Aflatoxins Binding by *Saccharomyces Cerevisiae* and *S. boulardii* in Functional Cereal Based Ice-cream

Eman M. Hegazy[1], Zeinab I. Sadek[2], Kawther El-Shafei[2] and Azzat B. Abd El-Khalek[2]

[1]Food Toxicology and contaminants Department and [2]Dairy Science Department, National Research Centre, Dokki, Cairo, Egypt

Abstract: The ability of *Saccharomyces cerevisiae* and *S. boulardii* (viable or nonviable) to bind aflatoxins (B_1, B_2, G_1 and G_2) in liquid medium, cereals extracts and ice-cream at different temperatures and times was detected. Viable *S. cerevisiae* showed the highest binding of aflatoxins (AFS). Highest AFS binding capacity (74.7%) was obtained by viable cells of *S. cerevisiae* when incubated at 8°C for eight hours. While, binding was not affected by the cells of *S. boulardii* (viable or nonviable) at 25°C. *S. cerevisiae* when inoculated in barley extract bound 80% of added total AFS, but it found to be 60% in wheat extract. In addition, that the *S. cerevisiae* binding AFS in chocolate and vanilla ice-cream supplemented with barely extract. Sensory evaluation appeared that the chocolate ice cream with barley extract and viable *S. cerevisiae* was highly accepted for appearance, texture, taste and odor. No changes were detected in microbiological examination in ice-cream after three months storage.

[Eman M. Hegazy, Zeinab I. Sadek, Kawther El-Shafei and Azzat B. Abd El-Khalek **Aflatoxins Binding by *Saccharomyces Cerevisiae* and *S. Boulardii* in Functional Cereal Based Ice-Cream].** Life Science Journal. 2011; 8(4): 75-81] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key words: aflatoxins binding, *Saccharomyces cerevisiae, S. boulardii,* cereal extracts, barley, wheat, ice-cream.

1. Introduction

Mycotoxins are secondary metabolites produced by various moulds of which *Aspergillus, Penicillium* and *Fusarium* are the most common genera. Fungal contamination of plants can occur in the field on contaminated seeds or during growth, or at transport and storage in certain environmental conditions. The level of mycotoxin contamination in fields varies according to the plants and depends of climatic conditions, which is explained by large differences between years **(Richard-Molard, 1999).**

Aflatoxins (B_1, B_2, G_1 and G_2) are group of closely related difuranocoumarin compounds produced by several fungi, mainly *Aspergillus flavus* and *A. parasiticus* **(Steyn, 1995).** These fungi produce aflatoxins, contaminating a number of crops bound to human consumption such as corn, sorghum, rice, wheat, and nut **(Cleveland *et al*., 2003)**. Aflatoxins contamination occurs by colonization of the fungus on susceptible crop, or may arise during harvesting, dying, storage or processing. Concerns related to the negative health impact of aflatoxins have led to the investigation of strategies to prevent, eliminate or reduce the presence of these toxins in contaminated products.

Saccharomyces cerevisiae, is the most common yeast used in food fermentation where it has shown various technological properties. Also, yeasts play a significant role in the spontaneous fermentation of many indigenous food products **(Jespersen, 2003)**, he also added several beneficial effects on human health and well-being. Moreover, *Saccharomyces boulardii* is the only yeast with clinical effects and the only yeast preparation with proven probiotic efficiency in double-blind studies **(Sazawal *et al*., 2006).**

Cereals have been investigated regarding their potential use in developing functional foods, which are grown over 73% of the total world harvested area and contribute over 60% of the world food production providing dietary fiber, proteins, energy, minerals and vitamins required for human health **(Charalampopoulos *et al*., 2002).**

New applications of probiotic microorganisms such as yeasts in foods have been introduced into the market or are still in the development phase, such as frozen yoghurt, soy yoghurt, dairy desserts, cheese, ice-cream, bread and chocolate **(De Vuyst, 2000).**

The aim of this work was to study the ability of *S. cerevisiae* and *S. boulardii* to bind aflatoxins at refrigerator and room temperature in phosphate buffered saline (PBS), barley or wheat extracts and ice-cream, also, detect the microbiological quality and sensory evaluation of fresh functional cereal based ice-cream supplemented with *S. cerevisiae* and barley extract then after three months of storage.

2. Materials and Methods

Yeast strains

Pure culture of *Saccharomyces cerevisiae* (Baker's yeast) was obtained from Al-Hawamdia Company fresh comprised yeast according to the Egyptian standard (191/2005).

Saccharomyces boulardii was obtained from Microbial Genetic Department, National Research centre, Dokki, Giza, Egypt. Strains grown in malt yeast extract glucose peptone (MYGP) tubes for 24h at 25°C.

Aflatoxins (B_1, B_2 G_1 and G_2) standard was obtained from Sigma chemical company. USA.

Production of yeast biomass

S. cerevisiae or *S. boulardii* were cultured in flasks containing a Yeast Peptone and Glucose (YPG) medium W/V (1% yeast extract, 2% bacteriological peptone and 2% glucose) at 30°C, shaken at 200 rpm for 24 h. Supernatant and pellet fractions were separated by centrifugation (4000 for 5 min) yeast cells were washed twice with buffer peptone solution (PBS pH 6.0), then cells of the two strains were divided into two parties viable and nonviable (heat-treated at 60°C for 10 min) (Bejaoui *et al*.,2004)

Cereals

Good quality of barley and wheat grains free from any toxin were obtained from the Agricultural Research Center Ministry of Agriculture, Egypt.

Barley and wheat grains were ground in a Laboratory Falling Number hanmer mill with a sieve of size 0.5mm, 50g, and mixed with 40ml distilling water, then centrifuged at 5000g for 30 min, finally the supernatant was collected and pasteurized at 70°C for 20 sec. (Charalampopoulos *et al*., 2002).

Detection of Aflatoxins

Aflatoxins (B_1, B_2, G_1 and G_2) were detected according to the method of AOAC (2007), qualitatively by thin layer chromatography following by quantitative method. Detection of toxin was performed using high performance liquid chromatography. Data were integrated and recorded using a Millennium chromatography. Manger Software 2010 (Waters, Milford MA 01757).

Ice-cream Preparation

Vanilla, chocolate, strawberry and mango ice-cream powder (full fat milk powder, sugar, stabilizer, emulsifiers, palm kernel and coconut oil) were prepared by adding 20% of barley or wheat extracts or milk and the mixtures were whipped with electric mixture at high speed within 3min. The whipped mixture was hardened in freezer at -18°C and stored for three months.

Binding ability of yeasts to aflatoxins in Phosphate Buffered Saline (PBS)

Two percent of *S. cervisiae* as well as *S. boulardii* viable or nonviable were placed in flasks with 20 µg/L aflatoxins (B_1, B_2, G_1 and G_2) with 100ml PBS buffer, then were incubated at temperature 8°C (refrigerator) or 25°C (room temperature). Aflatoxins were detected after 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h, according to the method performed by modifying of Shetty *et al*. (2007). After incubation, the flasks were centrifuged and the aflatoxins were detected in the supernatants.

Binding ability of *S. cerevisiae* to AFS in cereals extract

Two percent of *S. cerevisiae* (viable) with 20 µg/L of aflatoxins and 100 ml of barley or wheat extracts were incubated in refrigerator at 8°C for 12 h, then the residue of aflatoxins were detected by HPLC method and calculated the binding of AFS.

Binding ability of *S. cerevisiae* to AFS in ice-cream

Different flasks contain 500 ml barley or wheat extracts or milk were inoculated with 2% of *S. cervisiae* (viable) and 20ug/L of aflatoxins and were incubated in refrigerator at 8°C for 12 h, then, ice-cream powder (Vanilla, chocolate, strawberry and mango) were added, whipping with electric mixture at high speed within 3min then were hardened in freezer at -18°C to evaluate aflatoxins binding.

Microbiological examination

Total bacterial counts in fresh ice-cream mixtures and after three months of storage (at -18°C) were counted on plate count agar (Oxoid). Plates were incubated at 37°C for 48 h under aerobic conditions for mesophilic microorganism and at 7°C for 10 days for total aerobic psychrophilic microorganisms. Colony-forming units were counted (cfu / ml) and the results expressed as their $\log_{10}$ values (FDA, 1992). Yeast counts were determined on malt extract agar (Oxoid) supplemented with tetracycline at final concentration of 10mg/liter, plates were incubated at 25°C for 5 days (Sarais *et al*., 1996).Spore formers were determined by heating the sample dilution (10^{-1}) in water-bath for 10 min at 80-85°C as described by Meer *et al*. (1991), then plated on plate count agar supplemented with 0.1% soluble starch, incubated at 37°C for 18 h for mesophilic spore former and at 10°C for 7 days for psychrophilic sporformer .

Sensory evaluation of ice-cream

Samples of prepared ice-cream (Vanilla, chocolate, strawberry and mango) supplement by barley or wheat extracts or milk with 2% *S. cerevisiae* (viable) were evaluated by fifteen members of laboratory staff. Quantitative descriptive analysis (QDA) was used to determine differences in the sensory characteristics of the ice-cream with barley, wheat extract or milk. The panelists evaluated the texture, appearance, color, taste, odor and overall acceptability on unstructured 10 line scales verbally anchored at each end. The results from the linear scale were subsequently converted to numerical

values (from 0 to 10 units) by a computer. The panelists were also asked to evaluate the overall acceptability of the ice cream with barley, wheat and ice cream with milk on the basis of texture, appearance, color, taste, odor and overall acceptability. An unstructured graphical scale was anchored on both ends: not accept (0)-fully accept (10) (Meligaard *et al*., 1991).

Statistical analysis

Results of sensory evaluation of ice-cream with barley, wheat and milk were subjected to statistical analysis of variance and least significant differences (LSD) as described by Rao and Blane (1985).

3. Results and Discussion

Binding ability of yeasts to aflatoxins in PBS buffer

Binding ability of yeasts to aflatoxins in PBS buffer medium at 8°C and 25°C at different times were illustrated in Figs 1-3. Fig. 1 showed the AFS binding by viable cells of *S. cerevisiae* when incubated at 8°C for12 h was 74.70%. While non-viable yeast *S. cerevisiae* had low binding effect. Our results were not agreement with Shetty *et al*. (2007) who found that aflatoxin binding by *S. cerevisiae* was not affected by the cells grown at temperatures ranging from 20 to 37°C but was significant and reduced at 15°C, and added that bending seems to be a physical phenomenon with cells treated at 52, 55 and 60°C for 5 and 10 min or auto calving the cells at 120°C for 20 min (non-viable) which recorded 77.7% binding. On the other hand, total AFS binding by *S. boulardii* at 8°C in YPG medium, results showed that non-viable cells had no effect or binding AFS, but, viable cells binding 18% of AFS after incubated for 6h (Fig. 2). Regarding to the data in Fig. 3 shows that viable cells of *S. cerevisiae* could bind AFS at 25°C.

Appears specific nature of binding, after 4h binding 73.18% AFS but after 8h release the AFS or clean the cells from AFS binding. Fig.3 also shows that non-viable cells of *S. serevisia* binding 30.91% AFS after 8h. Finally, binding was not affected by the cells of *S. boulardii* (viable or nonviable) which grown at 25°C in YPG medium .These results due to the fact that, *S. boulardii* from a taxonomic point of view should not be recognized as a separate species, *S. boulardii* will in the following be referred to as *S. cerevisiae*. It is worth to notice that contrary to e.g., probiotic strains of lactic acid bacteria, apparently these seems not to be different strains *S. cerevisiae*. Based on the similarity in different molecular analyses, **Van der Aa Kiihle and Kiihle, (2003).**

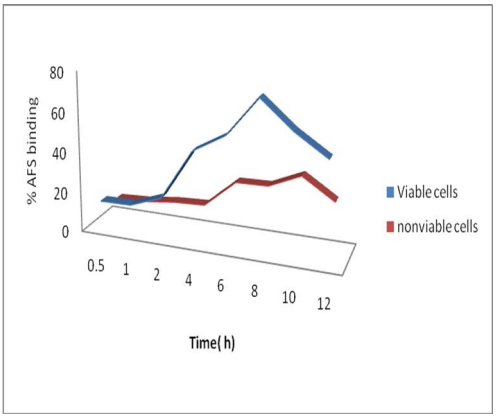


Fig (1). Total aflatoxins binding (%) by *S.cerevisiae* in YPG medium at 8 °C

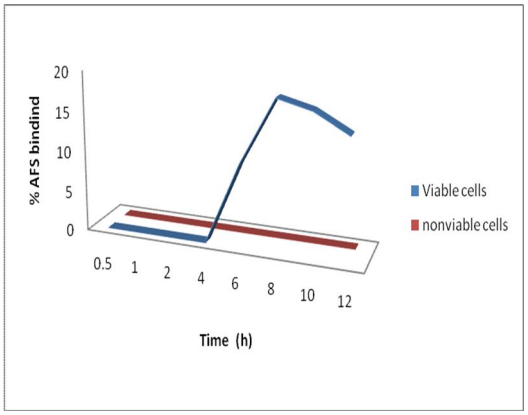
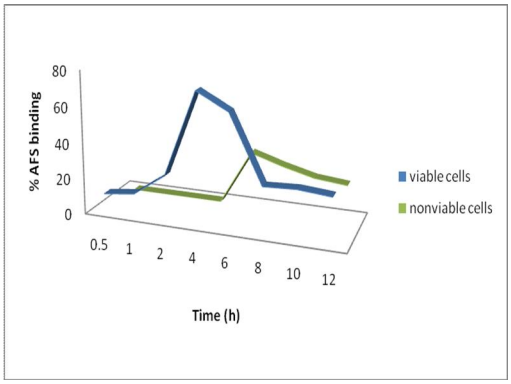


Fig (2). Total aflatoxins binding (%) by *S.boulardii* in YPG medium at 8 °C



Fig(3).Total aflatoxins binding (%) by *S.cerevisiae* in YPG medium at 25 °C

However, evidences from the poultry feeding experiments have shown that the yeast cell wall-aflatoxin complex can efficiently pass through the gut, resulting in protection from aflatoxin induced toxicities **(Santin *et al*., 2003)**. In these respect

Bueno *et al* (2007) studied the physical adsorption of aflatoxin B_1 (AFB_1) by lactic acid bacteria and *S. cerevisiae* from liquid medium the experimental results indicated the AFB binding to microorganisms was a rapid process and this binding involved the formation of a eversible complex the toxin and microorganism surface, considering that the binding (adsorption) and release desorption of AFB_1 to and from the site on the surface of the microorganism took place (AFB_1+S ⟷ S-AFB_1).

These observations were in agreement with **Guo *et al*. (2005)** who suggest that DNA in yeast *Saccharomyces cerevisiae* was damage tolerance pathways and are important in triggering AFB_1 associated recombination and mutation. So, recombinational or mutagenic pathways are selected in unclear. In addition, **(Shetty *et al*., 2007)** *S. cerevisiae* cells were capable of binding high amounts of alfatoxin B_1 at high concentration (20 µg/ml). The binding was still not saturated showing the high efficiency of strains. This indicates that *S. cerevisia* have a great-potential as aflatoxin binders in food and the nature of cell wall components involved in mycotoxin binding is still not clear and carbohydrate rich mannoproteins or glucans may be the likely candidates involved in the binding.

Binding ability of *S. cerevisiae* to AFS in cereals extract

Obtained results revealed that the best yeast was *S. cerevisiae* to bind AFS at 8°C for 8h in YPG medium then data obtained by HPLC showed that *S. cerevisiae* growth in barley extract bound 80% of added total AFS, with 60% in wheat extract .These observation were in agreement with **(Charalampopoulos *et al*., 2002)** who suggest this could be attributed to the simultaneous presence of considerable amounts of monosaccharide (glucose and fructose) and disaccharides (maltose and sucrose) in the barely medium**.** Also **Taillandier *et al*. (1996)** and **Elli *et al*. (1999)** found usually exhibiting poor growth in synthetic media without the addition of large amounts of supplements, such as yeast extract and peptone.

Binding ability of *S. cerevisiae* to AFS in cereal extracts ice-cream

As shown in Figures 4 and 5 *S. cerevisiae* binding AFS in chocolate and vanilla ice-cream with barely extract which may be due to the present of fat and protein in chocolate at high concentration than other types of ice-cream.

Determined 2.23 mg/ml AFM1 of ice-cream in Nigeria by **Atanda *et al*. (2007)**. He also, added the concentration of AFB_1 in feed which is transformed to AFM_1 in milk should be reduced by good manufacturing and good storage practices. Furthermore, there is need for stringent quality control during processing and distribution of these products**.**

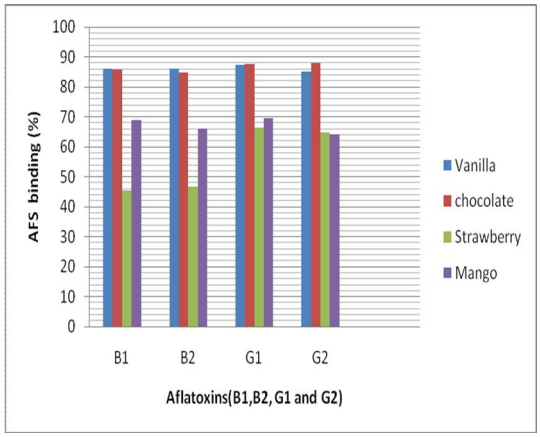


Fig (4). Binding ability of *S.cerevisiae* to AFS in barley extract ice- cream

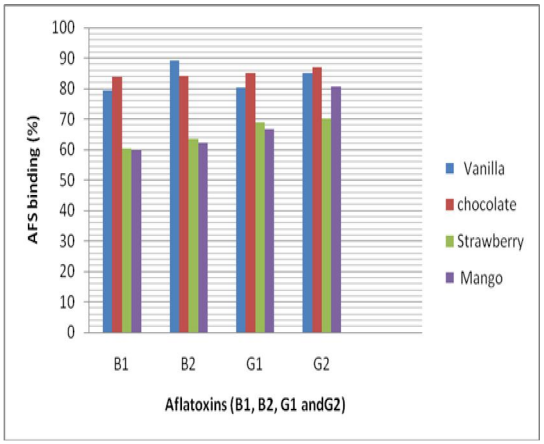


Fig (5). Binding ability of *S.cerevisiae* to AFS in wheat extract ice-cream

Microbiological examination

Fresh and stored samples of ice-cream supplemented with barley, wheat extracts as well as milk and inoculated with *S.cerevisiae* were analyzed for total bacterial count, psychrophilic bacterial, mesophilic and psychrophilic sporeformers and yeast counts (Figs. 6-9)

The obtained results in Figures 6, 7, and 8, showed similar trend of the results of total viable mesophilic aerobic bacterial and psychrophilic bacterial count .Also, the yeast count showed that these was not pronounced different counts among the all ice-cream samples. While, both mesophilic and psychrophilic sporeformers bacterial were not detected in any of ice-cream samples.

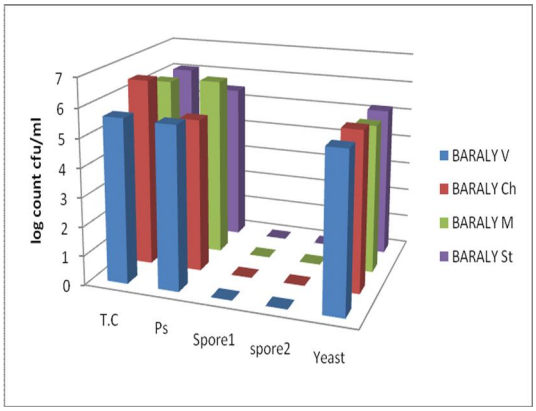


Fig (6). Microbiological examination of fresh barley ice-cream

T.C=Total bacterial count
Ps=Total psychrophilic bacterial count
Spore1= mesophilic sporeformers bacterial count
Spore2= psychrophilic sporeformers bacterial count

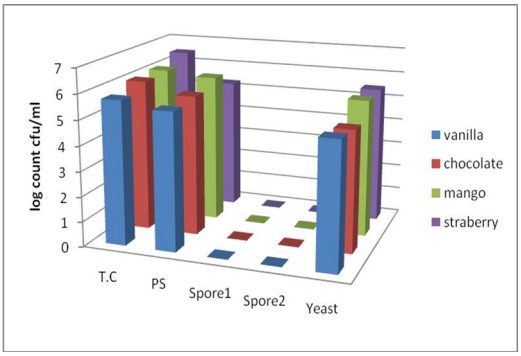
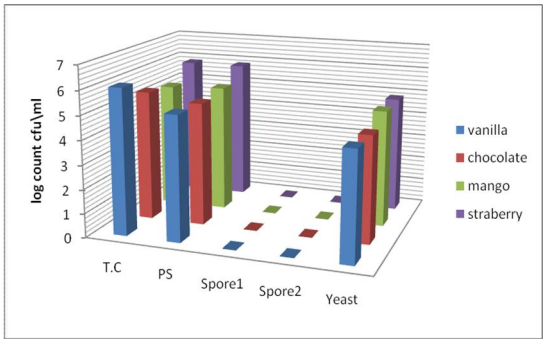


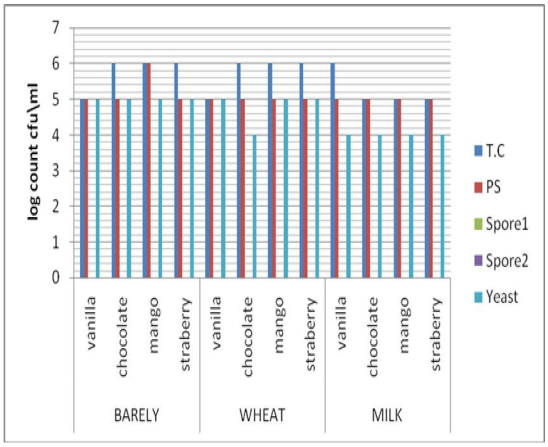
Fig (7). Microbiological examination of fresh wheat ice-cream

T.C=Total bacterial count
Ps=Total psychrophilic bacterial count
Spore1=mesophilic sporeformers bacterial count
Spore2= psychrophilic sporeformers bacterial count



Fig(8). Microbiological examination of fresh milk ice-cream

T.C=Total bacterial count
Ps=Total psychrophilic bacterial count
Spore1=mesophilic sporeformers bacterial count
Spore2= psychrophilic sporeformers bacterial count



Fig(9). Microbiological examination of barley, wheat extract and milk ice-cream after three months of storage

T.C=Total bacterial count
Ps=Total psychrophilic bacterial count
Spore1=mesophilic sporeformers bacterial count
Spore2= psychrophilic sporeformers bacterial count

Results as shown in Fig (9) revealed that the microbiological examination of barley, wheat extract and milk ice-cream after three months of storage in freezer (-18 ^{0}C) were had no changes.

Sensory evaluation of ice-cream:

The results in Table (1) indicated that chocolate ice-cream with barley or strawberry showed higher quality attributes especially texture, color, taste, appearance and overall acceptability, compared with the ice cream with wheat or milk. In addition, the ice cream with wheat received the lower color, taste and overall acceptability than the ice -Cream with barley and milk. The ice cream with barley was highly accepted for appearance, texture, taste and odor, respectively.

Conclusion

It could be concluded that viable *Saccharomyces cerevisiae* cells possess aflatoxins binding ability which can be incorporated into some cereal based dairy products this gives a new hope for safety cereal-based ice-cream where high aflatoxins level is potential health risk.

Corresponding author
Eman M. Hegazy
Food Toxicology and contaminants Department
National Research Centre, Dokki, Cairo, Egypt

Table (1) Sensory evaluation of ice-cream supplemented with barley or wheat extracts, milk and viable *S. cerevisiae*.

Ice-cream	Textures (10)	Color (10)	Odor (10)	Taste (10)	Appearance (10)	Overall Acceptability (10)
Ice-cream supplemented with barley extract and *S. cerevisiae*						
Vanilla	7.3±1.83^{ab}	7.4±0.84^{a}	7.3±0.61^{ab}	6.1±0.82^{ab}	6.9±0.02^{ab}	6.2±1.55^{b}
Chocolate	8.3±1.42^{a}	7.8±2.04^{a}	8.5±0.30^{a}	8.1±0.06^{a}	7.7±0.21^{a}	8.2±1.32^{a}
Strawberry	8.1±1.63^{a}	7.4±2.22^{a}	8.3±0.45^{a}	7.6±0.09^{a}	7.8^{a}±0.17	8.0±1.34^{a}
Mango	7.5±1.27^{ab}	7.2±1.84^{a}	7.2±0.53^{ab}	5.7±0.56^{abc}	6.6±0.38^{ab}	7.1±1.45^{ab}
Ice-cream supplemented with wheat extract and *S. cerevisiae*						
Vanilla	6.6±1.51^{abc}	7.6±1.71^{a}	6.9±0.72^{ab}	6.25±0.36^{ab}	6.6±0.02^{ab}	7.1±2.18^{ab}
Chocolate	6.9±2.02^{abc}	6.9±2.02^{a}	7.3±0.33^{ab}	6.5±0.11^{ab}	6.4±0.21^{ab}	7.0±2.26^{ab}
Strawberry	7.8±1.03^{a}	7.4±1.71^{a}	7.9±0.68^{a}	7.8±0.16^{a}	6.2±0.38^{ab}	7.6±1.96^{ab}
Mango	5.5±1.35^{c}	7.2±1.61^{a}	6.3±0.53^{b}	5.5±0.26^{abc}	6.8±0.17^{ab}	7.0±1.63^{ab}
Ice-cream with milk and *S. cerevisiae*						
Vanilla	7.6±1.96^{a}	7.2±1.03^{a}	7.0±0.61^{ab}	7.5±0.06^{a}	6.3±0.17^{ab}	7.4±1.35^{ab}
Chocolate	6.9±2.02^{abc}	7.1±1.69^{a}	6.5±0.30^{b}	5.5±0.26^{abc}	7.2±0.66^{a}	7.2±1.40^{ab}
Strawberry	7.2±1.27^{ab}	7.8±1.40^{a}	7.5±0.45^{ab}	5.7±0.56^{abc}	7.6±0.24^{a}	7.5±1.62^{ab}
Mango	7.15±1.63^{ab}	7.7±1.75^{a}	7.2±0.53^{ab}	6.1±0.82^{ab}	7.4±0.81^{a}	7.4±0.92^{ab}
LSD (0.05%)	1.59	NS	1.56	0.72	0.49	1.36

A, b: Mean in each raw followed by the differ letter are significantly different $p \leq 0.05$)
NS = Non significant

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10/2/2011