/cells dry weight			Total viable count x 10^9/g fresh yeast
			1st hour	2nd hour	3rd hour	Total	T. S. M.	Ash	Protein	
SH	8.3	25	375	725	920	2020	6.8	44.3	10.8	9.4
	7.1	22	400	860	875	2135	26.9	44.6	10.6	9.42
	6.1	20	425	875	850	2130	27.1	44.6	10.6	9.45
A1	8.1	24	350	775	850	1935	27.6	42.1	11.2	9.5
	7.2	21	380	840	810	2030	27.6	42.1	11.4	9.5
	6.3	20	415	860	800	2075	27.8	42.3	11.5	9.52

Table 3: Effect of inoculum size on the fermentation period, fermentative activity, yeast cells components (T.S.M, protein and ash) and total viable counts of two *S. cerevisiae* SH and A1

Yeast strain	% inoculum size (v/v)	Fermenta tion time (h)	alcohol % (v/v)	Produced Raising Power (cm^3CO_2/ha)**				Percent/cells dry weight			Total viable count x 10^9/g fresh yeast
				First hour	Second hour	Third hour	Total	T. S. M.	Ash	Protein	
SH	5	22	6.1	425	875	850	2130	27.1	44.6	10.6	9.45
	10	19	6.1	450	900	800	2150	27.5	43.8	9.7	9.45
	20	17	6.05	450	925	775	2140	27.4	73.8	9.6	9.51
A1	5	21	6.3	415	860	800	2075	27.8	42.3	11.5	9.52
	10	19	6.1	460	875	780	2115	27.6	42.8	11.3	9.56
	20	17	6.0	460	900	750	2130	27.6	42.1	10.9	9.61

Table 4: Effect of aeration the fermentation time, ethanol yield, yield, fermentative activity, yeast cells components (T.S.M, protein and ash) and total viable counts of two. S. cerevisiae SH and A1

Yeast strain	Aeration rate	Fermenta tion time (h)	Produced alcohol % (v/v)	Raising Power (cm^3CO_2/ha)**				Percent/cells dry weight			Total viable count x 10^9/g fresh yeast
				First hour	Second	Third	Total	T. S. M.	Ash	Protein	
SH	50rpm	24	6.6	375	750	950	2075	28.6	42.4	12.4	9.01
	100rpm	17	6.05	450	850	800	2100	27.3	44.2	10.6	9.45
	200rpm	14	5.8	475	875	775	2125	27.1	44.7	9.3	9.85
A1	50rpm	24	6.7	355	775	860	1990	29.3	40.8	14.3	8.95
	100rpm	17	6.0	460	900	750	2130	28.6	41.6	11.4	9.56
	200rpm	15	5.9	480	925	725	2140	28.3	42.1	10.2	9.64

Table 5: Effect of incorporation of diammonium phosphate (D) with urea (U) as nitrogen sources on the ethanol yield, fermentation activity, yeast cells components (T.S.M., protein and ash) and total viable counts of two industrial yeast *S. cerevisiae* SH and A1. The amount nitrogen in control experiment equivalent to that present in 2g/L was divided in a ratio of 1:1 or 2:1 in the from of the two salts used.

Yeast strain	Nitrogen source	Fermentation time (h)	Produced alcohol % (v/v)	Raising Power (cm^3CO_2/ha)**				Percent/cells dry weight			Total viable count x 10^9/g fresh yeast
				First hour	Second hour	Third hour	Total	T. S. M.	Ash	Protein	
SH	Urea	14	5.8	475	875	700	2050	27.1	44.7	9.3	9.85
	U: D2:1	14	5.8	500	810	850	2160	27.6	45.6	9.1	9.85
	U:D 1:1	14	5.8	500	825	815	2140	26.8	48.2	8.6	9.91
A1	Urea	15	5.9	480	925	725	2140	28.2	42.1	10.2	9.64
	U: D 2:1	15	5.9	500	875	775	2160	28.0	44.9	8.91	9.66
	U: D 1:1	15	5.8	500	875	775	2160	27.3	51.2	8.42	9.64

Table 6: Effect of successive washing of harvested cells after ethanol production on the fermentation activity of cells, their composition (T. S. M, protein and ash containing) and total viable counts of two selected yeast strain SH and A1

Yeast strain	Successive washing	Raising power cm^3 CO_2/h				T.S.M	% protein	%Ash	Total viable cells x 10^9/g fresh
		First hour	second hour	Third hour	Total				
SH	0	375	650	725	1750	27.6	44.2	11.4	9.80
	1	425	675	775	1875	27.8	44.3	10.7	9.85
	2	450	775	775	2000	27.8	45.1	9.9	9.85
	3	500	810	850	2160	27.6	45.6	9.1	9.85
	4	515	800	850	2165	27.6	45.6	8.75	9.85
	5	515	800	850	2165	27.2	45.3	8.60	9.75
A1	0	300	625	800	1725	28.4	44.4	12.6	9.24
	1	375	740	875	1990	28.3	44.8	10.8	9.56
	2	450	760	815	2025	28.0	44.9	8.96	9.66
	3	500	975	625	2100	28.0	44.9	8.84	9.54
	4	525	820	750	2095	28.0	44.8	8.64	9.54
	5	500	975	625	2100	27.6	43.9	8.20	9.52

Table 7: Effect of feeding by different concentrations of diluted molasses on the yeast productivity ethanol yield, fermentation activity, yeast cells components (T.S.M, protein and ash) and total viable cells of *S. cerevisrae* strain SH.

% Sugar (w/v)	Yeast yield g/L	% Ethanol yield (v/v)	Raising power cm^3CO_2/h				Total solid matter	% protein	%Ash	Total viable Cells x 10^9/g Fresh yeast
			First hour	Second hour	Third hour	Total				
0.0	15	0.0	475	775	750	2050	27.8	45.6	10.6	9.22
0.2	16	0.00	500	975	625	2100	28.2	45.5	10.6	9.34
0.4	16.8	0.01	515	800	850	2165	28.2	45.1	10.4	9.56
0.6	17.4	0.02	525	900	825	2250	28.4	44.6	10.1	9.87
0.8	18.1	0.03	550	875	800	2250	27.4	43.8	9.3	9.95
1.0	19	0.08	525	900	825	2250	27.8	43.3	8.9	9.60
2	18	0.1	515	850	825	2190	27.4	37.8	8.2	9.3
3	17.7	0.6	515	850	825	2190	27.4	38.6	8.4	9.3
4	17.5	1.1	500	850	825	2175	27.2	38.1	8.4	9.2
5	17	1.4	475	800	825	2125	27.6	39.3	8.6	9.0

Table 8: Effect of feeding by a medium containing molasses and nitrogen source on the yeast productivity, ethanol yield, fermentative activity, yeast cells, components (T.S.M, protein and ash) and total viable counts of *S. cerevisrae* SH

% Molasses (w/v)	% Ethanol yield (v/v)	Yeast yield (g/L)	Raising power cm^3CO_2/h				T.S.M.	% protein	%Ash	Total viable cells x 10^9/g fresh yeast
			First hour	Second hour	Third hour	Total				
0.0	0.0	15	425	775	850	2050	29.4	38.6	11.8	9.10
4	0.01	15.4	475	825	850	2150	29.0	41.3	9.2	9.4
8	0.02	16.2	525	850	852	2175	28.6	43.2	8.6	9.5
16	0.028	19.0	575	875	852	2272	28.2	44.1	7.8	9.7
24	0.030	18.9	550	875	852	2250	28.2	43.9	7.9	9.6

Table 9: Effect of cold storage (shelf life) period on the fermentative activity of the newly developed baker's yeast from fodder yeast by product of ethanol industry

Storage period (day)	Fermentative activity cm^3CO_2/h				Remaining fermentation activity
	First hour	Second hour	Third hour	Total	
0.0	650	950	900	2515	100%
2	650	950	900	2515	100%
4	625	950	900	2490	99.0%
6	625	925	875	2425	96.4%
8	600	900	850	2350	93.4%
10	575	875	825	2275	90.4%

Table 10: Summary of biochemical and fermentative baker's yeast properties acquired through the present studies by th fodder yeast byproduct of Ethanol Industry

Property modified	Original fodder yeast by product of ethanol industry	Newly developed baker's yeast	Increment percent
Raising pwer (cm^3O_2)			
First hour	375	650	73.3%
Total / 3 hours	1920	2500	30.2%
Protein content %	40.3	44.6	4.3%
Ash content %	12.3	7.7	59.7%
Total solid matter %	26.8	28.4	6.0%
Viability / gram fresh weight	$9.4x10^9$	$9.8x10^9$	4.2%

yeast strain SH and 6.3%. 7.2% and 8.1% for yeast strain A1 for these molasses concentrations tested respectively. On the other hand an inverse relation could be noted between resulting alcohol concentration and raising power of cells particularly to the first two hours the raising power test for both yeast strains under study. The obtained data can be discussed in the light of the harmful effect of alcohol on the enzymatic system in the yeast cells. The data also revealed also that the duration time of exposure to alcohol (fermentation time) as well as the alcohol concentration are of critical effects on the raising power of the yeast cells. Based on the obtained data a series of studies were conducted to modify some of the fermentation parameters used in the Egyptian distillation factory during the process of ethanol alcohol production. The reinvestigation of the fermentation parameters was aimed to the reduction of the alcohol formation period in the fermentation medium in order to decrease the time during which the cells are exposed to the toxic effects of alcohol resulting in deterioration of their raising power activity. Since the general alcohol productivity average in the factory is around 5.8 – 5.9% (v/v), which is suitable from economic point of view, the following studies were conducted taking into consideration this assigned figure. These studies included. Effect of inoculum size, changes in aeration rate and modification of the nitrogen source used. Effect of inoculum size on fermentation period of alcohol production. Quantity seed yeast used to inoculate the medium was varied between 5-20% (v/v), Duration time of fermentation to obtain ethanol yield was shorten from 22h to 17h and from 21h to 17h for strains SH and A1 respectively (Table 3) when the inoculum size was increased from 5% to 15% (v/v). The reduction of fermentation period from 22h to 17h gave promising results for raising power and decrease in ash content. Thus the raising power was raised during the first hour from 425 to 450 $cm^3 CO_2$ and from 415 to 460 $cm^3 CO_2$ / gram from SH and A1 strains respectively. Gradual decrease in ash content was shown by decreasing the fermentation time as a result for increasing the inoculum size in both strain tested.

Effect of aeration rate: Promising data was obtained using high level aeration (200 rpm mixed speeds). Under these conditions viable counts were markedly increased with 12% corresponding to 12% decrease in ethanol yield (Table 4). High aeration level resulted in reduction of fermentation period from 24 to 14h and from 24 to 15h for both SH and A1 strains respectively. Also raising power was increased from 375 to 475 $cm^3 CO_2$ and from 355 to 480 $cm^3 CO_2$ in the first hour for the two tested strains respectively. Further enhancing in raising power in the second hour as well as in the total raising power was also achieved. Furthermore a decrease in ash from 12.4% to 9.3% and from 14.3% to 10.2% for strains SH and A1 respectively. The increase in raising power obtained can be due to the improvement of cultural conditions and the marked decrease of fermentation period which decrease the time during which the yeast cells are exposed to the harmful effect of ethanol in the medium. In addition formation of new yeast cells takes place as a result of acceleration in yeast reproduction in the presence of excess aeration. Acceleration of yeast reproduction and increase in yeast productivity decrease the amount of minerals presents in the medium that can be deposited or adsorbed on the yeast membrane, consequently the ash percent decreases in the final products. These changes also increase cell protein content due to the fact that in the presence of oxygen more nitrogen can assimilate by yeast cell.(Jiranek *et al.*, 1991 and Sweere *et al.*, 1998).

Effect of nitrogen source used for ethanol production: Nitrogen source is cornidered as one of limiting nutrient during batch alcoholic fermentation by *S. cerevisiae*. The maximum fermentation rate during the process is closely related to the amount of assimilated nitrogen present in the medium (Bely *et al.*, 1990).

In the ethanol production process at the Egypt Distillation Factories urea is used as nitrogen source during the alcohol production at 0.2% final concentration in the industrial medium. In the preliminary stage of the present work level of inorganic nitrogen sources were tested instead of urea none of them were superior to urea with respect to alcohol productivity with the exception of diammonium phosphate salt. However, since this nitrogen source is much more expensive as compared to urea, combination with differentiation of urea and diammonium phosphate were tested taking into consideration the economic feasibility of application. Furthermore the incorporation phosphate in the

medium has saved the need for addition of calcium super phosphate used as phosphorus source in the original industrial medium .

Ethanol yield was not affected by nitrogen source in the fermentation medium (Table 5). Slight increase in fermentative activity and protein contents, and a decrease in ash content have been shown when urea to diammonium phosphate has been fed in the medium in ration 2:1. The two tested yeast strains SH and A1 exhibited a clear increase in protein percentages and decrease in ash contents as a result of increase of diammounm phosphate amount in the medium. The positive effects obtained by introducing diammonium phosphate along with urea in the fermentation medium can be attributed to the amount of phosphorus contained in diammonium phosphate, as the cane molasses are diffecient in this element.

The present finding agree with that reported by many workers. Bely *et al.* (1990) showed that ammomium salt additions dramatically decrease the fermentation duration. Nitrogen assimilation in complex media has been studied by different authors (Henschke and Jiranek, 1993). Jiranek *et al.*, 1991 and Manginot *et al.*, (1991) reported that the nitrogen demand differ according to yeast strain tested.

Further studies on harvested cells for improving raising power activities and their chemical composition: Additional studies were carried out on the yeast cells harvested after alcohol production. These studies were aimed to further enhancing the dough raising power of the cells and improving their protein percentage and lowering their ash content to approach those conventional levels present in standard baker's yeast used in baking purposes. These studies included effect of successive washing of cells for alcohol removal and improving of raising power and cell composition and stimulation of fermentation activity of cell by feeding with dilute molasses.

Effect of successive washing on improving fermentation activity and composition of yeast cells: In this experiment washing operations of yeast cells after the ethanol production processes have been carried out. The objective of this study was to evaluate possible recovery of cells activities and improving the characteristics of final product of fodder yeast. Table (6) shows that the raising power of the product yeast cells was increased by successive washings till the fourth run for the both tested yeast strains.

Significant decrease in ash content of cells was achieved by successive washings. The improvement in raising power was also evident. The positive effects of successive washings may be due to the lowering of ethanol concentration and elimination of fusil iol, pentanol and isobutanol and other inhibitory compounds found during ethanol formed during formation from inside the yeast cells consequently get rid off its repression effect on the yeast enzymes system and physiological activity of yeast cells (Walter, 1953 Nagodawithana and Steinfrans, 1976 and Reed and Nagodawithana, 1993).

The decrease in ash level in the washed yeast cells was also noted. The finding was obtained as a result of washing out the minerals precipitated on the cells membrane during fermentation course.

Stimulation of fermentation activity by feeding diluted molasses: The study was aimed to stimulate the physiological activities of the yeast cells harvested at the end of ethanol fermentation stage. In this experiment the cells harvested and washed with repeated centrifugation were resuspended in highly diluted molasses solution with final sugar concentration and incubated at 32°C for 24 h in the presence of moderate aeration (150 rpm).

Table 7 shows that raising power for strain SH was increased gradually and exhibited its maximum using diluted molasses containing 1.0% (w/v) total sugars. The protein content was also increased to its maximum as well as the decrease in ash were attained when diluted molasses containing 1.0% sugar was applied. Using high concentrations of molasses the yeast tended to yield ethanol and the yeast yield decrease and the raising power as well as total viable count were affected. The obtained data confirmed that reported by the sensors in baker's yeast production (Aiba *et al.*, 1976). Aerobic ethanol formation in the presence of high sugar concentration is known as the crabtree effect (Kazuo *et al.*, 1981). Afed – batch process generally has been empolyed in the baker's yeast industry so as to maintain the fermentor at a low sugar concentration. However, in the sugar feed is in excess, sugar accumulation in the medium will permit ethanol production even in the presence of sufficient oxygen. The formation of ethanol under these conditions results in lowering of maximum cellular yield. In the production of baker's yeast it is important that the a actual sugar concentration in the fermentor be maintained at low but optimal value all the time (Wang *et al.*, 1979).

Effect of incubation period in diluted molasses on raising power activity of cells and their yield and chemical composition: Suspension of yeast cells was carried out in diluted molasses containing1.0% total sugars. Samples were withdrawn periodically and various parameters including raising power a activities, protein percentage and ash content of cells were estimated. Data presented in Table (8) show that a gradual increase in yeast biomass with incubation time. Raising power for first and second hours as well as total raising power were also increase gradually up to 16h incubation period after which slight decrease in activity was evident. Maximum increase in protein content and total viable cells count were also noted. These changes were accompanied by lowering ash content to lowest value achieved also after 16h incubation. The data indicates the presence of active growth of the yeast cells during about 16h, then the yeast growth deereased due to accumulation of toxic and inhibitory metabolites resulting in reduction in yeast productivity and increase in rate of cells death and can explain the leveling off in total viable count beyond 16h incubation period.

Shelf life of the healthy new fodder yeast product: Shelf life of baker's yeast is considered one of the most important parameters from commercial view in baker's yeast technology. The newly prepared fodder yeast that acquired baker's yeast standard properties (high raising power and low ash content) was stored in the refrigerator at 4-8°C. Samples were withdrawn periodically and the raising power changes were followed. Table (9) shows that no significant changes in raising power of the new yeast product till the 8th day of cold storage. These results are confirmed with the standard features of the conventional baker's yeast produced from baker's yeast factories. In the present work detailed studies were conducted to convert fodder yeast a by product that results from ethanol industry, into baker's yeast by acquiring high raising power, low ash content and improvement of protein content percent (Table 10). These conversion processes are of high economic value since baker's yeast market value exceeds seven times that of fodder yeast per unit on dry weight bases. Furthermore the suggested approach, described in the present work, would provide the local market with more than 5000 tons of baker's yeast to meat the shortage in local production which now imported

from abroad with hard currency.

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