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Production of Prebiotic Milk and Investigation of Some Properties

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Abstract

Prebiotics are short chain carbohydrates that cannot be digested by human enzymes. They increase the activity of the colonic bacteria, thus provides beneficial physiological effects to the human body. The well-known prebiotics are inulin and fucrooligosaccharides. Those prebiotics are plant polysaccharides and have low-calorie. Prebiotics present several fruits, vegetables and cereals (jerusalem artichoke, onion, etc.) are used in functional food production. Another group of prebiotics, flavonoids, are antioxidants, and are also present in plant materials. Those compounds are responsible for the taste and color of the material. In this research, it was aimed that production of prebiotic milk by the addition of either *mentha suaveolens ehrh* extracts containing polyphenols or jerusalem artichoke juice containing fructooligosaccharides to its nutrient composition. Single parameter optimization of ultrasonic extraction of *mentha suaveolens ehrh.* was achieved with the parameters of temperature (45-50-60^{0}C), time (5-10-20-30-45-60-75 min) and 1g/10mL solid/liquid ratio by water as a solvent. At the optimum conditions (45^{0}C, 45 minute), 19.59mg gallic acid equivalents of polyphenols were extracted per gram of plant. When the extracts added into 5ml of milk drop by drop, it was determined that 3 drops of extracts changed the color of the milk and 9 drops caused a change of the taste. Also, by following the same procedure with jerusalem artichoke juice it was found that 15 drops caused a significant change in both taste and color of the milk. In the light of these preliminary observations, sugarless and sugar-added milk samples were prepared by mixing different kinds of milks (light, half-fat and whole, pasteurized) with either flavonoid or fructooligosaccharides or both, in two different concentrations (5 or 9 drops of flavonoid; 9 or 15 drops of fructooligosaccharide). Those samples were experienced by volunteers for taste and color acceptability. Addionally, the viscosities of milk samples were also measured and compared.

Keywords: Antioxidant, fructooligosaccharide, *Mentha suaveolens ehrh.*, milk, polyphenol, prebiotic

INTRODUCTION

To increase their quality of life, people prefer to take measures to prevent health problems before they become ill. In today's diet, functional foods that maintain the good state of the immune system, improve it, and reduce the risk of disease are preferred(Roberfroid, 2000).

Another prebiotic food ingredient that provides medical or health benefits, including prevention and treatment of the disease, is the flavonoids in plants. Functional food is a food component that contributes to the prevention and reduction of risk factors for various diseases or to the improvement of some physiological functions in the body (López-Varela, 2002). Inulin and fructooligosaccharides are well known prebiotics. The ground apple are a valuable inulin source with 14-19% inulin content (Lingyun Wei, et al., 2007). Prebiotics are short chain carbohydrates that can not be digested by digestive enzymes in humans, but selectively support the development of probiotics. These low calorie substances, which have beneficial physiological effects on the body, are found in many vegetables and fruits (banana, banana), cereals (oats), legumes (soybeans)(Roberfroid, 2002). Prebiotics have many benefits, such as reducing the risk of colon cancer, increasing calcium and magnesium absorption (Wollowski, et al., 2001; Pool-Zobel, et al., 2002). When added, it improves sensory properties such as taste and texture, and increases the stability of foams and emulsions in a wide variety of food applications such as dairy products (Slavin, Joanne, 2013).

Helianthus tuberosus, which is used as the best prebiotic source, also contains high amounts of vitamins A and B. It is a feathered, lumpy, perennial plant belonging to the family of *Helianthus*. The resistance of these materials to frost and drought can be explained by the fructan metabolism; because these plants synthesize compounds called fructans instead of starch. The ground apples do not contain the compounds of bitter taste, so it can be used appropriately in the food industry. It does not contain non-interfering components and can therefore be easily extracted(Yildiz, 2006). It strengthens the immune system, fighting harmful bacteria, preventing constipation. It also reduces the risk of diseases such as diabetes, cancer and bone loss. Since they have low calorie due to low glucose content, they can also be consumed by diabetic people(Brownawell, et al., 2012).

Flavonoids are belong to polyphenols and they are pigments as well as the most commonly used like chlorophyll and carotenoids(Constantine, 2007). These compounds act as strong antioxidants and metal chelators. They are also known to have antiallergic, antithrombotic, antiviral and anticarcinogenic effects (Tapas, 2008). Antioxidants act as radical scavengers, hydrogen and electron donors, peroxide scavengers, enzyme inhibitors (Shu-Huei, 2011). An antioxidant is a stable molecule that disassociates unpaired electrons by binding them to free radicals, thus reducing the cell's harmful capacity. These antioxidants retard or inhibit cellular damage by virtue of their free radical scavenging properties (Halliwell, 1995). Low molecular weight antioxidants interact safely with free radicals and terminate the reaction before the cells are damaged. Food antioxidants which cleave free radicals in the body are vitamin E, vitamin C (ascorbic acid) and B-carotene (Levine, et al., 1991). The body can not produce these micronutrients, so these component need to be taken separately.

In order to obtain the health benefits of prebiotics, they should be added into daily-life drinks. Thus, the production of prebiotic-enriched milk was investigated in this study. The prebiotics were extracted from different plant materials chosen by using water and then added into the milk samples. The consumer acceptance and some of the physical properties of prebiotic-containing milks were also evaluated.

MATERIAL and METHODS

In the study, the polyphenols intended to be added into a milk were extracted from *Mentha suaveolens* (mint) plant obtained from a regional herbalist (Gulluce et.al.., 2007). In order to obtain the maximum amount of polyphenol from the plant material, three different temperatures (45, 50, 60 ^{0}C) and seven different extraction times (5, 10, 20, 30, 45, 60 ve 75 minutes) were used in the extraction. In that, the solvent type and solid-to-liquid ratio were kept constants as water and 1g/10 ml, respectively. At the end of extraction by ultrasonic extraction device (Elmasonic S 30 H), the mixture was filtered through FilterLab Filter papers and the materials and extracts were separated. The total phenolic component in the extracts obtained was analyzed by the Folin_Ciocalteu method. The method was carried out with samples prepared by adding 400 μL extract, 100 μL water, 1.5 mL of 20% Na_2CO_3 solution and 5 mL of distilled water to 0.5 mL of Folin Reactant (Yigitarslan, 2017). These samples were subjected to a colorimetric reaction for 2 hours at 25 ℃ in the dark, then the absorbance values were measured at 765 nm by a spectrophotometer (Cary 60 UV-VIS) using pure water as a blank. The concentration values expressed as mg GAE/g *Mentha suaveolens* were calculated by inserting absorbances into the calibration curve equation (Abs = 0.01532 * Concentration (μg/ml), R^2 = 0.9989) (Balcı & Yigitarslan, 2017). The conditions providing the highest gallic acid equivalent polyphenol concentration were selected as optimum extraction conditions. Plant extracts of optimum extraction conditions were prepared freshly before adding them into a milk samples.

Another material that is added to a milk is the jerusalem artichoke juice, which is produced by pressing with Arçelik Solid Fruit Juicer. As noted in the literature, in order to have the maximum amount of fructooligosaccharides capable of the highest prebiotic properties, jerusalem artichoke samples were used after being washed and then dried and stored as tightly wrapped at +4°C for 20 days. (Rubel et.al., 2014). Since juices have fast browning reaction, care was taken to transfer them quickly into the 26 mM citric acid added solution (Yigitarslan, 2007).

Flavonoid (F), fructooligosaccharide (P) and flavonoid-fructooligosaccharide (FP) were added to different types of 10 ml milk (dietary (L), semi-oily (Y), whole (T)). Concentrations indicated by the codes 1, 2, 3 and 4 in the study are representing 10 drops and 18 drops of flavonoid, 18 and 30 drops of fructooligosaccharide, respectively. 48 different milk samples were formed in the study with sugar-free and 0.250 g sugar added samples (denoted as *). A control sample that doesnot contain any flavonoid and fructooligosaccharide was also included in the experiments. 25 consumers evaluated the milk samples in the sight of odor, color and taste over 5 full scores. The densities of the control groups and the obtained samples were measured by using a pycnometer and the viscosities were measured by Ostwald viscosimeter. The densities and viscosities were calculated by Equation 2 and 3, respectively. ρ_1 (g/ml) and ρ_0 (g/ml) represent the densities of the sample and water, t_0 (s) and t_1^* (s) represents the disposal time of water and samples, respectively.

$$\rho_{sample} = \frac{m_{sample}}{v_{sample}} \qquad \textit{(Equation 1)}$$

$$c = \frac{\rho_1 * t_1^*}{\rho_0 * t_0} \qquad \textit{(Equation 2)}$$

RESULTS and DISCUSSION

The summary of the sensory analysis of the milk samples were given in Tables 1, 2 and 3, and the results of physical analysis of the milk samples were given in Table 4. The most acceptable sample in terms of color in all of the dietary milk samples was the sample containing the maximum prebiotic (LP4). In terms of odor, the sample containing minimum flavonoid-and-maximum prebiotic content (LFP14) was found the best, whereas in terms of taste, the results showed that a sugary sample having less prebiotics (LFP13*) was accepted much more. Those who have 0 points on the samples submitted for the evaluation of 25 people on 5 full points didn't have got any points from them. The most unacceptable samples in terms of color were LP3 containing only minimal prebiotic and LF2* containing only maximum flavonoid and sugar. The sugary LP3* sample, which is intense in terms of prebiotic content, was found to be the least favored in terms of odor, while the LF1 sample with the minimum flavonoid content was found to be the least favored in terms of taste.

Table 1. The sensory analysis of dieatary milks

Sample	Color	Odor	Taste
LF1	8	8	1
LF1*	9	9	6
LF2	5	0	0
LF2*	3	5	10
LP3	3	9	7
LP3*	6	2	5
LP4	21	3	2
LP4*	9	6	11
LFP13	4	6	2
LFP13*	10	11	19
LFP14	0	14	11
LFP14*	4	13	13
LFP23	12	0	6
LFP23*	12	6	13
LFP24	10	6	9
LFP24*	16	3	14

As seen in the table below, YFP23, which is the sample containing maximum amount of flavonoid and minimum amount of prebiotic, was accepted highly in terms of color and odor in semi-oily milk samples. YFP13, containing minimal flavonoids-and-prebiotics, and its sugar-containing corresponding (YFP13*) were evaluated as the least favored samples in terms of color and taste within them. At the same time, the YFP14* sample with minimum flavonoid, maximum prebiotic and sugar content was not liked in the same manner as YFP13 sample in taste evaluations. The YF1* sample with flavonoid content was also found to be the least likable sample in terms of odor.

Table 2. The sensory analysis of semi-oily milks

Sample	Color	Odor	Taste
YF1	22	10	7
YF1*	15	4	10
YF2	7	16	11
YF2*	8	3	16
YP3	9	15	11
YP3*	11	15	10
YP4	6	9	7
YP4*	2	13	15
YFP13	10	13	6
YFP13*	1	17	7
YFP14	12	6	10
YFP14*	6	8	6
YFP23	23	18	9
YFP23*	12	8	23
YFP24	20	14	12
YFP24*	17	10	21

When the whole milk samples were evaluated in terms of color, TFP24 containing maximum flavonoid and maximum prebiotic was the most appreciated. When these samples were evaluated for odor, TFP14 containing the minimum amount of flavonoid and the maximum prebiotic was preferred. When evaluated in terms of taste, TFP24*, which was a sugary sample containing maximum amount in both components, was preferred by the volunteers as in the case of odor evaluation. The least preferred sample in terms of color and odor was the TFP13* sample containing the minimum flavonoid, maximum prebiotic and sugar; while in terms of taste the least favored sample was found as TFP14* sample containing minimum flavonoid maximum prebiotic and sugar.

Table 3. The sensory analysis of whole milks

Sample	Color	Odor	Taste
TF1	15	9	13
TF1*	12	9	17
TF2	4	13	9
TF2*	11	9	17
TP3	9	5	3
TP3*	9	17	17
TP4	2	12	7
TP4*	12	7	7
TFP13	6	0	0
TFP13*	1	4	7
TF14	6	20	9
TFP14*	12	8	2
TFP23	9	6	6
TFP23*	7	6	9
TFP24	18	0	4
TFP24*	9	15	25

Table 4. The results of physical analysis of the milk samples

Sample	Density (g/ml)	Viscosity (cp)
LF1	0.946	2.004
LF1*	0.979	2.13
LF2	0.965	2.072
LF2*	1	2.177
LP3	1.014	2.266
LP3*	1.042	2.298
LP4	0.936	2.227
LP4*	0.965	2.35
LFP13	0.912	2.064
LFP13*	1.025	2.588
LFP14	1.023	2.315
LFP14*	1.063	2.56
LFP23	0.952	2.099
LFP23*	0.966	2.288
LFP24	1.036	2.405
LFP24*	1.054	2.447
Control (dietary milk)	1.032	3.055
YF1	0.968	2.191
YF1*	0.972	2.2
YF2	0.998	2.288
YF2*	1.021	2.37
YP3	0.976	2.181
YP3*	1.034	2.40
YP4	1.012	2.49
YP4*	1.027	2.53
YFP13	0.954	2.16
YFP13*	1.065	2.35
YFP14	1.009	2.372
YFP14*	1.020	2.42
YFP23	0.985	2.086

YFP23*	1.025	2.17
YFP24	0.960	2.20
YFP24*	1	2.32
Control (semi-oily milk)	1.035	3.24
TF1	1.037	2.73
TF1*	1.073	2.865
TF2	1.018	2.599
TF2*	1.047	2.613
TP3	1.004	2.593
TP3*	1.047	2.643
TP4	1.039	2.533
TP4*	1.065	2.59
TFP13	1.027	2.593
TFP13*	1.033	2.638
TFP14	1.025	2.379
TFP14*	1.098	2.517
TFP23	1.053	2.261
TFP23*	1.147	2.73
TFP24	1.033	2.34
TFP24*	1.081	2.415
Control (whole milk)	1.062	2.39

As seen in the table above, it has been determined that the densities of milks changed significantly depending on flavonoid, prebiotic and sugar contents and the amount of these ingredients. The viscosity of the experimental groups was found to be lower than that of the control groups and it was determined that milk samples containing sugar had higher viscosities than sugar-free milk samples. Since prebiotics are also sugars, the viscosities of the milk samples were found as increasing with an increase in prebiotic amount. A similar effect was also observed when the amount of flavonoid was increased. It has been determined that the addition of prebiotic and flavonoids individually or together lead density to increase.

CONCLUSIONS

In this study aiming to add the prebiotic and antioxidant properties to an ordinary milk, the antioxidant feature was supplied from *Mentha suaveolens ehrh.*, while the color was obtained from the jerusalem artichoke extracts. In general, the color coming from jerusalem artichoke was appreciated. Some people declared the taste and odor of the mint extracts were found heavy, while the others declared them as preferable in terms of aroma. The acceptability increased with reducing the extract concentrate in the sample somewhat and when consumed with sugars. Samples with the same content that was not liked on the diet milk samples could found as favorable in semi-oily and whole milk samples. It has been found that the acceptability of the sugary samples containing both flavonoid and prebiotic together was found quite good according to 25 people presented. Finally, it was found in this study that prebiotic and antioxidant enriched milk can be consumed, and if it is needed the taste can also be increased with the usage of some sugar. Thus, by this way, beneficial physiological effects will be provided for the human body.

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