

## Effects of sugar beet fiber and inulin on viability of *Bifidobacterium bifidum* and physicochemical and sensory properties of red grape juice

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### Abstract

**Background and objective:** Dietary fibers are of prebiotic compounds which can increase viability of probiotic microorganisms in food products during storage and have healthful effects on the consumers. The aim of this study was to investigate the effect of different concentrations of inulin and sugar beet fiber on physicochemical and organoleptic properties of red grape juice and their stimulatory impact on viability of *Bifidobacterium bifidum* in the beverage.

**Materials and methods:** Amounts of 0.7, 1.4, and 2.1% w/v sugar beet fiber and 1, 2 and 3% w/v inulin were added to red grape juice inoculated with  $10^8$  CFU/ml *Bifidobacterium bifidum*. Viability of *Bifidobacterium bifidum* by enumeration on MRS agar, colorimetric evaluation, and study of sensory attributes by 5-points hedonic test were done on days 0, 5, 10, 15, 20, and 25. Moreover, pH and brix were determined during the storage time.

**Results and conclusion:** By increasing the concentration of inulin and sugar beet fiber, brix and pH of red grape juice increased and decreased, respectively. After 25 days of storage, the highest survival (7.05 log CFU/ml) was observed in the treatment containing 3% w/v inulin and 2.1% w/v sugar beet fiber and the least survival (5.03 log CFU/ml) was observed in control. Number of probiotic in all the treatments containing dietary fiber was higher than  $10^6$  CFU/ml after 25 days of storage, but lower than  $10^6$  CFU/ml was enumerated in control. The highest brightness and the least redness and yellowness was observed in control. By increasing the concentration of inulin above 1% w/v and sugar beet fiber above 1.4% w/v, palatability of grape juice samples decreased significantly. Therefore, red grape juice fortified with 1.4% w/v sugar beet fiber and 1% w/v inulin is a desirable beverage which can support survival of probiotic bacteria within the recommended range.

**Keywords:** *Bifidobacterium bifidum*, Inulin, Probiotic, Red grape juice, Sugar beet fiber

### 1. Introduction

There is an increasing tendency in development of functional foods. They promote health and

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provide nutrients for the body. In this regard, addition of probiotics to foods, especially dairy products, is one of the most popular approaches [1]. Some dietary fibers such as inulin are known as prebiotic which improve viability of probiotics. Daily intake of 38 g and 25 g dietary fiber is recommended for men and women, respectively [2]. A direct correlation between consumption of high-fiber diet and reduced risk of some chronic diseases such as colon cancer, constipation, obesity, diabetes, and cardiovascular diseases is reported. Dietary fibers are selectively metabolized by intestinal flora and change the microbial population in favor of beneficial microorganisms. Unfortunately, a large quantity of dietary fibers is lost through industrial wastes annually. They may have technological importance and can be used as raw material in functional food industry. For example, sugar beet fiber is obtained from sugar beet pulp as a byproduct of sugar extraction process. Processing of 100 tons sugar beet results in 6-10 tons dry pulp [3].

Presence of living organisms in probiotic foods is a difference between these products and other functional foods containing chemical agents [4]. Probiotics can improve and balance the GI microflora, stimulate the immune system, prevent cancers, treat lactose intolerance, prevent/treat diarrhea, and lower blood cholesterol. Resistance of probiotics in foods is a big deal because many factors affect cell viability during processing and storage [5]. Dairy products containing *Bifidobacterium bifidum* or Lactobacillus species fortified with fructo-oligosaccharide or inulin have been marketed over 10 years [6]. However, fruits and vegetables are free of some allergens found in dairy products and also contain beneficial compounds of minerals, antioxidants, dietary fibers, and vitamins. Therefore, they are good candidate in formulation of probiotic drinks [7]. Viability of Bifidobacterium and Lactobacillus species in orange, apple, pineapple, green apple, blueberry, lemon, peach, strawberry,

mango, pear, grape, kiwifruit, and pomegranate juices has been studied [8].

Optimization of fermentation process and stimulation of probiotics in the intestine by increasing their metabolic capacity is of interest worldwide [9]. Due to the health properties of probiotics, development of novel functional formula available in the market is necessary. Our investigation has revealed that no study has been done about synergistic effect of sugar beet fiber and inulin in probiotic grape juice. Therefore, sugar beet fiber and inulin were added to red grape juice containing *B. bifidum* to evaluate their stimulatory impact on viability of the bacterium during 25 days of storage. In addition, physicochemical and sensory properties of the product were studied.

## 2. Materials and methods

### 2.1. Materials

Grape juice concentrate and sugar beet fiber were prepared from Sunich company (Iran) and Hegmatan sugar company (Iran). Lyophilized *B. bifidum* (ATCC 29521) was purchased from Iranian Research Organization for Science and Technology (IROST). Short-chain inulin (8 degree of sterilization and 99.5% purity) was purchased from Roosendaal company (Netherlands). All other chemicals were obtained from Merck (Germany).

### 2.2. Preparation of samples

#### 2.2.1. Preparation of sugar beet fiber

100 g frozen sugar beet pulp was defrosted and cleaned manually. Then, 50 ml ethyl alcohol (69% v/v) was added to the 100 g pulp and the mixture was homogenized until a colorless pulp was achieved. The mixture was passed through a sieve (pore size of 100-150  $\mu\text{m}$ ) and the retentate was heated in oven at 50  $^{\circ}\text{C}$  for 12 h. The dried pulp was grounded and filtered by a sieve with mesh size of 250-300  $\mu\text{m}$  [10].

#### 2.2.2. Preparation of *B. bifidum* suspension

To activate the lyophilized bacteria, they were transferred to MRS broth. Then, the inoculated

medium was incubated at 37 °C for 24 h under micro-aerophilic condition. The bacterial suspension was further centrifuged at 8000 ×g and the precipitate was dissolved in 0.1% peptone water. Bacterial density of  $1.5 \times 10^8$  CFU/ml was prepared by MC Farland method and used for further analysis [11].

### 2.2.3. Preparation of synbiotic beverage

Through preliminary study, different concentrations of sugar beet fiber and inulin to red grape juice and amounts of 1, 2 and 3% inulin and 0.7, 1.4 and 2.1% sugar beet fiber were selected for our further studies (Table 1). Homogenization of the formula was done by a laboratory homogenizer (Fan Azma, Iran) at 20 Mpa. Then, the beverages were inoculated with  $10^8$  CFU/ml *B. bifidum* and kept at 4 °C.

Table 1- the prepared formula of synbiotic red grape juice

Treatments	Inulin (% w/v)	<i>B. bifidum</i> (CFU/ml)	Sugar beet fibre (% w/v)
T1	1	$1.5 \times 10^8$	0.7
T2	1	$1.5 \times 10^8$	1.4
T3	1	$1.5 \times 10^8$	2.1
T4	2	$1.5 \times 10^8$	0.7
T5	2	$1.5 \times 10^8$	1.4
T6	2	$1.5 \times 10^8$	2.1
T7	3	$1.5 \times 10^8$	0.7
T8	3	$1.5 \times 10^8$	1.4
T9	3	$1.5 \times 10^8$	2.1
T10	-	$1.5 \times 10^8$	-

### 2.3. Physicochemical study

Brix and pH of the functional red grape juice were measured according to Iranian national standard no. 3414 [12]. The experiments were done on days 0, 5, 10, 15, 20, and 25.

### 2.4. Viability of *B. bifidum*

Serial dilution was firstly prepared by addition of 1 ml homogenized grape juice to 9 ml peptone water (1%) followed by addition of 1 ml of the last dilution to 9 ml peptone water (1%). Then, the suspensions were cultured in MRS agar by pour plating method and were incubated under micro-aerophilic condition at 37 °C for 72 h. After incubation, the observed colonies were

enumerated [13]. The experiments were done on days 0, 5, 10, 15, 20, and 25.

### 2.5. Colorimetric study

Color was evaluated by colorimeter (Model D25-DP9000, Germany) equipped with standard black and white plates as reference. A white sample of 1.5 m height was placed in standard plates, X=81.2, Y=83.32, and Z=98.03. CIE system was used [14]: L\* (lightness) blackness (0)/whiteness (100); a\* (greenness/redness); b\* (blueness/yellowness) (-120 to +120). The experiments were done on days 0, 5, 10, 15, 20, and 25.

### 2.6. Sensory evaluation

Sensory attributes of the juice samples including taste, flavor, color, mouthfeel, consistency, and overall acceptance were evaluated by 25 semi-trained panelists in Karaj agricultural engineering research institute by 5-points hedonic text [15]. The experiments were done on days 0, 5, 10, 15, 20, and 25.

## 3. Results and discussion

### 3.1. Brix

According to Figure 1, brix of the samples increased significantly by increasing the concentration of inulin and sugar beet fiber. The highest and the least brix were calculated on day 25 for the samples containing 3% w/v inulin + 2.1% w/v sugar beet fiber (T<sub>9</sub>) and control (T<sub>10</sub>), respectively. The increase can be attributed to the inclusion of carbohydrates such as starch, pectin or other fibers in the product [16]. Brix of all treatments decreased significantly on day 25 especially in control and the samples containing lower amounts of inulin and sugar beet fiber. It was due to the consumption of carbohydrates by probiotic bacteria during storage [17]. Mesbahi et al. added skin and seeds of tomato to ketchup to improve its nutritional quality and sensory properties. In agreement, they found that addition of tomato pomace increased the brix of ketchup [18]. Similar result was observed by Bahmani et al. after inoculation of pineapple juice with *L.*

*casei* which resulted in decreased brix of the samples during storage [19].

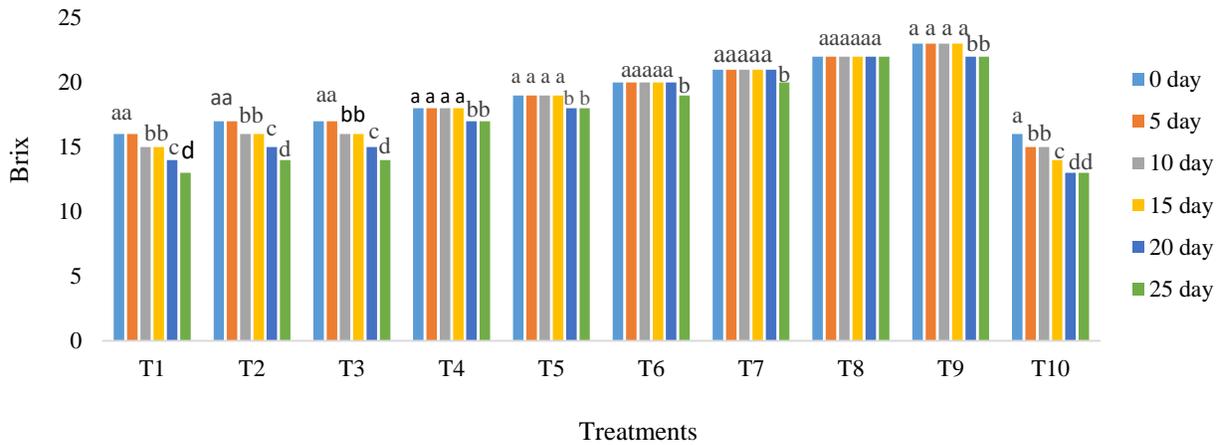


Figure 1- Brix of red grape juice samples containing different concentrations of inulin and sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

**3.2. pH**

Figure 2 shows that pH of the samples decreased significantly by increasing the concentration of inulin and sugar beet fiber. The highest (2.38) and the least (1.72) pH were observed on day 25 for control (T<sub>10</sub>) and the sample containing 3% w/v inulin + 2.1% w/v sugar beet fiber (T<sub>9</sub>), respectively. As seen in the figure, the changes are more significant in the samples containing

higher concentration of both fibers because they are used by probiotic bacteria through which organic acids are produced and accumulation of the organic acids leads to pH decline in the environment [20]. In agreement, pH of probiotic set yogurt containing red grape concentrate decreased during storage in study of Nadali et al [21].

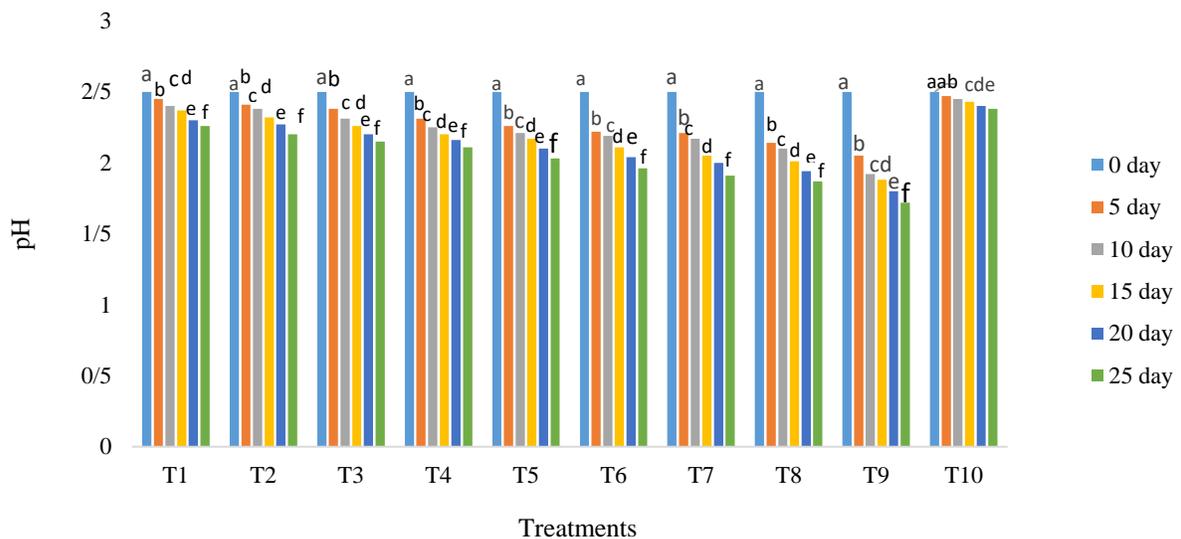


Figure 2- pH of red grape juice samples containing different concentrations of inulin and sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

### 3.3. Viability of *B. bifidum*

Monitoring the viability of probiotic bacteria in products is important for their qualification. The results showed that number of *B. bifidum* decreased significantly in all the treatments during storage (Table 2). The decrease was lower in the samples containing higher concentrations of inulin and sugar beet fiber than the others. On day 25, the highest (7.05 log CFU/ml) and the least (5.03 log CFU/ml) number of probiotics were found in the treatment containing 3% w/v inulin + 2.1% w/v sugar beet fiber and control, respectively. Count of probiotic bacteria in all the treatments containing inulin and sugar beet fiber was higher than  $10^6$  CFU/ml as the least acceptable number [22], while it was calculated lower than  $10^6$  CFU/ml in control likely due to

shortage of nutrients in the environment. Inulin and sugar beet fiber as nutrient provide energy for the bacteria and enhance their growth and viability during storage [19, 23, 24]. In another study, number of *L. acidophilus* in pomegranate juice decreased from 7.34 to 4.43 log CFU/ml within one week. The authors stated that survivability of the bacteria depends on their adaptation to the environment and prebiotic content in the food [25]. Similar results were observed by Ghorbani et al. in monitoring other probiotic bacteria in pomegranate juice [26]. Although, other environmental parameters are also of concern. They include pH, acidity, oxygen level, water activity, salt and sugar, additives, and processing parameters such as pasteurization, rate of cooling, packaging, and storage method [27].

Table 2- Viability of *Bifidobacterium bifidum* (log CFU/ml) in grape juice samples containing different concentrations of inulin + sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

Treatments	Storage time (days)					
	0	5	10	15	20	25
T <sub>1</sub>	8.00 ± 0.00 <sup>aA</sup>	7.51 ± 0.05 <sup>dB</sup>	6.89 ± 0.01 <sup>dC</sup>	6.49 ± 0.15 <sup>gD</sup>	6.19 ± 0.09 <sup>dE</sup>	6.06 ± 0.08 <sup>gF</sup>
T <sub>2</sub>	8.00 ± 0.00 <sup>aA</sup>	7.63 ± 0.08 <sup>cB</sup>	7.08 ± 0.09 <sup>cC</sup>	6.58 ± 0.17 <sup>fD</sup>	6.32 ± 0.14 <sup>cE</sup>	6.14 ± 0.10 <sup>gF</sup>
T <sub>3</sub>	8.00 ± 0.00 <sup>aA</sup>	7.68 ± 0.11 <sup>cB</sup>	7.18 ± 0.08 <sup>cC</sup>	6.71 ± 0.15 <sup>eD</sup>	6.47 ± 0.05 <sup>cE</sup>	6.27 ± 0.04 <sup>fF</sup>
T <sub>4</sub>	8.00 ± 0.00 <sup>aA</sup>	7.64 ± 0.06 <sup>cB</sup>	7.26 ± 0.03 <sup>cC</sup>	6.89 ± 0.16 <sup>dD</sup>	6.56 ± 0.21 <sup>bE</sup>	6.33 ± 0.10 <sup>eF</sup>
T <sub>5</sub>	8.00 ± 0.00 <sup>aA</sup>	7.70 ± 0.17 <sup>abB</sup>	7.31 ± 0.09 <sup>bC</sup>	6.95 ± 0.12 <sup>dD</sup>	6.65 ± 0.15 <sup>bE</sup>	6.45 ± 0.07 <sup>dF</sup>
T <sub>6</sub>	8.00 ± 0.00 <sup>aA</sup>	7.75 ± 0.13 <sup>abB</sup>	7.36 ± 0.18 <sup>bC</sup>	7.01 ± 0.06 <sup>dD</sup>	6.73 ± 0.20 <sup>bE</sup>	6.62 ± 0.03 <sup>cF</sup>
T <sub>7</sub>	8.00 ± 0.00 <sup>aA</sup>	7.77 ± 0.09 <sup>abB</sup>	7.39 ± 0.17 <sup>bC</sup>	7.17 ± 0.09 <sup>cD</sup>	6.81 ± 0.15 <sup>bE</sup>	6.70 ± 0.11 <sup>bF</sup>
T <sub>8</sub>	8.00 ± 0.00 <sup>aA</sup>	7.89 ± 0.16 <sup>aB</sup>	7.47 ± 0.12 <sup>bC</sup>	7.31 ± 0.06 <sup>bD</sup>	6.94 ± 0.14 <sup>bE</sup>	6.81 ± 0.10 <sup>bF</sup>
T <sub>9</sub>	8.00 ± 0.00 <sup>aA</sup>	7.91 ± 0.14 <sup>aB</sup>	7.77 ± 0.08 <sup>aC</sup>	7.55 ± 0.04 <sup>aD</sup>	7.15 ± 0.13 <sup>aE</sup>	7.05 ± 0.04 <sup>aF</sup>
T <sub>10</sub>	8.00 ± 0.00 <sup>aA</sup>	7.18 ± 0.21 <sup>eB</sup>	6.56 ± 0.06 <sup>eC</sup>	6.16 ± 0.12 <sup>hD</sup>	5.54 ± 0.16 <sup>eE</sup>	5.03 ± 0.05 <sup>hF</sup>

- Different small letters at each column show significant difference ( $P \leq 0.05$ ).

- Different capital letters at each row show significant difference ( $P \leq 0.05$ ).

### 3.4. Color measurement

According to Figure 3, L\* index for all the treatments decreased significantly during storage. On the other hand, by increasing the concentration of inulin and sugar beet fiber, L\* decreased significantly. The highest L\* was observed for control (T<sub>10</sub>) followed by the sample containing 1% w/v inulin and 0.7% w/v sugar beet fiber (T<sub>1</sub>). It was likely due to the presence of fibers in the product which prevented the light transmittance. In agreement, a decreased lightness was also

reported for Barbari bread containing sugar beet pulp [28]. In addition, Shuorideh et al. used inulin as sucrose substitute in formulation of dark chocolate and reported that treatments containing higher inulin content showed lower lightness. They stated that inulin absorbed water which resulted in decreased light scattering and lightness in the products containing high inulin content [29]. Rodrigues et al. also used inulin as fat substitute in formulation of ground meat and reported that inulin at concentrations above 2%

w/w reduced the lightness [30]. A reduced lightness in inulin-based dessert as a carrier of

probiotic bacteria was also reported by Mantzouridou et al. [31].

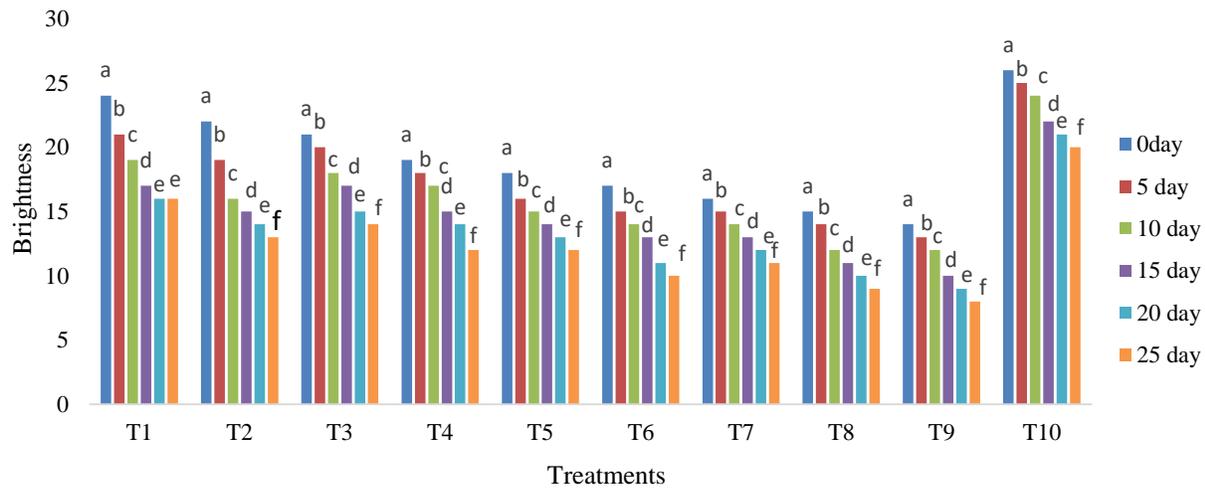


Figure 3- Lightness ( $L^*$ ) of grape juice samples containing different concentrations of inulin and sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

According to Figure 4, time had no significant effect on redness ( $a^*$ ) of the treatments. By increasing the concentration of sugar beet fiber,  $a^*$  increased significantly which might be due to the presence of red pigment in the fiber. In

comparison, addition of inulin to the samples did not change the  $a^*$  significantly. The least  $a^*$  was observed in control while the highest was found in the treatments containing 2.1% w/v sugar beet fiber (T<sub>3</sub>, T<sub>6</sub>, and T<sub>9</sub>).

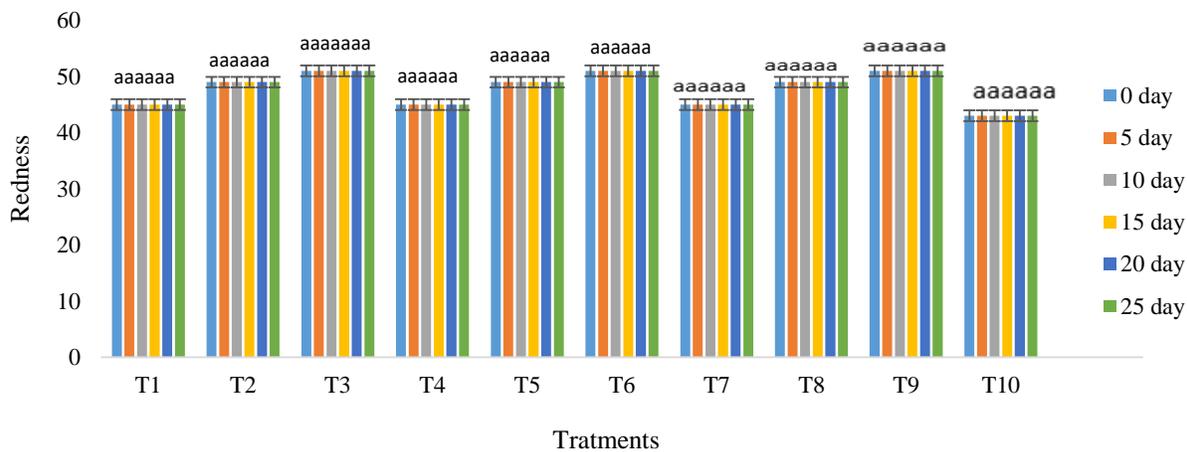


Figure 4- Redness ( $a^*$ ) of grape juice samples containing different concentrations of inulin and sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

As seem in Figure 5, time had no significant effect on yellowness ( $b^*$ ) of the treatments. By increasing the inulin content,  $b^*$  increased significantly which might be due to the long chains of inulin affecting the light transmission. Similar result was observed by Afshin pajooh et

al. by addition of inulin to pasta dough [32]. Addition of sugar beet fiber to the samples had no significant impact on  $b^*$ . The least and the highest  $b^*$  were found in control and the samples containing 3% w/v inulin (T<sub>7</sub>, T<sub>8</sub>, T<sub>9</sub>), respectively.

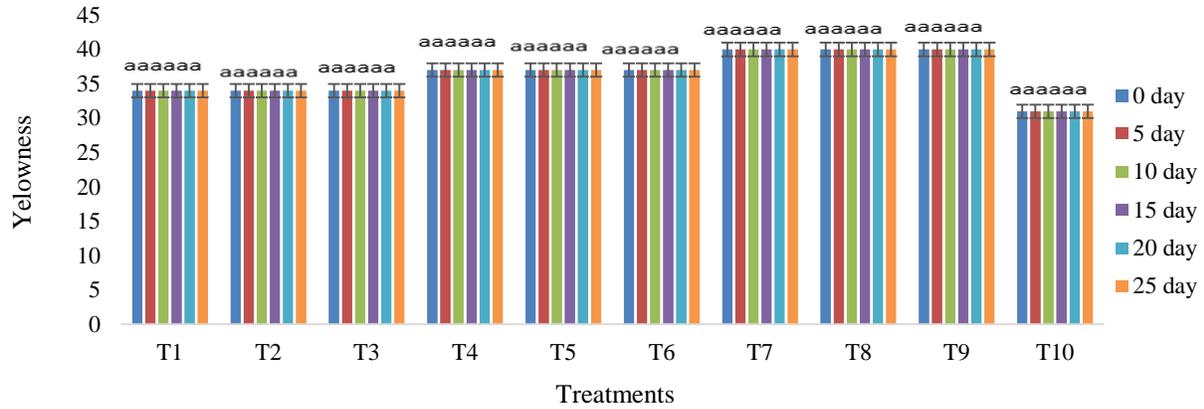


Figure 5- Yellowness ( $b^*$ ) of grape juice samples containing different concentrations of inulin and sugar beet fiber ( $T_1$ - $T_9$ ) and control ( $T_{10}$ ) during 25 days of storage

**3.5. Sensory evaluation**

**3.5.1. Flavor**

The results showed that by increasing the time of storage, score of flavor decreased significantly (Figure 6). It was more significant in the samples containing inulin above 1% w/v and sugar beet fiber above 0.7% w/v. It might be due to production of undesirable gases by *B. bifidum* in the samples during storage. Majozoobi et al. reported that high concentration of sugar beet fiber in Barbari bread had adverse effect on flavor and also the activity of starter yeast [28]. In agreement, Damanafshan et al. found a worse flavor of the cake formulated with inulin [33].

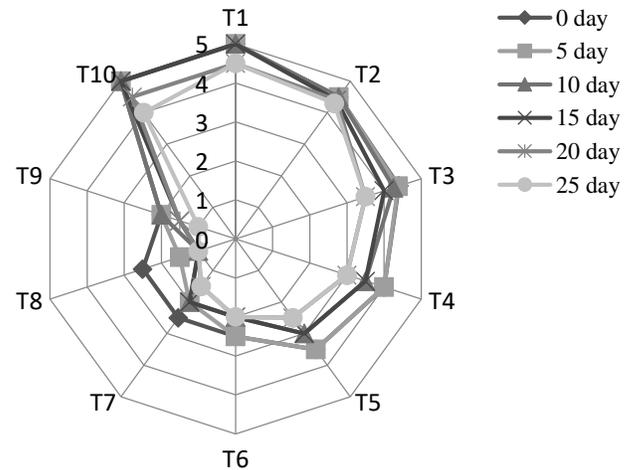


Figure 6- Flavor of grape juice samples containing different concentrations of inulin and sugar beet fiber ( $T_1$ - $T_9$ ) and control ( $T_{10}$ ) during 25 days of storage

**3.5.2. Color**

Storage time had negative impact on color score of the treatments (Figure 7). The changes were more significant in the samples containing inulin above 1% w/v and sugar beet fiber above 1.4% w/v. High concentration of carbohydrate in foods exposed to high temperatures negatively affects the color due to development of Millard reaction [28]. The highest apparent color score was calculated for control and the sample containing 1% w/v inulin + 0.7% w/v sugar beet fiber showed no significant difference with control.

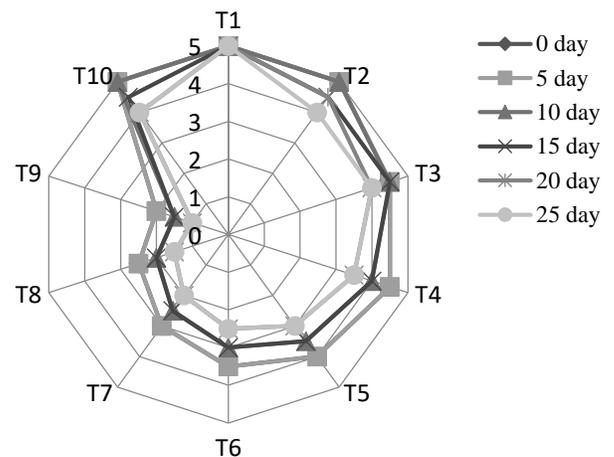


Figure 7- Color of grape juice samples containing different concentrations of inulin and sugar beet fiber ( $T_1$ - $T_9$ ) and control ( $T_{10}$ ) during 25 days of storage

### 3.5.3. Mouthfeel

Increasing the inulin concentration above 1% w/v and sugar beet fiber above 1.4% w/v decreased the mouthfeel scores significantly, especially under prolonged time of storage (Figure 8). It might be due to the oily mouthfeel caused by high concentrations of inulin and also metabolization of the carbohydrates by *B. bifidum* and production of undesirable compounds. Saadatmandi et al. used sugar beet fiber in formulation of tortilla chips and reported decreased mouthfeel scores at concentrations above 3% w/w due to the texture hardness [34]. In comparison, Homayounirad et al. found that inulin at concentration of 2% w/w can significantly increase the mouthfeel scores in milk chocolate [35].

### 3.5.4. Consistency

By increasing the inulin concentration above 1% w/v, the consistency scores decreased significantly in all the treatments (Figure 9). The highest consistency score was observed for control and the treatments containing 1% w/v inulin + 0.7% and 1.4% w/v sugar beet fiber (T<sub>1</sub> and T<sub>2</sub>) had no significant difference with control. Increasing the concentration of inulin above 1% w/v and sugar beet fiber above 1.4% w/v significantly decreased the consistency score. In agreement, Homayounirad and Hojat Ansari reported that using 1.5% and 2% w/v inulin in formulation of apple juice decreased the consistency score significantly [36]. The observed results were possibly due to the viscosifying effect of the fibers so that their increased concentration leads to undesirable sense of consistency by the consumers.

### 3.5.5. Overall acceptance

According to Figure 10, increased concentration of inulin (above 1% w/v) and sugar beet fiber (above 1.4% w/v) decreased the overall acceptance score in all the treatments. The highest score was observed in control on day 1 and the treatments containing 1% w/v inulin + 0.7% and 1.4% w/v sugar beet fiber (T<sub>1</sub> and T<sub>2</sub>)

showed no significant difference with control. Homayounirad and Hojat Ansari studied the sensory properties of apple juice containing inulin (1.5% w/v), (2% w/v), during one month at ambient temperature. They found that the samples containing inulin alone had higher overall acceptability [36].

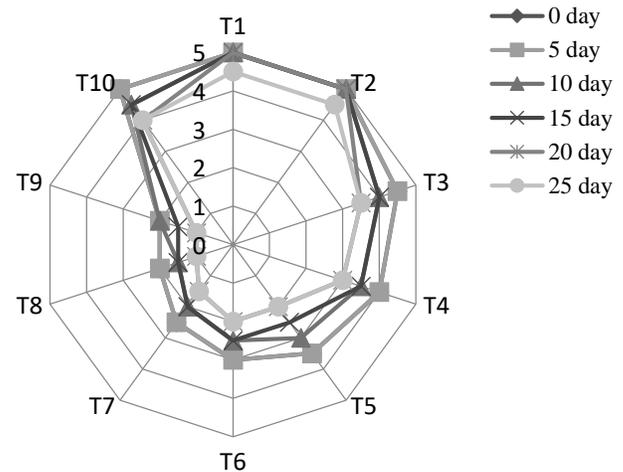


Figure 8- Mouthfeel of grape juice samples containing different concentrations of inulin and sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

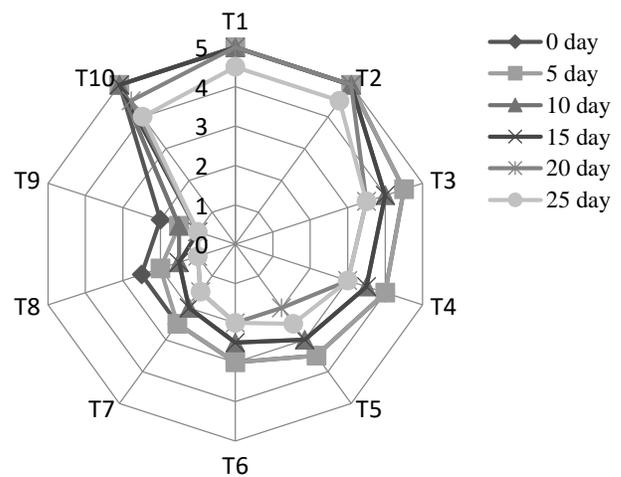


Figure 9- Consistency of grape juice samples containing different concentrations of inulin and sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

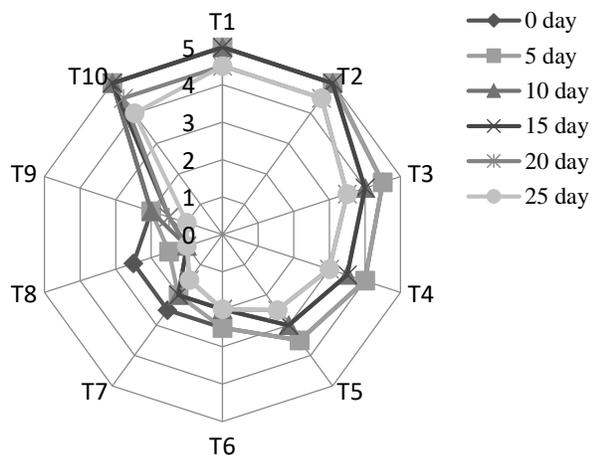


Figure 10- Overall acceptance of grape juice samples containing different concentrations of inulin and sugar beet fiber (T<sub>1</sub>-T<sub>9</sub>) and control (T<sub>10</sub>) during 25 days of storage

#### 4. Conclusion

By increasing the concentration of inulin and sugar beet fiber in probiotic red grape juice, brix and acidity increased. Both fibers could improve viability of *B. bifidum* in the formula so that all the treatments containing inulin and sugar beet fiber contained the probiotic bacteria at concentrations more than  $10^6$  CFU/ml until the end of storage (day 25). In comparison, probiotic count was lower than  $10^6$  CFU/ml in control after day 20. Addition of inulin more than 1% w/v and sugar beet fiber more than 1.4% w/v decreased the sensory attributes significantly. Therefore, formulation of probiotic red grape juice containing inulin up to 1% w/v and sugar beet fiber up to 1.4% w/v is suggested to benefit from its healthful properties.

#### 5. Conflict of interest

The authors declare no conflict of interest.

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