Mannan extract from *Saccharomyces cerevisiae* used as prebiotic in bio-yogurt production from buffalo milk

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**Abstract**

Four strains of the dry yeast *Saccharomyces cerevisiae* were obtained from different sources. The identification of yeasts was confirmed by morphological and physiological properties. Mannan extracts from yeast cell walls ranged between 25.80-34.40% of the dry weight of yeast cells. Five different concentrations of mannan extract were mixed with buffalo milk, to be used for bio-yogurt production. Probiotic starter culture contained *Lactobacillus acidophilus, Bifidobacterium* sp. and *Streptococcus thermophilus* used. After fermentation, pH values decreased to 4.6-4.8, while total acidity was 0.89-1%. The cells viability of *Lactobacillus acidophilus* was 6.8-7.85 Log CFU/g, 5.8-7.15 Log CFU/g of *Bifidobacterium* sp. and 6.74-7.22 Log CFU/g of *Streptococcus thermophilus* after fermentation. At 28 days (the end of the storage period), pH values decreased to 3.7-4.2, while total acidity increased to 1-1.3%, The cells viability of starter bacteria decreased with *Streptococcus thermophilus* being affected. Consequently mannan extract added to the yogurt increase the viability of probiotic bacteria after production.

**Introduction**

Yeast cell wall is a non-specific stimulator of the immune system in both human and animals which is generally composed of of 30-60% polysaccharides (beta-glucan and mannan sugar polymers), 15-30% proteins, 5 - 20% lipids and a small amount of chitin. Most of the protein is linked to the Mannan-OligoSaccharides (MOS) and is referred to as the Mannoprotein complex (Aguilar-Uscanga and Francois, 2003; Huang *et al*., 2005), (Klis *et al*., 2006). The mannoproteins contain 80-90% mannose sugar which is linked with 5-20% proteins. The molecular weight of mannoproteins ranging from 20000 to 200000 Daltons. The backbone of complex chains in mannoproteins is composed of α-1,6-linked mannoside which is 83% branched at O2 within oligosaccharide side chains which is mostly found in the form of di-, tri- and tetramers (Izabela *et al*., 1974). The production of mannan is significantly affected by the mannan content in *Saccharomyces cerevisiae*, changes in the physiological state of yeast cells and interactions within the cells such as addition of alpha-fator (alos known as sex factor) or transfer to the non-permissive temperature (Diaz *et al*., 1992).

Prebiotics, known as non-digestible carbohydrate compounds, do not affect the host by reducing the activity and the number of bacteria in the colon, and thus improve the health of the host (Gibson and Roberfroid, 1995; Niamah *et al*., 2016). Short-chain carbohydrates including transgalactooligosaccharides, polydextrose, galactooligosaccharides, banana psyllium, wheat dextrin, whole grain wheat, acacia gum, and whole grain corn also have prebiotic effects (Slavin, 2013). Mannan oligosaccharide content is an important biological function of yeast cell walls and it’s because of its prebiotic activity. The activity come from Prebiotics, known as non-digestible carbohydrate compounds, do not affect the host by reducing the activity and the number of bacteria in the colon, and thus improve the health of the host (Gibson and Roberfroid, 1995). Prebioticc activity which have a vital role that can serve as a nutrient source for the growth of beneficial bacteria (probiotic bacteria) in the colon of warm-blooded animals (Kneifel *et al*., 2000; Halas and Nocthe, 2012) and fish (Akrami *et al*., 2013). The aim of the present work is to provide an appropriate approach to obtain mannan extract from yeast cell walls and study the effect of add on the acidity of yogurt and the viability of bacteria starter during storage time.

**Materials and Methods**

*Saccharomyces cerevisiae* strains

Many types of dry cultures of *Saccharomyces cerevisiae* were collected from the local markets of Basra city in Iraq which included Saf-instant.
red (S) production coda 31150/Milwaukee, USA, Lallemand BRY-97, European (E)/ UK, Natu (N)/ arbin Fit Chemical Co., Ltd., China and Yuva-Maya (Y)/Yuva Company, Istanbul, Turkey. It was grown on yeast extract peptone dextrose medium (YPDA) agar composed of 20 g/L glucose, 20 g/L peptone, 10 g/L yeast extract and 20 g/L agar and kept at 4°C before use. Identification tests of yeast types included morphological, microscopic and biochemical tests: carbon source fermentation, carbon source assimilation, decomposition of urea, acid production and growth at different temperatures (Lodder, 1970; Barnett et al., 1990).

**Chemicals**

All chemicals used in the study were analytical grade. The glucose, yeast extract, peptone, phenol, agar, nalidixic acid, neomycin sulphate, lithium chloride LiCl and paromomycin sulphate were obtained from BDH Company, UK. Sodium hydroxide NaOH, Hydrochloric acid HCl fructose, sucrose, maltose, galactose, Lactose and raffinose from Sigma Company, Germany. Ethanol absolute, diethyl ether and sulfuric acid H$_2$SO$_4$ from Scharlau company, Spain.

**Extraction of crude mannan oligosaccharides**

The water-soluble mannan oligosaccharides were obtained from 5 g dry yeast by extraction with 1% NaOH (50 ml) at 100°C for 2h, cooling and neutralizing at pH7 with dilute HCl solution. After filtration, the mannan oligosaccharides were precipitated by adding 200 ml (4 volumes) of ethanol absolute. The precipitate was washed with ethanol absolute and diethyl ether redissolved in water, dialyzed against 2 changes of water and subsequent drying (Huang et al., 2010).

**Determination of mannan concentration and yield**

The mannan concentration in the extracts (ME) was determined by the phenol-sulfuric acid method using mannose as standard method followed by (Dubois et al., 1956). The yield value was estimated by dividing the weight of mannan obtained on total weight of the yeast.

**Mannan production**

Old yeast (age 18h) stimulated on the YPDA medium (Hi-media, India) was transferred to 100 ml conical flasks containing 50 ml of yeast extract peptone dextrose medium broth (YPDB) and incubated at 30°C temperature for 72h in a shaking incubator at 150 rpm/min (Liu et al., 2009). Thereafter, the cells were separated by centrifuging (Damon/IEC division, USA) at 6000 × g /min for 15 minutes after which, they were washed with distilled water and then estimated the biomass by the quantity of mannan extract.

**Measurement of yeast biomass**

The method described by DeSous et al. (2006) was followed for the measurement of the yeast biomass. Biomass was collected at the end of the fermentation period by centrifuging at 6000 × g / min at 4°C and washed two times with distilled water. The precipitate was dried in aerobic oven at 105°C temperature for 4h and the yeast biomass was measured with an analytical balance.

**Probiotic starter culture**

Probiotic starter was obtained from Chr. Hansen Middle East and Africa, UAE. It contains Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium sp. which was grown in de Man Rogosa Sharpe broth (Hi-media, India) at 37°C for 24h and used as a probiotic starter in yogurt production.

**Bio-yogurt production**

Buffalo milk (Animals station of Agriculture College / Basrah University) was used in yogurt production (Tamime and Robinson, 1999). After heating of the milk at 90°C temperature kept at 4°C for 5min made it cold. The concentrations of mannan extract (0.5, 1.0, 1.5, 2.0 and 2.5%) (W/V) were mixed respectively with milk and then 3% probiotic starter was added. After that prepare mixture was incubated at 40°C temperature for 4h. After fermentation, yogurt products were refrigerated at 4°C temperature for 28 days.

**Bio-yogurt tests**

Total acidity and pH of yogurt were estimated by the method of Nielsen (2010) described as followed: Cells viability count of the probiotic starter cultures were done on selective media: M17 (Hi-media, India) for S. thermophilus, de Man Rogosa Sharpe (MRS)-raffinose for L. acidophilus and MRS-nalidixic acid-neomycin sulphate-LiCl-paromomycin sulphate (NNLP) for Bifidobacterium sp. (Shah, 2000; Ashraf and Shah, 2011). Tests were conducted at 0, 7, 14, 21 and 28 days of storage.

**Results and Discussion**

Saccharomyces cerevisiae characteristics

Morphological and biochemical analysis of Saccharomyces cerevisiae strains included white or
cream colored colonies, circular or diagonal in shape colonies when grown on solid media. These colonies had regular and smooth edges and were elevated on the agar surface. Yeast cells appear blue in color when stained by blue methylene dye, spherical to coccii in shape with clear nucleus. The presence of buds were found more in all parts of the cells but the lack of mycelium was found under the microscopic examination.

The biochemical tests showed the ability of the yeast to ferment carbohydrates including glucose, fructose, sucrose, maltose, galactose and raffinose (Table 1). All strains were not able to ferment lactose. Ammonia was not produced from urea because it does not possess urease enzyme. These results also showed the ability of yeast strains to grow excellently at 30°C temperature while the growth was weak at 37°C. All tests confirm that the strains belong to Saccharomyces cerevisiae (Barnett et al., 1985).

The yield of mannan

The yields of mannan extracted were ranged between 25.80-34.40% of the dry weight of yeast cells (Table 2). Higher yield was attributed to the presence of a protein linked with mannan. All mannan in yeast cells is supposed to exist in the complex form with protein and were found in wall cell (Maier et al., 1993). The results were agreed with many studies which reported that mannan in the wall of yeast cells ranges between 30-50% (Aguilar-Uscanga and Francois, 2003; Klis et al., 2006).

The ability of yeast strains to grow on media culture was studied for the purpose of knowing the amount of biomass production, which ranged between 0.47 to 0.73g/100 ml, while mannan extracted ranged between 24.43-38.44% (Table 2). The higher yield of biomass and mannan production in the fermentation medium was attributed to carbon content and energy sources readily metabolized by Saccharomyces cerevisiae such as monosaccharides. In media culture these can be directly used for production (Dynesen et al., 1998). The highest amount of mannan extract (36.51%) was obtained from the European yeast which was found suitable for the mannan production as prebiotic in the present study.

Table 1. Biochemical tests used to diagnose yeast

<table>
<thead>
<tr>
<th>Kinds of yeast</th>
<th>Fermentable carbon sources</th>
<th>Diagnostic tests</th>
<th>Carbon Assimilation</th>
<th>Decomposition of urea</th>
<th>Growth at different temperatures</th>
<th>Acid production</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>G +, T - , M - , Ga - , Ra - , L +</td>
<td>30°C</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E</td>
<td>G - , T + , M + , Ga + , Ra + , L +</td>
<td>37°C</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>G - , T - , M - , Ga - , Ra - , L +</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Y</td>
<td>G + , T + , M + , Ga + , Ra + , L +</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


Table 2. Yield of mannan extracted and mannan content in yeast isolates at the media of growth (YPD)

<table>
<thead>
<tr>
<th>Yeast kinds</th>
<th>Mannan g/yeast</th>
<th>Mannan yield %</th>
<th>Dry cell (g) /100 ml media</th>
<th>Mannan % of dry cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>1.58±0.01</td>
<td>31.60±0.05</td>
<td>0.56±0.00</td>
<td>27.87±0.10</td>
</tr>
<tr>
<td>E</td>
<td>1.72±0.01</td>
<td>34.40±0.03</td>
<td>0.73±0.03</td>
<td>36.51±0.06</td>
</tr>
<tr>
<td>N</td>
<td>1.42±0.02</td>
<td>28.40±0.05</td>
<td>0.55±0.01</td>
<td>30.44±0.08</td>
</tr>
<tr>
<td>Y</td>
<td>1.29±0.03</td>
<td>25.80±0.10</td>
<td>0.47±0.05</td>
<td>24.43±0.05</td>
</tr>
</tbody>
</table>

S: Saf-instant red, E: Lallemand European, N: Natu, Y: Yuva-Mayy, SD±: standard deviation

pH and total acidity of yogurt

pH and total acidity value of control yogurt without mannan extract concentrations were 4.81 and 0.81% respectively after fermentation. Low acidity found in control yogurt because of weak growth probiotic bacteria in the milk and it need to increase of fermentation time suggested by (Lourens-Hattingh and Viljoen, 2001). With the addition of mannan extract to yogurt, the pH found to be decreased and the percentage of total acidity increased with increasing the concentration of added ME. The pH and percentage total acidity values were 4.6, 4.6, 4.55, 4.53, 4.45 and 0.89, 0.9, 0.95, 0.96, 1% for 0.5, 1, 1.5, 2, 2.5% mannan extract, respectively, after fermentation (Figure 1 and 2). The pH levels further decreased during storage, while percentage total acidity increased during storage for control sample and all mannan extract concentrations (namely pH 4.63, 4.2, 4.14, 4.1, 3.9, 3.7 and %total acidity of 0.9, 1, 1.2, 1.22, 1.28, 1.3% for the different mannan
extracts 0, 0.5, 1, 1.5, 2, 2.5% respectively) after 28 days of storage (Figure 1 and 2).

Growth ratio and acidifying activity of the probiotic bacteria in products were variable depending on the type and concentration of the prebiotics. Mannan and other prebiotics bioavailability in media improve growth of probiotic bacteria (Aryana et al., 2007; Gustaw et al., 2011). Prebiotics tend to increase viability and activity of probiotic bacteria (Lenoir-Wijnkoop et al., 2007). Similar studies on results of the pH values of commercial yogurt containing probiotic bacteria during their storage were reported by (Shah et al., 2000). The decline in pH was assumed because of the continuation growth of lactic acid bacteria during storage (Kailasapathy, 2006).

Survival of bacteria starter

The viability of *Lactobacillus acidophilus* was 6.8 Log CFU/g after fermentation time and the numbers of viable cells decreased to 4.9 Log CFU/g under storage conditions at 4°C for 28 days. Added mannan extracts to yogurt increased the cells viability of *Lb. acidophilus* after fermentation time compared to the control without mannan extract (Fig. 3). After storage time, the viability of *Lb. acidophilus* was 6, 6.09, 6.21, 6.6, 6.09 Log CFU/g for 0.5, 1, 1.5, 2, 2.5% mannan extract, respectively (Table 3). After fermentation, the cells viability of *Bifidobacterium* sp. in yogurt production was 5.8, 5.86, 6.1, 6.6, 7.1 Log CFU/g for 0, 0.5, 1, 1.5, 2, 2.5% mannan extract, respectively. The cells viability of *Bifidobacterium* sp. in yogurt without mannan extract (control) was 4.6 Log CFU/g while addition of mannan extract into yogurt lead to increased values of 5, 5.13, 5.11, 5.5, 5.2 Log CFU/g for the respective ME concentrations (0, 0.5, 1, 1.5, 2, 2.5% mannan extract) respectively after storage time (table 3). *Lb. acidophilus* and *Bifidobacterium* have β-mannanase (EC 3.2.1.78) enzyme which do mannan analysis into simple units and utilize it for growth (Hammes and Hertel, 2009; Kulcinskaja et al., 2012).

Table 3 indicates the lack of a significant effect on the *Streptococcus thermophilus* cells viability in yogurt production within mannan containing extracts or without (control yogurt). The cells viability of St. thermophilus was 6.74, 6.79, 6.82, 7.01, 7.19, 7.22

Table 3. Viability of starter bacteria (Log CFU/g) in yogurt with mannan extract concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5%) after fermentation and storage days.

<table>
<thead>
<tr>
<th>ME</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0%</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
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<tr>
<td>0.5%</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
<td>5.86</td>
</tr>
<tr>
<td>1.0%</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
<td>5.90</td>
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<tr>
<td>1.5%</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
<td>5.94</td>
</tr>
<tr>
<td>2.0%</td>
<td>5.98</td>
<td>5.98</td>
<td>5.98</td>
<td>5.98</td>
<td>5.98</td>
<td>5.98</td>
<td>5.98</td>
<td>5.98</td>
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<td>5.98</td>
</tr>
<tr>
<td>2.5%</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
<td>6.02</td>
</tr>
</tbody>
</table>

ME: Mannan extract
Log CFU/g for the 0, 0.5, 1, 1.5, 2, 2.5% mannan extracts respectively after fermentation. The viability decreased during storage to values of 4.5, 4.51, 4.55, 4.53, 4.58, 4.5 Log CFU/g for the respective ME concentrations (0, 0.5, 1, 1.5, 2, 2.5%) mannan extract respectively after 28 days.

In previous studies, the loss of cells viability of probiotic bacteria in yogurt during storage, were attributed to the acidity and low temperature (Shah et al., 1995; Vinderola et al., 1999). Prebiotics such as lactulose, inulin and β-glucan significantly improved the cells viability of probiotic bacteria (Ozer et al., 2005; Akalin and Erisir, 2008; Sahan et al., 2008).

In general, the cell numbers of probiotic bacteria in yogurt production containing mannan extract concentrations are among the numbers allowed by FAO/WHO protocols which were reported to be 10⁶-10⁹ CFU/g.

Conclusion

Mannan content of wall cell is different between strains of yeast. Mannan oligosaccharides work as prebiotic when it adds to milk fermentation products. The relationship between probiotics and prebiotics in food fermentation are called symbiotics. Add mannan extracted to yogurt product from buffalo milk led to increase the viability of probiotic bacteria in yogurt and enhancement of the survival of probiotic bacteria during storage. Viability of Lb. acidophilus and Bifidobacterium were within the allowable limits, despite low pH and increase the percentage of total acidity after storage time.

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References


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