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EFFECT OF CARBOXY METHYL CELLULOSE (CMC) AND PECTIN ON PHYSICAL AND STABILITY PROPERTIES OF MILK MIXTURE WITH JUJUBA CONCENTRATE

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ABSTRACT

Jujube concentrate may be used in products such as jam, cakes, and milk as flavoring or additive due to its antimicrobial and antioxidant properties with the aim of improving nutritional and therapeutical effects. It can play a significant role in promoting the consumers health as a functional food. In this study the formulation and physical properties including stability, total solid, pH , sensory properties of jujube concentrate – milk as a function of type and concentration of hydrocolloids pectin, carboxy methyl cellulose (CMC) and the concentration of jujube concentrate were investigated. To do so jujube concentrate–milk treatments containing CMC and pectin at concentrations of 0.4, 0.3, 0.2, 0.1 and a mixture of pectin and CMC in ratios of 75:25, 33.4:66.6, 66:35 were prepared. The results showed that the amounts of gum and jujube concentrate individually had no significant effect of the pH and amount of gum and jujube concentrate did not show any significant difference. pH showed a decrease at 1, 7 and 14 th days of storage at 4ċ but not to the extent that would make total solid to precipitate, pH values of the samples varied (5.9-6.44), did not show any insignificant difference. The content of concentrate and hydrocolloids had significant effect on total solid and biphasication ($p<0.01$). Total solid content significantly affected the rate of precipitation ($P<0.01$). precipitation exhibited a significant decrease as total solids content increased in a way that the treatments containing pectin, CMC and 2% concentrate had greater stability ($p<0.05$)

Keywords: Flavored milk, Jujuba concentrate, Hydrocolloids, Stability, total solid

INTRODUCTION

In many societies, more attention is given to the health effects of food (i.e. mixed fruit juices and milk) [42, 21]. Milk can be added to fruit beverages in order to improve iron bioavailability [20].

Milk drinks may be classified into two main groups: fermented and unfermented. The former includes dairy products where the action of lactic acid bacteria (LAB) increases the shelf-life as well as health effects of the product [43]. The latter represents milk drinks which are not exposed to fermentation, e.g. flavored milks containing sugar, flavoring and stabilizer [7]. Flavored milks are those drinks with modified fat or nutrients which are formulated to provide a savory product [35, 43].

Flavored milks are made using different flavorings for attracting consumers especially children. Milks flavored with cacao, vanilla, coffee, strawberry, chocolate, and banana are among the most important such products [43, 7].

pH drop is caused by fruit pulps, juices, juice concentrate and / or edible acids such as citric, tattaric, ascorbic, etc [2, 3]. Low pH value makes casein unstable, so in such products, stabilizers are needed to avoid casein aggregation and achieve a desirable mouth feel [9]. Food formulations are complex and colloidal systems for which their organoleptic properties are adjusted to agree with current fashions by means of structure-forming additives, flavorings, and colorings [36, 56, 49, 19, 29].

Jujuba fruits, as fresh and / or dried and processed are used as food additive or flavoring with the aim of improving nutritional and therapeutical properties in jam, cake, jelly etc [57, 44, 40]. Jujuba (*Ziziphus Jujube miller*) belongs to Rhamnacea family [44]. Zizyphus species are commonly used as folklore medicine for the treatment of various diseases such as digestive disorders, weakness, diabetes, skin infection, loss of appetite, fever, anaemia, cancer, heart diseases [41, 37]. Jujuba is a good source of ascorbic acid as its vitamin C content is 20 times as vitamin C contained in citrus [44, 58]. Sugars contained in jujube are saccharose, glucose, with fructose showing the highest content (42.9%) being useful for diabetics [32]. Jujuba is rich in potassium and calcium [32]. Studies have revealed that calcium is effective on factors lowering blood pressure [60, 32]. Jujuba, containing phenolics especially flavonoids has multiple biological effects including antioxidant activity, antimicrobial activity [44, 33, 58, 60] and preventing platelets aggregation, cancer and cardiovascular diseases, it also has analgesic effect [41, 59, 52]. Jujuba extract contains alkaloids, flavonoids, glycosides, saponin, providing anti – diarrhea effect inhibiting sympathetic nerves function and stain quinon colon [10]. Also jujuba boiled in water or milk exerts a laxative and sedative effect [18]. The use of two or more hydrocolloids in the formulation of a food is to achieve a synergistic effect [1, 25], and to improve rheological properties to the product, which may decrease cost during manufacture [53]. Carboxy methlyl cellulose (CMC) is a typical anionic polysaccharide and has been widely used as a stabilizer in food. CMC chains are linear *B* (1-4) linked glucopyranose residues. Electro sorption of CMC caused CMC layer to be adsorbed on the surface of casein and prevent flocculation of casein micelles by stric repulsion.

In addition, the non-adsorbed CMC increased the viscosity of serum and slowed down the sedimentation of casein particles [11, 27].

Since CMC has lower cost than pectin and in same concentration it creates more viscous solution; therefore, pectin is going to be substituted with CMC in food industries [8].

Pectin is widely used in many dairy products as gelling/thickening agent (acid and non acid milk desserts) and as a stabilizer ingredient (acid milk drinks milk/juice blends) [15]. High-methoxy pectin is used to avoid flocculation of milk proteins.

Pectin directly interacts with the casein micelles and reintroduces a steric repulsion among the casein micelles [7].

In the present study the feasibility of manufacturing Jujube – milk drink with a proper formulation as well as its physical properties including pH, total solid, stability properties as a function of concentrate content were investigated in order to describe the characteristics of the product and provide useful applicable indicators.

MATERIALS & METHODS

Hydrocolloids used in this study were pectin (high – methoxyl grade SS200, Danisco, Denmark) and sodium carboxy methyl cellulose (FCC No. 2012082301, Sinochem Fhaghai, chaina). Jujuba (Variety *Ziziphus miller)* was purchased from gardens in south Khorasan province. Skimmed milk (Choopan, 1.5% fat, and total solid 9.5±0.2) was obtained from local stores.

Preparation of Jujuba concentrate

Ten g of Jujuba was washed, destined, and grinded by a home grinder and then boiled in 100 ml water for 5 min [38, 30]. It was kept in the boiled water in an erlene under aseptic conditions at 4ċ for 72 h to obtain jujuba extract. The extract then was filtered through a filter paper under vacuum conditions. The obtained extract was concentrated by rotary system (IKA (RV 10 Basic/ Germany) at 50 ċ, 45 rpm iNt: 20 S for 120 min [58].

Preparation of jujube Concentrate – milk mixture

To prepare Jujuba concentrate – milk mixture a certain amount of the hydrocolloid was mixed with a certain amount (w/w) of sugar, added to 80ċ water and mixed thoroughly, then the temperature was lowered and the mixture was gradually added to milk. Jujuba concentrate was added to milk – stabilizer while stirring and then homogenized by lab homogenizer (Iran Benabsazan) at 120 bar. It was then pasteurized by a pasteurizer (Model FSA, Iran Fanavaran Sahandsaz) at 75 ċ for 25 second and immediately cooled in water-ice mixture [50, 58]. Finally, the samples were poured into sterile test tubes and capped by parafilm and aluminium foil and kept at 5 ċ for following experiments. As shown in Table 1 and Table 2, treatments were coded according to Jujuba concentrate and gum contents.

Table 1: Treatments coding

Gums	Codes of treatments
CMC	*C1,C2,C3*
Pectin	*P1,P2,P3*
CMC/Pectin	*CP1,CP2,CP3*
Jujuba concentrate	*A2,A5,A7*

Table 2: Composition of the used hydrocolloids (gums) of concentrated Zizyphus jujube-milk samples

Treatments	CMC%	Pectin%	Z.Jujuba concentrate (%)
C1	0.1	-	2
C2	0.2	-	2
C3	0.3	-	2
P1	-	0.2	2
P2	-	0.3	2
P3	-	0.4	2
CP1	0.15	0.05	2
CP2	0.1	0.2	2
CP3	0.15	0.25	2
A2	0.15	0.25	2
A5	0.15	0.25	5
A7	0.15	0.25	7

Measurement of pH and total solid of samples

In order to measure pH value of milk – jujuba concentrate mixture (Metrohm/Swiss), Brix value of Jujuba concentrate by refractometer (RF 10/11/EXTECH/America) and milk total solid by Sartorious (Germany Co), the respective methods of AOAC and National standards were used [23].

Sedimentation measurement in Jujuba concentrate – milk mixture

To investigate the rate of sedimentation Jujuba concentrate – milk mixture was poured into sterile test tubes and capped with parafilm and aluminum foil and kept at 5ċ for 14d. Biphasication was investigated during this period and reported as percent. To calculate biphasication, serum phase (upper phase) was measured by a ruler and divided by the amount of mixture contained in the tube multiplied by 100.The drink was considered unstable if any biphasication would be observed [5, 16, 26, 34].

RESULTS AND DISCUSSION

Physicochemical properties of Jujuba concentrate

As the results showed, pH value of Jujuba concentrate was 3.12 – 3.46 and total solid content was 46.34.

Total solid variations of Jujuba concentrate – milk samples

Total solid content in milk drinks containing Jujuba concentrate varied from 16.58 to 22.31 showing significant difference between the formulations ($P<0.01$). According to finding RAMÍREZ-SUCRE *et al*., (2011) total solid content in caramelized jam drinks was in the range of 14.8-21.5 and Yanes *et al*., (2002) reported that commercial cacao milk containing K – Carrageanan had 17.50-18.40 % total solid.

As shown in table 3, P1 Containing 2% concentrate and 0.2% pectin, had the lowest total solid content. The highest amount of total solid was observed for A7 containing 7% concentrate, 0.15% CMC and 0.25% pectin showing good stability as compared to other samples. According to diagram 1, biphasication reduced as total solid content increased. Precipitation percentage showed a significant decrease as total solid increased, it may be due to reduced ion strength and increased water content as total solid increased requiring more pectin to provide stability. Koksoy & Kilic (2004) also reported an increase in biphasication in Ayran drink samples as total solid content increased. It could be said that the stability of acid and non – acid drinks depends on the interaction of casein and poly saccharides affected by concentrations protein polysaccharides, pH ion environment composition of milk proteins as well as the rate of heating [24].

pH variations of Jujuba concentrate – milk samples

According to diagram 1, On the 1 st, 7 th and 14 th day of storage at 4ċ, pH value of samples showed a slight drop. As shown in table 3 pH value varied from 5.9 to 6.44 showing no significant difference between samples. The lowest pH value was observed for A2 containing 2%, concentrate 0.15% CMC and 0.25% pectin. In a study on the effect of pH and low – methoxyl pectin on rheological and physicochemical properties of acidified milks, Françoise *et al.*, (2009) concluded that pH value of milk dropped following storage at 4ċ showing no significant difference among the samples which is consistent with our results.

Table 3: Total solid content and pH value of samples 9 (Mean ±SD)

Samples	pH	Total solid (w%)	Sedimentation in 14 day storage (%)
Control	**6.42±50.05^{a}**	**14.187±0.201^{b}**	**43.27±0.94^{ab}**
C1	**5.9±50.05^{a}**	**17.187±0.201^{b}**	**13.67±0.94^{a}**
C2	**6.023±0.311^{a}**	**18.567±0.021^{b}**	**14.67±0.94^{a}**
C3	**6.047±0.333^{a}**	**19.753±0.167^{b}**	**19.08±0.94^{a}**
P1	**6.173±0.06^{a}**	**16.583±0.222^{ab}**	**14.67±0.94^{b}**
P2	**6.243±0.04^{a}**	**17.540±0.289^{ab}**	**7.33±0.94^{b}**
P3	**6.210±1.00^{a}**	**18.670±0.102^{ab}**	**1.00±0.94^{b}**
CP1	**5.940±0.541^{a}**	**19.523±0.131^{a}**	**5.00±0.94^{cd}**
CP2	**6.093±0.221^{a}**	**20.660±0.565^{a}**	**1.33±0.94^{cd}**
CP3	**6.260±0.06^{a}**	**20.273±0.200^{a}**	**0.00±0.94^{cd}**
A2	**6.440±0.98^{a}**	**21.717±0.201^{a}**	**0.00±0.94^{b}**
A5	**6.217±0.129^{a}**	**22.717±0.41^{a}**	**1.75±0.94^{b}**
A7	**6.240±0.129^{a}**	**23.317±0.08^{a}**	**1.89±1.36^{b}**

Different letters represent significant difference at 99% (p<0.01) c1...c2 refer to Table1

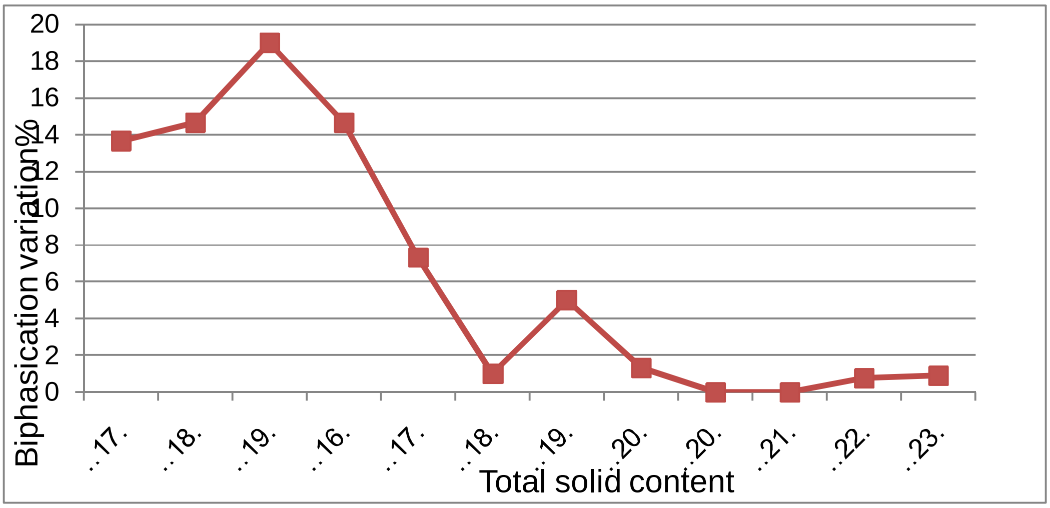

Diagram 1: Biphasication variations vs. dry matters jujuba concentrate- milk mixture

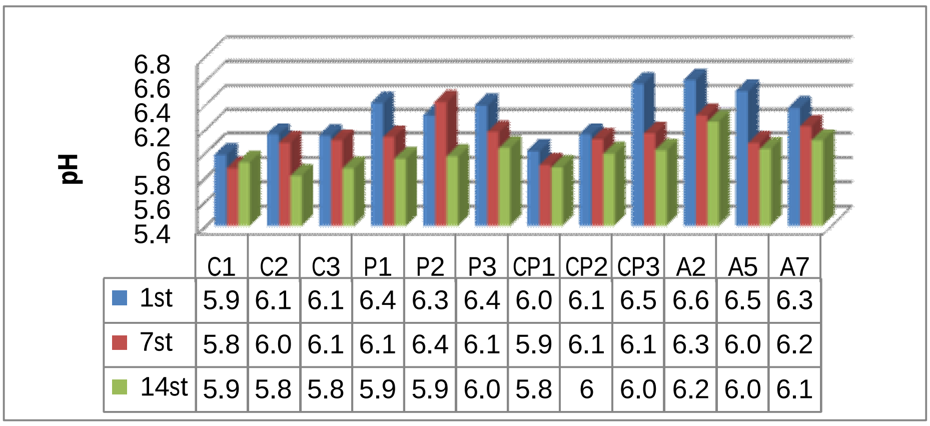

Diagram 2: pH value of different samples in 14 days storage

pH effects on sedimentation of Total solid variations of Jujuba concentrate – milk samples

In milk samples flavored with Jujuba concentrate even those with the concentrate amount as high as 5-8%, pH drop was not too much to make dry matters precipitate. In a study Gastaldi *et al*., (1996) concluded that at pH = 6.7 and 5.8-6 micelle demineralization begins and finally casein micelle lose their individuality and aggregate at pH= 5.8. On the 1 st , 7 th and 14 th days, pH slightly decreased likely due to the growth of lactic acid – producing bacteria (LAB), resulting in increased lactic acid and reduced pH value which cause some precipitation of dry matter and heterogenous formulations for flavored milk at the end storage period. RAMÍREZ-SUCRE *et al*., (2011) studied the physicochemical properties of milk drink flavored with caramelized jam and showed that dropped pH caused by growing LAB increased lactic acid production and lowered pH resulting in precipitation of dry matters and heterogenous formulations.

Effect of different concentrations of gums on the stability of Jujuba concentrate – milk samples

According to table 3 the results of measuring the precipitation of 12 treatment of jujuba concentrate – milk drink over 14 days storage P3, CP3 and A2 had the greatest stability showing significant difference (P<0.01). Other nine treatments were significantly different in the stability of Jujuba concentrate – milk drink (P<0.01). In samples containing CMC biphasication increased as the concentration of CMC exceeded 0.1%. C1 had the greatest stability so when it uptakes CMC it may cause electrostatic and steric repulsion between casein micelles and make the drink stable. Our results are in agreement with the findings of Du *et al*., (2009) who reported that at pH=6.7 the samples containing low level of CMC were stable and high concentration of CMC resulted in reduced stability and that CMC absorption may results in phase separation by discharge flocculation between non – adsorbant polysaccharides and casein micelles. Thus, at low pH value CMC may cause electrostatic repulsion between casein micelle preventing the micelles from aggregation [54, 45]. At higher concentrations of polysaccharides, repulsive interactions between casein micelles become excessive so the re-aggregation of the particles may increase biphasication, thus; at pH= 5.2-6.8, both casein and CMC are negatively charged repulsing each other [13] and the only effect of CMC in the studied samples is increased viscosity. It does not make the structure any more ordered [24].

The results of treatments using high – methoxyl pectin revealed that in P1 containing 0.2% considerably decreased from 43% in control to 14% and in P3 containing 0.4% pectin perfect stability occurred in dairy drink. Pectin directly interacts with the casein micelles and reintroduces a steric repulsion among the casein micelles [7]. It has well established that it is impossible to prevent the aggregation of casein micelles being prone to biphasication unless stabilizers and/or sufficient pectin would be added. Therefore, lack of sufficient pectin to cover all casein micelles makes the system very unstable because of discharge flocculation [48]. However, excessive amount of pectin also may cause a highly unstable system due to over – covering the surfaces of particles [48]. Nakamura *et al.*, (2006) found that primary absorption in non-acidified milks with an increase in pectin concentration at neutral pH is probably due to the presence of calcium ions, a finding which is in consistency with the results obtained by other researchers [15, 39]. The results of evaluation the use of CMC – pectin hydrocolloids in CP1, CP2 and CP3 showed that in CP3 containing 0.25% pectin and 0.15 % CMC, biphasication considerably decreased from 43% in control to 0.00% providing perfect stability in dairy products.

The results of experiments using different amounts of jujube concentrate showed that (Table 3), Also 2% Jujuba concentrate and 0.25 and 0.15 pectin and CMC caused a significant decrease in biphasication from 43% in control to 0.00% and at 7% concentration phase separation increased to 1.89%, thus; perfect stabilization of dairy product requires a greater amount of hydrocolloids.

The use of combination of stabilizers including absorbent and nonabsorbent hydrocolloids (Pectin and CMC), pectin is as absorbent hydrocolloid that is absorbed on to the surface of casein micelles and creates electrostatic repulsion there by reducing the phase separation. Nonabsorbent hydrocolloids do not interact with casein but they increase viscosity and develop a desirable texture through increasing the viscosity of serum phase [26, 14].

In acidified dairy drink protein – polysaccharide complexes are formed between positively charged casein and anionic hydrocolloids such as pectin tragacanth and CMC [14], while in dairy drinks with neutral pH two hypotheses are proposed which justify the interaction of polysaccharides and proteins at neutral pH providing stability;1) via gel formation where inter-molecular connections between polysaccharides results in gel formation which suspends casein micelle and prevent biphasication. Cations such as Ca^{2+} and K^{+} are needed for gelation; 2) via complex formation which occurs because of interactions between proteins and polysaccharides through electrostatic reaction. In dairy drinks thus bridges are formed between cationic groups such as calcium or carboxyl groups of proteins and sulphte groups of polysaccharides forming complex [47, 46]. The second mechanism may be developed by whey proteins and casein micelles at neutral pH [12].

CONCLUSION

As studies show that, Jujuba concentrate may be added to milk in order to provide a functional food. Given the results of variance the amount of concentrate has a significant effect on biphasication ($P<0.01$). According to the results from the present study, optimum concentration of pectin and CMC individually or in combination 0.25% and 0.15% respectively and addition of 2% Jujuba concentrate make the Jujuba concentrate – milk drink stable for 14 days. Addition of > 7% Jujba concentrate results in biphasication being not justifiable economically.

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