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Microbiological Quality of Active Dry and Compressed Baker's Yeast sold in Egypt

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The microbiological quality for nine brands of active dry yeast (ADY) - indigenous and imported- sold in Egypt was investigated and compared with the Egyptian compressed yeast. In addition, the viability of the yeast cells was estimated. The results showed that the mean counts (log_{10} cfu/g) for the total viable count ranged from 8.70 to 9.59 while enterococci were not detected in any sample. The compressed yeast recorded the worst microbiological quality regarding to its very high microbial load; total coliforms (5.38 log cells/g), Faecal Coliforms (5.25 log cells/g) and *Salmonella* (detected in 50% of the tested samples). On the other hand, ADY recorded better result as percentage of unacceptable samples as following; total coliforms (23.3%), faecal coliforms (17.8%) and *Salmonella* (4.4%) most of the unaccepted samples were those made or packed in Egypt. However, ADY recorded a bit higher unaccepted samples regarding to moulds (35.6%), *Bacillus cereus* (18.9%) and *Staphylococcus aureus* (34.4%) while the results of the compressed yeast was for moulds (30%), *Bacillus cereus* (10%) and *Staphylococcus aureus* (20%). Concerning yeast cell viability, the compressed yeast revealed the highest viability (96.9%) while the viability of ADY brands ranged from 23% to 78.3%. All samples of the English ADY met the standards with high microbiological quality and viability (78.3%). This study indicates that baker's yeast could represent notable hazards to humans and maybe a cause for public health concerns.

Key words: ADY - *Saccharomyces cerevisiae*, quality, viability and microbiology.

Baker's yeast (*Saccharomyces cerevisiae*) is the common name for the strains of yeast generally used as a leavening agent in baking. It is still one of the most important fermentation products based on volume of sales and its use for bread-making which is a staple food for large section of world's population. It is also one of the most important biotechnological products because it has several industrial applications (Daramola and Zampraka, 2008) such as the commercial production of beverages, industrial ethanol, antibiotics, industrial enzymes, chemicals, foods and nutritional supplements.

Baker's yeast as a commercial product has several formulations that can be grouped into two main types: compressed yeast, called fresh yeast, and dried yeast (Beudeker *et al.,* 1990). Compressed yeast is the traditional formulation of baker's yeast, and is ready for immediate use. Dried yeast is available in two forms: active dry yeast (ADY) and instant dry yeast (IDY) which normally sold in airtight packages, vacuum seal or filled with an inert gas such as nitrogen.

There is an increasing demand for such products in order to satisfy the needs of an ever growing population. This necessitates that efforts be made to ensure their hygienic suitability. The presence of marker groups such as coliforms and

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Escherichia coli in processed products demonstrates possible process related contamination (Blood and Curtis, 1995), and may indicate poor manufacturing practices and inadequate factory hygiene standards (Jay, 2000). Many moulds including *Penicillium* and *Fusarium* grow readily on the surface of compressed yeast if it is not stored at 1–5 °C (Irvin, 1954; O'Brien *et al.*, 2004). In addition, Baker's yeast has also been implicated as a source of bacterial contaminants for example, *Bacillus* spores (Bailey and von Holy, 1993) and lactic acid bacteria (Reed and Nagodawithana, 1991; Viljoen and Lues, 1993) in commercial bread production.

No information concerning the microbial content of these products in Egypt is available. This study was, therefore, executed to determine the microbial content of baker's yeast taken from retail markets in an attempt to gain some insight into potential microbial related problems associated with these products.

MATERIAL AND METHODS

Samples

Nine brands of active dry yeast (ADY) made in different countries (two Egyptian made, two packed in Egypt, one made in China, three Turkish and one made in the U.K.) and one brand of compressed yeast made in Egypt. Ten samples of each brand aforementioned were purchased from different retailers and bakeries around Cairo city during December 2008; throw August 2009 to evaluate the microbiological quality by testing the incidence of different pathogenic bacteria as well as to determine the viability of the baker's yeast cells.

Microbiological analysis

Samples were examined for total bacterial count (TVC) using trypticase soy agar medium (APHA, 1978) with pour-plate method (cfu/g) and the plates were incubated at 30 ºC for two days. For Moulds, Rose Bengal chloramphenicol agar medium (APHA, 1978) was used and the plates were incubated at 25 °C for 5 – 7 days. For Counting *Staphylococcus aureus,* Vogel-Johnson agar medium (Vogel and Johnson, 1960) was used and the plates were incubated at 37ºC for 18 to 24 hr. MacConkey broth purple medium (World Health Organization, 1963) and MPN technique (AOAC, 1997) was applied for counting the total coliforms (TC) at 37 ºC for 24 hr and for counting faecal coliforms (FC) at 44.5 ºC for 24 hr. Counts of enterococci were determined by means of Buffered Azide Glucose Glycerol broth (BAGG) broth medium (Hajna and Perry, 1943) and MPN technique (AOAC, 1997) at 45 ºC for 48 hr. *Bacillus cereus* was enumerated using *Bacillus cereus* selective agar medium (Mossel *et al.*, 1967) and pour plate technique (cfu/g) at 37ºC for 24 hr. the procedure of Taylor, (1965) was used for detecting *Salmonella* spp.

Microscopic yeast counting and viability test

One gram of each sample was suspended and homogenized for 2 min in 100 ml of phosphate buffer (pH 7) then incubated for 30 min at 30 °C. After incubation, samples were diluted with sterile saline by serial 10-fold dilutions (10^{-3}, 10^{-4}) and 1 ml of each sample was transferred into a test tube and mixed with 1 ml of methylene blue solution (0.01% in distilled water) (Trevors *et al.,* 1983) then incubated at 30 °C for additional 15 min. Microscopic examination of stained cells was performed on a Zeiss research microscope with appropriate filters and a haemocytometer counting chamber for enumerating cells. Stained cells were counted as dead cells while unstained cells considered alive cells.

pH measurement

The pH – value of the samples was measured using a digital pH – meter (Jenway 3020 pH meter, the UK); calibration was done with buffers of pH 4.00 and pH 7.00. One gram of each sample was suspended and homogenized for 2 min in 100 ml of phosphate buffer (pH 7) then incubated for an additional 30 min at 30 °C before measuring.

Statistical analysis

In order to compare the different brands of baker's yeast, the results were expressed as the mean value ± the standard deviation (SD). All microbiological counts were converted to the base-10 logarithm of colony forming units per gram of baker's yeast samples (log cfu/g), and from these, means and their standard deviations were calculated. Data were tested for statistical significance at the 5% level ($p < 0.05$) by the analysis of variance (ANOVA) using SPSS, an IBM Company software (SPSS 11.0.1 for Windows).

RESULTS AND DISCUSSION

Microbiological survey of different baker's yeast samples

The industrial production of commercial baker's yeast is carried out in large fermentation vessels that practically can not be kept completely sterile, allowing microbial contamination to occur (Barrette *et al*., 1999). Therefore, ninety samples of different active dry yeast (ADY) brands sold in Egypt and 10 samples of the compressed yeast which is manufactured and sold in Egypt were collected and tested to determine their microbiological quality. The qualities of the tested samples were evaluated according to standards of The Bakery Yeast Manufacturers Committee of the European Union (COFALEC, 2009) and International Organisation of Vine and Wine (OIV, 2009).

As deducted from the data summarized in Table 1, total coliforms were detected in high numbers (5.38 ± 1.02 log cell/g) in brand J (compressed yeast) while none was detected in brand D. Brand F also recorded number of cells above the standards limits (3.50 ± 0.48 log cell/g) but significantly less than coliforms counted in brand J. On the other hand, total coliforms were detected within the limits of both standards OIV 2009 and COFALEC 2009 with no statistically significant differences ($P > 0.05$) in brands G (1.5 ± 1.14 log cell/g), H (1.32 ± 1.56 log cell/g) and I (1.24 ± 1.39 log cell/g). In addition, very limited number of total coliforms was detected with no statistically significant differences ($P > 0.05$) in brands C (0.24 ± 0.76 log cell/g), A and B (0.20 ± 0.62 log cell/g) and E (0.16 ± 0.5 log cell/g). The results are consistent with previous studies which noted that, coliforms have been found in a variety of dried food products including dehydrated soup, dried milk and dried egg (Jay, 2000). In this respect, O'Brien *et al.,* (2004) suggested the possibility of packing line equipment (conveyers and dosing cups), packing material and personnel contributing to dry yeast contamination with coilforms.

Faecal coliforms (FC) (Table 1) were detected in very high numbers (5.25 ± 0.99 log cell/g) in brand J which was compressed yeast wile brand F recorded remarkably high numbers (2.80 ± 0.46 log cell/g) which were above the standard limits but significantly less than what were detected in brand J. No significant differences ($P > 0.05$) were observed between the FC counts in brands G (0.68 ± 0.88 log cell/g) and H (0.39 ± 0.82 log cell/g) which are considered within the limits according to COFALEC 2009 however they do not meet OIV 2009 standard. On the other hand, FC was not detected in the other brands A, B, C, D, E, and I. O'Brien *et all* (2004) reported that, drying of yeast to 4% moisture content and subsequent vacuum packing may contribute to the decrease in *E. coli* counts. Infact, *E. coli* and coliforms are very important as indicators of sanitation (Jay, 2000). More consistent cleaning practices may help to reduce the risk of yeast product contamination during processing. Consequently, good manufacturing practices (GMP) and good hygiene practice (GHP) should be applied in baker's yeast manufacturing plants.

The results in Table 1 show that, enterococci were not detected in any sample. However, this finding is not in line with many studies which reported that, Enterococci are heat resistant and survive adverse environmental conditions in dried products. So, it was found with high numbers in dry yeast samples (Jay, 2000 and O'Brien *et al*., 2004).

Means of total viable count (TVC) ranged between 8.70 ± 0.47 log cfu/g and 9.59 ± 0.27 log cfu/g with significant differences ($P < 0.05$) between some brands (Table 2). However, the total count is normally the total achieved on a suitably rich medium agar plate. In the case of baker's yeast, the total plate count will include the yeast cell count, which will overwhelm all other counts. So unless special measures are taken to suppress the growth of the yeast cells, this result is with little meaning. Even when the growth of yeast cells is suppressed this cell count is not very informative because the vast majority of the cell count is usually due to lactic acid bacteria that are harmless (COFALEC, 2009).

Moulds were detected in all brands with mean number ranged between 3.09 ± 2.80 log cfu/g and 0.81± 1.12 log cfu/g. There were 2 brands - I (3.09 ± 1.71 log cfu/g) and D (3.04 ± 0.80 log cfu/g) - recorded above the limits of OIV, 2009 standard. While, the results of the other 8 brands were recorded within the limits and no significant differences ($P > 0.05$) were observed. In this

Table 1. Means of microbiological profile of 100 samples of 10 baker's yeast brands

Brand [A] (n = 100)	Origin	Log cell/g (mean ± SD)			Log cfu/g (mean ± SD)				*Salm.*
		TC	FC	Entero.	TVC	Moulds	*B. cereus*	*Staph.*	
A (n = 10)	Turkey	0.20± 0.62^{d}	0.00± 0.00^{d}	0.00± 0.00^{a}	9.06 ± 0.21^{b}	1.33± 1.42^{b}	0.00± 0.00^{b}	0.00± 0.00^{c}	ND [C]
B (n = 10)	Turkey	0.20± 0.62^{d}	0.00± 0.00^{d}	0.00± 0.00^{a}	9.47± 0.22^{a}	0.90± 1.46^{b}	0.10± 0.32^{b}	0.72± 1.54^{bc}	ND
C (n = 10)	Turkey	0.24± 0.76^{d}	0.00± 0.00^{d}	0.00± 0.00^{a}	9.06± 0.27^{b}	1.16± 1.90^{b}	0.60± 1.35^{ab}	0.80± 1.69^{bc}	ND
D (n = 10)	China	0.00± 0.00^{d}	0.00± 0.00^{d}	0.00± 0.00^{a}	8.70± 0.47^{c}	3.04± 0.80^{a}	0.43± 0.91^{ab}	1.90± 2.09^{b}	ND
E (n = 10)	UK	0.16± 0.5^{d}	0.00± 0.00^{d}	0.00± 0.00^{a}	9.08± 0.18^{b}	0.81± 1.12^{b}	0.00± 0.00^{b}	0.00± 0.00^{c}	ND
F (n = 10)	Egypt	3.50± 0.48^{b}	2.80± 0.46^{b}	0.00± 0.00^{a}	9.59± 0.27^{a}	2.22± 1.96^{ab}	0.96± 1.60^{ab}	3.86± 0.79^{a}	2/10^{a} [D]
G (n = 10)	Egypt	1.51± 1.14^{c}	0.68± 0.88^{c}	0.00± 0.00^{a}	9.49± 0.26^{a}	2.31± 2.05^{ab}	0.75± 1.23^{ab}	2.04± 1.85^{b}	ND
H (n = 10)	Egypt [B]	1.32± 1.56^{c}	0.39± 0.82^{cd}	0.00± 0.00^{a}	8.98± 0.47^{bc}	2.02± 2.22^{ab}	1.53± 2.03^{a}	1.35± 1.76^{bc}	2/10^{a} [D]
I (n = 10)	Egypt [B]	1.24± 1.39^{c}	0.00± 0.00^{d}	0.00± 0.00^{a}	8.98± 0.51^{bc}	3.09± 1.71^{a}	0.78± 1.65^{ab}	0.85± 1.81^{bc}	ND
J (n = 10)	Egypt	5.38± 1.02^{a}	5.25± 0.99^{a}	0.00± 0.00^{a}	9.56± 0.10^{a}	1.15± 1.85^{b}	0.57± 1.79^{ab}	0.74± 1.63^{bc}	5/10^{b} [E]

TC, total coliform; FC, faecal coliform; Entero., enterococci; TVC, total viable count; *Staph.*, *Staphylococcus aureus*; *Salm., Salmonella* spp.

n, number of samples

[a,b,c] Means bearing different superscripts in the same column differ significantly ($p < 0.05$)

[A] A-I represent different brands of active dry yeast while J represents the compressed yeast

[B] brand imported from outside Egypt but packed in Egypt

[C] Not detected; [D] Detected in 2 samples; [E] Detected in 5 samples

respect, Irvin (1954) reported that, airborne contamination was the main source of mould spores.

Regarding pathogenic microorganisms, *Bacillus cereus* was detected - with no statistically significant differences ($P > 0.05$) - in 8 brands with mean count ranged between as high as 1.53 ± 2.03 log cfu/g in brand H and as low as 0.10 ± 0.32 cfu/g in brand B while it not detected in 2 brands A and E. These results agree with Bailey and von Holy (1993) who reported that, yeast has been implicated as a source of *Bacillus* spores. Both standard were mentioned earlier did not have limits for *Bacillus cereus* although, it seems important to have limits for such pathogenic microorganisms since it was detected in the vast majority of brands. Quite similar to *Bacillus cereus* results, *Staphylococcus aureus* was detected in 8 brands with significant differences ($P < 0.05$) between brand F (3.86 ± 0.79 log cfu/g) and the other 7 brands which ranged between (2.04 ± 1.85 log cfu/g and 0.72 ± 1.54 log cfu/g) while it was not detected in brands A and E. On the other hand, the presence of *Salmonella* was detected in 5 samples out of 10 in brand J (which is compressed yeast) and 2 samples out of 10 in brands F and H. In this respect, it has previously been reported that packaging may contribute to the contamination of foods with gram negative bacteria (Georgnaras *et al*., 1996). On the other hand, the absence of *Salmonella* in a sample of 25g was checked in the other 7 brands.

Comparison between the Microbiological quality of different local and imported brands of active dry yeast versus the Egyptian compressed yeast

Tables (2) and (3) represent the percentage of positive samples of 6 microbiological tests in order to compare the microbiological quality of the different brands of ADY as well as comparing the ADY against the compressed yeast. Results reported as a percentage of positive samples for Faecal Coliform (FC), *B. cereus, Staph. aureus* and *Salmonella* counts $\geq 10^2$ cfu/g for Total Coliform (TC) as well as counts $\geq 10^3$ cfu/g for moulds. The total viable count (TVC) test was excluded due to its irrelevance on the quality of baker's yeast while the enterococci test was excluded since it was absent in all samples.

Table 2. Microbiological comparison between different local and imported brands of active dry yeast

Origin	Percentage of positive samples (%) in different origin groups					
	TC $\geq 10^2$ cfu/g	FC	Moulds $\geq 10^3$ cfu/g	*B. cereus*	*Staph. aureus*	*Salmonella*
Egypt(n = 20)	70.0	70.0	60.0	30.0	80.0	10.0
Egypt(n = 20)	30.0	10.0	65.0	30.0	30.0	10.0
China(n = 10)	0.0	0.0	60.0	20.0	50.0	0.0
Turkey (n = 30)	3.3	0.0	23.0	10.0	13.3	0.0
England (n = 10)	0.0	0.0	0.0	0.0	0.0	0.0

TC, total coliforms; FC, faecal coliforms; *n,* number of sample

Table 3. Microbiological comparison between different brands of active dry yeast and the Egyptian compressed yeast

Origin	Percentage of positive samples (%) in different origin groups					
	TC $\geq 10^2$ cfu/g	FC	Moulds $\geq 10^3$ cfu/g	*B. cereus*	*Staph. aureus*	*Salmonella*
Active dry (n = 90)	23.3	17.8	35.6	18.9	34.4	4.4
Compressed (n = 10)	100.0	100.0	30.0	10.0	20.0	50.0

TC, total coliforms; FC, faecal coliform; n, number of samples

As shown in table (2), samples of ADY made in Egypt represent the incidence of TC and FC in 70% of the tested samples as well as Moulds in 60%, 30% of *B. cereus,* 80% of *Staph. aureus* and 10% of *Salmonella*. In other words, ADY manufactured in Egypt recorded the poorest microbiological quality by representing all groups of tested microorganisms including *Salmonella* spp. Conversely, all tested samples of ADY made in the United Kingdom appear either free of any microbial groups or within the limits. Brands of ADY imported from outside Egypt and packed by Egyptian companies reserved the second position of poor microbiological quality after the Egyptian ADY with 30% TC, 10% FC, 65% moulds, 30% *B. cereus,* 30% *Staph. aureus* and 10% *Salmonella*. The Chinese ADY brand was rather better than brands made or packed in Egypt with 60% positive samples for moulds, 20% positive samples for *B. cereus* and 30% positive samples for *Staph. aureus*. The Turkish ADY recorded good results which TC indicated above the standard in 3.3% of the tested samples, moulds recorded $\geq 10^3$ cfu/g in 23% of the tested samples. *B. cereus* was detected in 10% of the tested samples and *Staph. aureus* was detected in 13.3% of the tested samples. Furthermore, all tested Chinese and Turkish samples were free of faecal coliforms and *Salmonella.*

Data in table (3) represents a comparison between the Egyptian compressed yeast and the overall results of all brands of ADY. As expected, the compressed yeast shows obvious higher loads of TC (100%), FC (100%) and *Salmonella* (50%) than the samples of ADY which recorded 23.3% for TC, 17.8% for FC and 4.4% for *Salmonella.* These results are consistent with the study of O'Brien *et al*. (2004) which noted that microbial counts of compressed yeast samples were generally higher than those of dried yeast samples. Considering risk assessment, it is worthy to mention that most of unaccepted samples of ADY were regarded to brands made or packed in Egypt. On the other hand, ADY yielded slightly higher positive samples than compressed yeast for moulds (35.6%), *B. cereus* (18.9%) and *Staph. aureus* (34.4%) while the results of compressed yeast show the following; moulds (30%), *B. cereus* (10%) and *Staph. aureus* (20%). Thus, the role of brands packed or made in Egypt as well as the Chinese brand can not be ignored in increasing the overall percentage of the unaccepted samples of the ADY.

Microscopic yeast counting and viability test

The results shown in table (4) represent significant differences ($P < 0.05$) between the various brands of commercial baker's yeast for its content of yeast cells which all ranged between 10.36 ± 0.03 log cell/g and 10.05 ± 0.04 log cell/g. However, the total count of yeast cells per gram has little meaning especially for ADY because not all these cells are alive. Therefore, using a

Table 4. Microscopic count and viability for different brands of baker's yeast

Brand* (n = 100)	Origin	Total	Live	Viability (%)	pH
		Log_{10} cell/g (mean ± SD)			
A (n = 10)	Turkey	10.26 ± 0.03^{c}	10.00 ± 0.03^{d}	54.20 ± 1.69^{e}	6.55 ± 0.10^{cd}
B (n = 10)	Turkey	10.33 ± 0.04^{ab}	10.13 ± 0.03^{a}	61.60 ± 1.84^{c}	6.58 ± 0.12^{bc}
C (n = 10)	Turkey	10.30 ± 0.04^{b}	10.01 ± 0.03^{cd}	50.80 ± 2.25^{f}	6.48 ± 0.09^{d}
D (n = 10)	China	10.11 ± 0.04^{d}	10.01 ± 0.04^{cd}	78.30 ± 1.49^{b}	6.65 ± 0.05^{b}
E (n = 10)	UK	10.25 ± 0.03^{c}	10.14 ± 0.03^{a}	78.30 ± 1.89^{b}	6.51 ± 0.09^{cd}
F (n = 10)	Egypt	10.14 ± 0.06^{d}	9.68 ± 0.05^{f}	35.00 ± 2.54^{g}	6.63 ± 0.08^{b}
G (n = 10)	Egypt	10.36 ± 0.03^{a}	10.06 ± 0.04^{b}	50.00 ± 1.49^{f}	6.54 ± 0.05^{cd}
H (n = 10)	Egypt**	10.05 ± 0.05^{e}	9.42 ± 0.05^{g}	23.30 ± 1.83^{h}	6.54 ± 0.05^{cd}
I (n = 10)	Egypt**	10.14 ± 0.04^{d}	9.90 ± 0.04^{e}	56.60 ± 3.57^{d}	6.49 ± 0.11^{d}
J (n = 10)	Egypt	10.05 ± 0.04^{e}	10.04 ± 0.04^{bc}	96.90 ± 1.97^{a}	6.93 ± 0.05^{a}

* A-I represent different brands of Active dry yeast while J represents the compressed yeast

** Brand imported from out outside Egypt and packed in Egypt

n, number of samples

a,b,c Means bearing different letters in the same column differ significantly ($p < 0.05$)

method for counting the live cells and calculating the viability is of prime importance. Live cells which are the actual cells active in dough appeared in significantly difference amount ($P < 0.05$) in the tested brands and ranged between 10.14 ± 0.03 and 9.42 ± 0.05 log cell/g. The viability gives a clear indication of the quality of the drying method which has its impact on the overall role of yeast cells in dough. The compressed yeast recorded the best viability (96.90 ± 1.97) while brand (H) recorded the worst (23.30 ± 1.83). The British and the Chinese brands came in the second with viability (78.30 ± 1.89) and (78.30 ± 1.49) respectively. Moreover, brands of ADY made in Turkey -in general- reported higher viability than those brands made or packed in Egypt.

The pH value of all brands of baker's yeast ranged between 6.93 ± 0.05 for the compressed yeast (brand, J) and 6.48 ± 0.09 for brand C. these results are compatible with COFALEC, 2009 standard ($pH = 6 \pm 2$).

CONCLUSION

The results of this study confirm the high levels of the microbiological contamination (including Total Coliforms, *E. coli*, Moulds, *B. cereus, Staphylococcus aureus* and *Salmonella*) in the compressed baker's yeast as well as the ADY which made or packed in Egypt and some imported brands. This fact makes baker's yeast - especially the compressed yeast- a concern for suppliers, consumers and public health officials worldwide. On the other hand, the viability of the ADY varied between brands and sometimes was less than 50% which is due to the poor drying methods. Therefore, it is highly recommended that hygienic practices and regulations during production, drying, packing and handling should be introduced to facilitate the production of a high quality baker's yeast that is safe for consumption. In addition, the microbiological content and cell viability must be mentioned on the product's label and checked by the authorities. Moreover, further studies are needed for the complete characterization of baker's yeast.

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