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PREPARE A FUNCTIONAL DRINK OF ACID WHEY FORTIFIED WITH BEET JUICE AS A NATURAL SOURCEOF IRON AND FOLIC ACID

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ABSTRACT

Background: Nowadays, functional food are the most consumed products and became over widespread in the world as a part of healthy lifestyle as a supportive care and preventive of disease. **Objective:** the aim of this study was development a healthy and nutritious whey–based drink, fortified with beetroot juice as a natural source of important vitamins (such as folic acid and vitamin C) and minerals (such as iron and potassium), as a novel procedure to increase natural folic acid and iron levels in the drink. **Method:** A mixing ratio of acid whey and beetroot juice was used (1:1) and the physicochemical properties of the prepared drink (pH), (Brix) and the content of folic acid and iron were evaluated, as well as a sensory evaluation was conducted in terms of taste, smell, texture, color and consumer acceptance. **Results:** The prepared drink showed good physicochemical and sensory properties (Brix = 13.3%, pH 3.94, folic acid (286 µg/100ml) and iron (1.37 mg/100 ml)), also high acceptability. Therefore, as a result of increasing interest in the nutritional value and potential health benefits of food, whey- based beverages can be nutritious and interesting products in today's developing functional food markets, also can be used in supplement of diet programs, especially to pregnant and lactating mothers, to prevent anemia and folate deficiency which can cause neural tube defects and other related diseases.

KEYWORDS: Functional drink; Acid whey; Beetroot; Folic acid; Iron.

1. INTRODUCTION

Whey is the greenish-yellow liquid, free of casein and fat, that remains after the milk has been curdled and strained as a by-product of the dairy industry (Castelli et al., 2013).

Conventionally, two type of whey can be distinguished, according to the technology used in manufacture of dairy products, sweet whey that is a by-product of making hard cheesesusing rennet enzyme (such as cheddar cheese and Swiss cheese), and acid whey which produced during the manufacture of acidic types of dairy products (e.g. strained yogurt or Greek yogurt, ricotta and cream cheese) (Castelli et al., 2013; de Wit, 2001). In general, acid whey contains less protein, lactose, and lower pH, but more calcium, phosphorous and lactic acid than sweet whey(Schmidt et al., 1984; Pintado et al., 2001; Narendra, et al., 2016), this results in significant differences in sensory, nutritional and technological properties, as well as different strategies for the use and processing of the two types of whey, due to differences in the demeanor of lactose and other acid whey components during preparing, which making the processing process very difficult (Dec and Chojnowski, 2006). Notably, acid whey has a lower protein content than the other type, due to the depletion of whey proteins resulting from the prolonged heat treatment used to make yogurt (Gyawali and Ibrahim, 6102). The dairy industry is recently facing an extra challenge regarding the use of acid whey, given of the current consumer trends and market requirements, with growing Greek yogurt and cottage cheese production (Elliott, 2013; Zotta et al., 2020). Currently, nutritionists unanimously agree on the importance of whey in the food industry, because it contains ingredients with high nutritional value and desirable functional properties, and because its consumption positively affects body functions (Khan et al., 2015; Charo and Dan, 2017). Serumcomponents of bioactive proteins and peptides such as lactoferrin and lysozyme have antimicrobial properties (Kasim, 2015; Khan et al., 2015). A body of research has also indicated that serum proteins can reduce the risk of certain diseases, such as cancer and cardiovascular disease (Ha and Zemel, 2003; Charu and Dhan, 2017). It is also possibleto improve the sensory and functional properties of products prepared from whey by incorporating plant ingredients in their composition, so that this mixture provides important nutrients such as vitamins, minerals and dietary fiber. (Kedaree) et al., (2021) have used lemon (5.5%) and sugar (12%) successfully in preparing lemon whey drink with the best quality and acceptable sensory quality. Also (Larionov) et al., (2020) have found that the best result in terms of sensory indicators was obtained for mint whey drink, as a source of additional vitamins and mineral salts, when adding (1 g) of mint per (1 liter) of whey.

While (Kilavan) et al., (2015) have studied the development of a healthy energy drink from sweet whey with barley water and green tea as sources of many micronutrients (vitamins and minerals) and important polyphenols that give the body energy.

Moreover, the root vegetable (B. vulgaris rubra), better known as beetroot, have more interested by nutrition researchers as a health-promoting functional food (Ninfali and Angelino, 2013). Beetroot (Beta vulgaris L.) is grown all over the world and is regularly consumed as part of a normal diet, and commonly used in food processing as a natural colorant (Georgiev et al., 2010; Zielińska-Przyjemska et al., 2009). It is one of the richest plant sources of folic acid, and an important source of iron, fiber, potassium, manganese, vitamin C, and a number of other vitamins and minerals (Rauha et al., 2005). In addition, beetroot contains a unique class of water-soluble non-phenolic antioxidants, the betalains, comprising two groups of compounds, red beta-cyanin (mainly betanin) and yellow beta-xanthin (Kanner et al., 2001). The results of several in vitro studies have shown that the betalain compounds from beetroot possess potent anti-free radical and antioxidant activity (Kujala et al., 2002). Wejdan (2018) has conducted a study in which beetroot was used to increase hemoglobin in the blood of adult patients suffering from iron deficiency anemia. Also, Hatlin Sugi (2014) found that taking beetroot extract helps reduce anemia among pregnant woman.

In view of these valuable properties of whey and beetroot, the aim of the study was to prepare a functional drink of acid whey and enhanced it with beetroot juice as a natural source of many nutrients, especially iron and folic acid, and evaluating the physicochemical and sensory properties of the prepared drink.

2. MATERIALS AND METHODS

2.1 Preparation of acid whey and beetroot juice

The acid whey had obtained from strained yogurt, which prepared by heating the milk to a temperature of (95°C) for (5 min), cooling to (42°C), inoculating with a yogurt starter, obtained from the previous day's preparation, for (3 hr). After coagulation, it should be quickly cooled, then it was strained using cloth bags, and the resulted acid whey was collected (Tamime and Robinson, 2006; Chammas et al., 2006; Chandan et al. 2013). In the otherwise, beetroots were collected from the local market, sliced followed by cutting into small pieces and grinded in a mixer (Moulinex, France). The resulting mixture was passed through a cloth to extract the juice. Then the juice was heated up to 75°C for 30 seconds in a water bath to eradicate the microbes.

2.2 Preparation of mixtures

Three mixtures of (whey: beet juice) were prepared with the following volume ratios (25:75), (50:50), (75:25) as an attempt to reach the optimal addition ratio based on the observed changes in (Brix) and (pH) values for the drink during heat treatment at (75°C) for different periods (30, 60, 90) sec.

2.3 Preparation of drink

A mixing ratio of acid whey with beetroot juice (1:1) was adopted, and the sucrose added to improve the taste and increase the concentration of total solids, that increase the shelf life of the product. Citric acid also used as a preservative, while pectin was applied to improve the texture. A pasteurization process was carried out, according to results in section (2.2), by heat the mixture at (75 °C) for (30 seconds), then immediately cooled, and strawberry flavor added. The prepared drink, which composition is showing in table (1), was packaged in airtight glass bottles and kept in the refrigerator at (4 ± 1)°C.

Tabe 1: Composition the prepared drink.

Ingredients	Quantity
Acid whey	100 ml
Beet juice	100 ml
sucrose	10 g
pectin	1 g
Citric acid	1 g
strawberry flavor	2 drops

2.4 Analysis of the physicochemical properties of whey, beetroot juice and the prepared drink

2.4.1 Determination of titratable whey acidity (% lactic acid)

The titratable acidity of whey was determined according to IS: 1949, Part I (1960), by taking (100 ml) of the sample into a titration flask, then adding (1 ml) of phenolphthalein solution, and titrated with sodium hydroxide solution (0.1 N).

The titratable acidity was calculated as follows:

$$Lactic\ acid\ \% = \frac{(0.1\ N\ NaOH \times vol.\ of\ NaOH(in\ liter) \times 90.8) \times 100}{Weight\ of\ sample}$$

Where, the molecular weight of lactic acid is (90.8 g/mol).

2.4.2 Determination of pH

The pH value of the whey, beetroot juice, and the prepared drink was measured using a digital pH meter (Milwaukee, USA).

2.4.3 Determination of Brix

The total solids (Brix) concentration of whey, beetroot juice and the prepared drink was measured using a refractometer (ABBE Digital Refractometer, India).

2.4.4 Analysis of whey and drink using lactoscan apparatus

Two samples of the whey, the first unpasteurized and the second pasteurized, were analyzed at a temperature of (75°C) for a period of (30 seconds), to study the effect of heat treatment of the whey on its physical and chemical properties. The prepared drink was also analyzed on a lactoscan apparatus (MilkAnalyzer, Bulgaria).

2.4.5 Determination of folic acid and iron contents in the prepared drink

The content of folic acid in drink was measured by High-Performance Liquid Chromatography Method (HPLC) as described by (Lawrance, 2011), while the determination of iron was performed using flame atomic absorption spectroscopy (AAS) according to (Tautkus, et al. 2004).

2.5 Sensory evaluation of the prepared drink

Sensory properties were evaluated in a consumer preference test performed, after 1 day of refrigerated storage of the drink. Sensory evaluation included evaluation of the appearance, texture, smell, taste and final degree of acceptability of the drink. Eight semi-trained faculty and student members were included in the sensory assessment. A whey drink was served in clear plastic cups, and all samples were marked with a digital code. Product acceptability wrated on a scale of points, with each sensory factor receiving a score of 1 (very poor quality), 2 (acceptable quality), 3 (good quality), and 4 (very good quality), to 5 points (excellent quality), and use the average score for each quality factor assessment to calculate the overall sensory quality and final acceptability score of the drink (Watts et al., 1989; Ruicong et al., 2016).

3. RESULTS AND DISCUSSION

3.1 pH and Brix of drink

Brix and pH values are important physicochemical properties for the quality of drinksand juices, in which Brix values affect the taste and shelf life of products, and pH is one of the main factors affecting flavor, shelf life and spoilage rate of juice (Hezron, et al., 2014), and figure 1 shows the effect of heat treatment (75 °C) time on the (Brix) and (pH) values of the mixtures prepared from the drink.

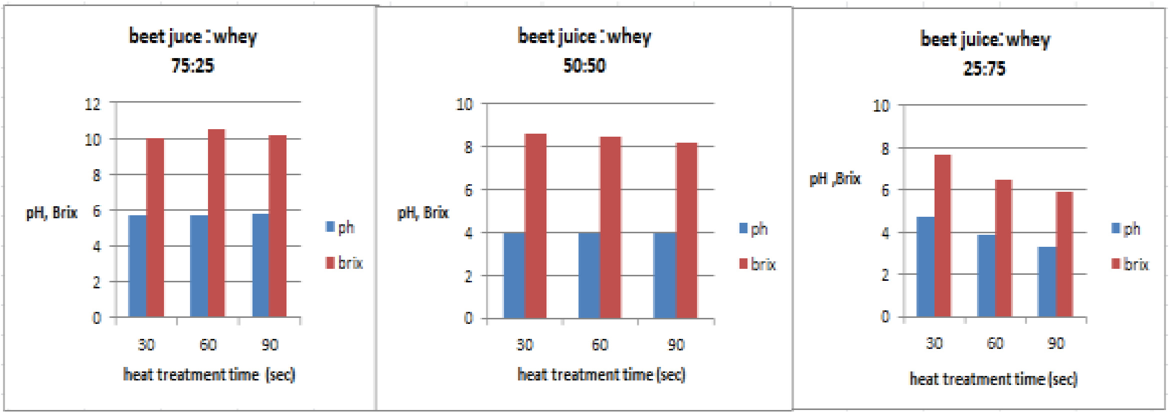

Fig 1: Effect of heat treatment (75 °C) time on the (Brix) and (pH) values of the mixtures preparedfrom the drink.

As noticed in the figure, the higher percentage of whey had caused the decrease of Brix and pH values of the drink with the increase in the heat treatment time on (75°C), this is due to the possibility of coagulation of whey proteins (Gyawali and Ibrahim, 2016), and since the whey percentage is higher in mixture, the Brix value will be affected and a portion loss will occur of solids, also the sensory properties of this drink were checked and it was found that the taste was unpalatable and the color was dull red. On contrary, when using equal proportions of whey and beet juice, the Brix and pH values were fairly stable in the different times, the taste was acceptable and the red color desirable. While, using a higher percentage of beetroot juice, the Brix and pH values was relatively higher, due to their high percentage mainly in beetroot juice, as well as it was noted that the taste of beet juice was clearly obvious in the drink, unpalatable, and the color was dark red.

Based on the above, a mixing ratio (1:1) of acid whey and beet juice was selected, as shown in Figure (2), and heat treatment at (75°C) for (30 sec) to maintain the best proportion of solids, in addition the lower pH of the final drink increases the stability the product during the storage by inhibiting the growth of undesirable microorganisms including coliform bacteria (Mahmoud, et al., 2009).

Fig 2: A drink prepared in a (1:1) ratio of whey and beetroot juice.

3.2 Physicochemical properties of acid whey

The physicochemical properties (titratable acidity, pH, and total solids) of acid whey are presented in Table (2).

Table 2: The physicochemical properties of acid whey.

Type of whey	(ml) of NaOHconsumed by titration	titratableacidity %	pH	Brix
Acid whey	32	0.58	4.35	6.3

It can be seen from the table that these properties are typical of acid whey (FAO, 2013), where the values of (pH) and titrated acidity were (4.35 and 0.58%, respectively). In general, the pH of acid whey is less than (5), and the titrated acidity (calculated based on lactic acid) is higher compared to sweet whey, this depends on the type of milk used and the method of treatment and acidification, whether using organic or mineral acids or a lactic acid bacteria starter (Narendra, et al., 2016), and since the whey used in this study resulted from the treatment of milk with lactic acid bacteria starter, these values, in addition to the Brix value, were consistent with researchers mentions (Rosalie and James, 2007). It has been found in previous studies that the composition of whey varies with season and type of cheese produced (Johansen, et al., 2002), also affected by the heat treatment of milk (Zorana, et al., 2016), and Table (3) shows the results of whey analysis on a lactoscan apparatus.

Table 3: The results of analysis the whey on Lactoscan apparatus.

	Non fat-solids %	Fat %	Protein %	Lactose %	Densityg/cm^3	Temperature °C
Acid whey	6.45	0	2.40	3.44	0.0234	31.3

As noticed in the table that the values of non-fat solids, fat, protein and lactose sugar, expressed as a percentage, are within the typical values of whey (FAO, 2013), and are consistent with researchers mentions (Rosalie and James, 2007) and (Katarzyna, et al., 2019).

3.3 Physicochemical properties of beetroot juice

The physicochemical properties of beetroot juice (total solids and pH) were studied and the results are presented in Table (4).

Table 4: Physicochemical properties of beet juice.

	pH	Brix
beetroot juice	5.57	7.8

It was shown that the values of (pH) and total solids are (5.57 and 7.8, respectively), and these results are agreement with previous studies (Dambalkar, et al., 2015) and (Kale, et al., 2018). In which, the total dissolved solids content is greatly influenced by thecombined effect of maturation stages and growing conditions (Tasnim et al., 2010).

3.4 Results of analysis on lactoscan apparatus

The results of analysis the drink on lactoscan are listed in Table (5).

Table 5: The results of analysis the drink on Lactoscan apparatus.

	Non fat-solids %	Fat %	Protein %	Lactose %	Densityg/cm^3	Temperature °C
Prepared drink	13.35	0	4.95	7.11	0.0487	30

Where an increase was observed in all physicochemical properties (non-fat solids, proteinand lactose) compared to serum alone, due to the high percentage of solids in beetroot juice, and it also contains a percentage of protein estimated at about (1.35%) according to (Kale, et al. al., 2018), and thus the process of combining it with the serum contributed to doubling these percentages in the prepared drink.

3.5 Folic acid and iron contents

The amount of folic acid in the drink was (286 µg/100ml) and iron (1.37 mg/100 ml), which consider a good amount compared with acid whey composition, in which, iron andfolic acid present in tiny quantities (Wong et al., 1978; Reif et al. 1975). So adding beetroot juice to acid whey have improvement in folic acid and iron levels, and this correspond with (Nyamete et al., 2017), suggesting beetroot can be a good choice for enhancing folate and iron contents of acid whey.

3.6 Sensory evaluation of the prepared drink

Sensory evaluation allows characterizing and evaluating the acceptance level and quality conditions of products. It is also used to estimate the shelf life of products where the organoleptic properties of the product decrease before microbial quality. Thus, sensory evaluation gives assurances for launching products into the market.

Table (6) shows the results of the sensory evaluation of the prepared whey drink.

Table 6: Sensory evaluation results of the whey-based drink.

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	Average
Texture$	5	5	5	5	5	5	5	5	5
Color	5	5	5	5	5	5	5	5	5
Taste	4	5	4	4	3	4	4	3	3.87
Smell	4	4	3	5	4	4	4	5	4.12
Final acceptance score									**4.49**

Where it relied on the average of points for each quality factor, texture and color were acceptable to all tasters, while the taste and color ranged between good and excellent, andthe average points for each of these characteristics (5, 5, 3.87 and 4.12, respectively), where it was observed High acceptability (4.49) by the appraisers of this drink, the taste was refreshing between sweet and sour, and its consistency was a liquid with a low viscosity, the smell was acceptable and not very strong, and its color was homogeneous red. The stability of the drink was also observed when stored at refrigeration temperature (4 C^{o}) during the study period, which amounted to (15) days.

4. CONCLUSION

In general, human health necessitates more continues research and development theproducts containing natural biologically active substances avoiding of the synthetic combinations, in which, there are increased trends currently to functional food to meet the consumer requirement for healthy foods. whey- based drinks, as functional foods, offer a variety of vital activities, such as anti-allergic, anti-inflammatory, antimicrobial, and antioxidant, and enhance health of immune and nervous systems. In this study, whey is successfully applied to prepare a functional drink containing beetroot juice with important sensory and nutritional properties. This drink which prepared by mixing of acid whey, beet juice and sugar in appropriate proportion, is an important source of vitamins and minerals, especially folic acid and iron, in addition possesses perfect color, flavor and taste. In view of the functional properties from bioactive constituents present in beetroot and whey, it is proposed that whey-based beetroot juice drink with excellent nutritional, sensory properties could be an interesting product in the constantly growing market for promising functional foods and can be taken daily as supplement of necessary essential nutrients. However, much work is still required to search and modify in using variety of whey- based plant beverage to meet the necessary needs of various nutrients with significantly acceptable.

This could potentially lead to an increased interest in whey as a functional food rather than considered as a by-product.

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