Effect of Addition of Jerusalem Artichoke (Helianthus Tuberosus) Tubers Powder, and Inulin on Lactobacillus Reuteri Activity and Recovery After Freezing Injury

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Abstract

This study indicated that the addition of 2% of Jerusalem artichoke (Helianthus tuberosus) tubers powder (JTP) exceeded the activity of Lactobacillus reuteri in comparison with inulin and control treatment. The number of bacteria after the activation period 24 hours/37°C increased in the skim milk added 1, 2 and 5% JTP to 70 × 10⁶, 210 × 10⁸ and 119 × 10⁷ Colony Forming Unit (cfu)/ml respectively. While the number of bacteria in the skim milk supported by addition 1, 2 and 5% Inulin was 56 × 10⁶, 83 × 10⁷ and 74 × 10⁷ cfu/ml. While in the control treatment was 44 × 10⁶ cfu/ml. The results indicated that the addition of 2% of the JTP contributed well to the preservation bacterial viability during period of storage at 4°C/7 days and frozen storage at -18°C/60 days. The number of bacteria in the fermented skim milk was supported by 1, 2 and 5% JTP 90 × 10⁷, 200 × 10⁷, 63 × 10⁷ cfu/ml respectively after cooling period, while it reached 30×10⁵, 22×10⁶ and 6 × 10⁵ cfu/ml respectively after freezing period, while the numbers of bacteria in the control treatment were 44 cells/g only. The results indicated that the addition of 2% of the inulin was superior in keeping the numbers of bacteria during the frozen storage period at -18°C/60 days compared to the refrigerated storage at 4°C/30 days, the number of bacteria in the fermented milk and supported by 1, 2 and 5% of the inulin 72×10⁷, 83×10⁷ and 63×10⁷ cfu/ml after the cooling period respectively, while in frozen milk was 36×10⁵, 47×10⁵ and 50×10⁵ cfu/ml. The results also indicated the positive role of the addition of 2% JTP in the recovery of injure cells after the end of the frozen storage period -18°C/60 days where the number of cells at activation period following the freezing increased from 44 cells/ml to 71×10⁴ cfu/ml and in the was control 57×10⁴ cfu/ml.

Keywords: Lactobacillus reuteri, Jerusalem artichoke, Inulin.

1. Introduction

The Jerusalem artichoke belongs to the Sunflower family [1]. This crop is characterized by its ability to withstand low temperatures, which can sometimes reach frost, as well as the ability to resist droughts. This plant is a naturally grown plant in the North American continent, and many researches have pointed to the health benefit resulting from the use of this plant [2]. many researches have pointed that the tubers of this plant contain significant quantities of anulin, which made it enjoy the status of substances supporting the beneficial microbes prebiotic; which are types of food that host’s digestive system cannot digest or absorb and has at the same time a health effect on it through specialized support and / or reactivation of bacteria with health or therapeutic properties present in the digestive tract with a negative effect on harmful bacteria in the intestinal cavity of the host [3]. The Jerusalem artichoke (Helianthus tuberosus) tubers has a prebiotic capacity due it contains significant amounts of inulin, Studies have indicated that the dry weight of Jerusalem artichoke (Helianthus tuberosus) tubers may contain 68-83% inulin as well as 15-16% proteins, 13% insoluble fiber and 5% ash. All of these materials provide an excellent support for the growth of microorganisms that make up the human intestinal flora [4]. The references confirmed the effect of harvest time and storage conditions of tubers on the degree of polymerization of inulin [5].
Inulin is defined as a poly fructose sugar which has structure \((C_6H_{12}O_{10})_{3-35}\), often consisting of 3-35 fructose units bound to a terminal glucose molecule and sometimes up to 60 units of \(\beta\)-Fructose type, depending on the length of its carbon chain can be classified as oligo or polysaccharides [6], where fructose molecules are bound by \(\beta\)-1-2 that binds \(\beta\)-D-fructosyl residues with \(\alpha\)-D-glucose end group [7]. Some plants store inulin as the only source of energy, It is stored either in roots or tubers and is stored as crystals rather than granular [8]. Numerous studies have indicated the supportive role of inulin in the growth and activity of bacteria with therapeutic properties Probiotic, [9-11], pointed to the positive role of this sugar in the support and activity of *Streptococcus salaverus spp. thermophiles* and Bifidobacterium bacteria in milk fermentation.

*Lactobacillus reuteri* belongs to the genus Lactic acid bacteria, which classified as gram positive, with oxygen trace requirements and heterogeneous fermentation, a temperature of 37°C is the optimum temperature for growth [12,13], were found this bacteria has a positive effects, healthy and therapeutic in the host. This bacteria was shown the ability to survive and colonize in the digestive tract due high acidity resistance of the stomach and high concentration of bile salts [14-16]. Through its ability to reduce the acidity of the lumen Gastrointestinal and production *reuterin* (a bacteriocin with a wide range of effects on the growth of harmful microorganisms) this bacteria control the pathogenic bacteria in the IG. [17] and its ability to secrete several enzymes which improve digestibility [18], and it’s effective to reduce glucose and cholesterol value in the blood [19], as well as its positive role in improving host immunity [17] and host resistance to HIV [20].

### 2. Materials and methods:

The Jerusalem artichoke (*Helianthus tuberosus*) tubers was obtained from the local market in Ramadi between 15 November 2018 and 15 December 2018. The tubers were washing by water, peeling and slicing. Slides were immediately submerged with boiling water for 5 minutes followed by submerging them in a cold solution of 1% citric acid, dried at 50-55°C by electric oven model YCO-L01037 made in Taiwan until neat weight stability. Dried slices were grind and storage in polyethylene bag at -18°C until use [21]. Inulin was extracted from powdered tubers according to the method previously described by [1]. The amount of anulin was estimated according to the method described by [22]. Moisture, total solids, ash content of tubers, fat, fiber and protein ratio were estimated according to the methods described by [23].

#### 2.1 *Lactobacillus reuteri*

Pure isolation from *Lactobacillus reuteri* was obtained from the laboratories of the College of Engineering for Agricultural Sciences-University of Baghdad, and biological tests did to confirm the viability of the bacteria and the purity of strain. Bacteria was activated by transferring twice in MRS broth for 48 hours at 37°C, and then was grown in sterilize skim milk 121°C / 5 minutes and incubated at 37°C for 24 hours, then the colony was cooled until it used.

#### 2.2 Effect of Jerusalem artichoke powder and inulin on the bacterial activity

*Lactobacillus reuteri* was inoculated in two groups, first sterilized skim milk supplemented with tubers of Jerusalem artichoke powder, 2nd with pure inulin at 1, 2 and 5% respectively and incubated at 37°C for 24 hours. Then the living cells were counted as Described by [24]. Bottles were distributed to two groups for each treatment. The first group was stored at 4°C for 14 days, the second was stored at -18°C / 60 days. Then total count of bacteria was enumerated. Bacteria were reactivated in skim milk and skim milk with 1, 2 and 5% Jerusalem artichoke powder and inulin.

### 3. Results and discussion

#### 3.1 Chemical composition of Jerusalem artichoke tubers

Heating of Jerusalem artichoke tubers inhabited the activity of polyphenoloxidase and inulinase enzyme [25], and denatured some proteins which is approximately 5.5% of wet weight In tubers. This steep was important to improve the solubility of polysaccharides [26]. Table 1, shows the chemical composition of the Jerusalem artichoke tubers powder estimated as wet weight, a 100g of tuber powder contained 28.25 g solids, while the ash content was about 1.88 g. The total amount of carbohydrates was about 17.15 grams, where fructons were 87% of the total carbohydrates, this is consistent with [27].
### Table 1. Chemical Composition of Jerusalem artichoke tubers.

| Chemical composition (100 g) | wet  | Dry  |
|-----------------------------|------|------|
| Total Solids                | 28.25| 6.5  |
| Ash                         | 1.88 | 0.75 |
| Total carbohydrates         | 17.15| 5.1  |
| Total Fructons              | 14.9 | 4.25 |

#### 3.2 Effect of tubers and inulin powder on bacteria growth

The results in Figure 1 show that the addition of Jerusalem artichoke tubers powder has more effect on bacterial count in compared with the inulin or control. The bacterial numbers increased from $48 \times 10^5$ to $70 \times 10^6$ and $56 \times 10^6$ c.f.u./g in milk contains 1% of Jerusalem artichoke tubers powder and inulin respectively. While the bacterial numbers were $210 \times 10^5$ and $83 \times 10^6$ c.f.u./g in milk containing 2% of Jerusalem artichoke tubers powder and inulin respectively. The bacteria account was $53 \times 10^6$ and $36 \times 10^6$ c.f.u./g in milk containing 5% Jerusalem artichoke tubers powder and inulin respectively. While the number of bacteria was $44 \times 10^6$ c.f.u./g in the control. These results agreed with [28], that inulin supports the growth of lactic acid bacteria. The increase in bacterial numbers due to the presence of inulin comes from its ability to break down this sugar into fructose, a carbon source that supports cell’s growth during the incubation period [29,30], and the analyze inulin increases with low pH of medium [31].

#### Figure 1. Effect of Jerusalem artichoke tubers and inulin on bacterial growth.

The superiority of tuber powder because it containing inulin as well as quantities of amorphous fructones that are easy to consume from bacteria as well as significant amounts of proteins and other growth factors [2,21]. The decrease number of bacteria in the control treatment was due to the competition between cells on the limit materials in growth media. The results showed that the addition of 2% tubers powder or inulin gave the best growth of the bacteria, while 5% caused a decrease number of bacteria due to the association of solids with free water in the media which leads to increased osmotic pressure on the bacteria adversely affecting their growth rate [32,33].

#### 3.3 Effect of tubers powder and inulin on bacterial survivability under cooling:

The results in Figure 2. showing the positive role of Jerusalem artichoke tubers powder and inulin to support bacterial growth during 14 days at 4°C, compared with control. The results also indicated that the addition of tubers powder contributed more than only inulin. A count of bacteria after refrigerated period increased from $104 \times 10^6$ to $90 \times 10^7$ and $200 \times 10^7$ and $104 \times 10^6$ c.f.u./g in supported milk by 1%, 2% and 5%, Jerusalem artichoke tubers powder, and to $72 \times 10^5$, $83 \times 10^7$, $82 \times 10^6$ c.f.u/g in milk with 1%, 2% and 5%, respectively. While the number of bacteria decreased to $43 \times 10^6$ c.f.u/g in the control.
This is because tuber powder contains many growth factors as well as significant quantity of inulin which serve as a prebiotic for bacteria [21]. Inulin alone played as prebiotic role to bacteria, but to a lesser extent [34,35].

The decrease in the number of bacteria in the control is due to the consumption of most of the nutrients in the medium culture during storage. The decrease of bacteria in milk containing 5% of tuber powder it became due to the increase of total solids in the media.

3.4 Effect of tubers and inulin on bacteria survivability during frozen storage

The results in Figure 3. indicate that the addition of Jerusalem artichoke tubers powder and inulin contributed well in bacterial viability during the freezing period -18°C/ 60 days compared to the control while the number of bacteria was significantly reduced. The results showed that the numbers of live bacteria decreased in the control from $33 \times 10^8$ cfu/g on the first day to 44 cells after the end of the storage period. This is due to the mechanical action of ice crystals which developed during the slow freezing period, as well as decrease in water activity during water transferring from liquid to solid so that it became difficult for the cells to use water for growth.

The results indicated that the presence of 1, 2 and 5% of Jerusalem artichoke tubers powder contributed well to the stability of bacterial viability, especially during the first week of frozen storage, but during increase the period it was decreasing from $33 \times 10^8$ to $54 \times 10^8$, $63 \times 10^7$, $48 \times 10^6$ cfu/g during the first week and to $30 \times 10^7$ and $22 \times 10^6$ $6 \times 10^2$ cfu/g, respectively in the end of frozen storage period. This may be due to the positive role of solid powder components, especially fats, proteins and complex sugars, to protect bacteria from the mechanical action of ice crystals formed during the storage period, but their association with free media`s water and transformation of that water from liquid to solid which caused decreasing of water activity and increased the osmotic pressure of the medium which has impacted negatively of bacterial viability during the late frozen storage period. The addition of inulin gives a good chance for the bacteria to survive during the first week of storage. After the first week of frozen storage, the numbers of bacteria were $60 \times 10^6$, $32 \times 10^7$, $74 \times 10^6$ cfu/g in milk was fortified by 1 , 2 and 5% inulin respectively, these results due the role of inulin played in supporting activity of bacteria [34].
Figure 3. Effect addition of Jerusalem artichoke tubers powder and inulin on the viability of \textit{L. reuteri} during frozen storage.

In addition to provide protection to the cells by surrounding them by capsulation [36], which reduces the destructive mechanical action of ice crystals formed during the slow freezing period [37]. The number of bacteria decreased through frozen storage to $36 \times 10^3$, $47 \times 10^3$, $50 \times 10^2$ cfu/g in the fortified milk by adding 1, 2 and 5% inulin, respectively.

3.5 Role of Jerusalem artichoke tuber powder and inulin on the recovery of injure bacteria after freezing.

The results in Figure 4. indicated that the presence of 2% Jerusalem artichoke powder tubers and 2% inulin contributed to improve a recovery of injure bacteria during freezing after the end of the frozen storage period, compared with the control.

Figure 4. Effect of Jerusalem artichoke powder tubers and inulin on the recovery of injure bacteria after freezing.

The number of \textit{L. reuteri} bacteria increased after 24 hours from 44 cfu/g to $57 \times 10^3$ cfu/g when grown in milk sorting only, while the number reached $52 \times 10^3$ and $71 \times 10^4$ and $83 \times 10^3$ cfu/g in milk fortified with Jerusalem artichoke tubers powder 1, 2 and 5%, respectively. The addition of inulin to medium contributed a good recovery of strained bacteria after freezing compared to the control treatment, but it was lower when it’s compared with milk fortified with tubers powder. The number of bacteria after the end of the incubation period increased to $40 \times 10^3$, $48 \times 10^3$, $25 \times 10^3$ cfu/g In milk fortified by 1, 2 and 5% inulin, respectively.

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