Utilization of Milk Protein Hydrolysate in Functional Beverages

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ABSTRACT

The objective of this study was to utilize milk protein concentrate hydrolysate (MPCH) in special drinks as functional beverages and to study the acceptably of such drinks with fresh fruits and flavors. The milk protein concentrate was hydrolyzed by trypsin at pH 7.5 for 20 h. Protein hydrolysis was evaluated by SDS-PAGE and RP-HPLC. Milk protein was completely hydrolyzed by trypsin after 20 h of incubations as there were no bands observed on the SDS-PAGE and broad peptides were separated by RP-HPLC as a source of bioactive peptides. The bitterness of milk protein hydrolysate by trypsin was eliminated by adding sweeteners (sucrose, fructose and sucralose). The reduction of bitterness was highly observed in fresh strawberry and mango juices when compared to flavored juices. However, the best score of sensory evaluation was in MPCH when it was treated by sucrose, fructose and sucralose without flavor compared to MPCH with strawberry flavor. MPCH that utilized in fresh mango juices and sweetened by sucrose, fructose and sucralose received the highest acceptability scores and lowest bitterness compared to all other treatments.

Key words: Milk protein, bioactive peptides, functional beverages.

INTRODUCTION

Milk proteins represent an excellent source of both functional and nutritious proteins. Due to their unique benefits for human health, protein hydrolysates have been developed as a kind of novel ingredient for nutraceutical food (Chen *et al.* 2006). Protein hydrolysates rich in peptides with low molecular weight possess high digestibility and low immunogenicity. They also show some specific biological properties such as opioid, antibiotic, antioxidant, mineral-binding, and antihypertensive. However, the unpleasant bitter flavor and hygroscopicity of protein hydrolysates hinder their direct utilization in food systems (Lin *et al.* 1997).

Milk proteins have received increasing attention as potential ingredients of health-promoting functional foods targeted at diet-related chronic diseases, such as cardiovascular disease, diabetes type two and obesity (Korhonen., 2009). Furthermore, milk proteins are considered as the most important sources of bioactive peptides. These remain inactive within the sequence of the parent protein until they are released by either gastrointestinal digestion or food processing. Their beneficial health effects are classified as antimicrobial, immunomodulatory, blood-pressure lowering (ACE inhibitory), antithrombotic, antioxidant and opioid like, in addition to cholesterol lowering and enhancers of mineral absorption/ bioavailability (Korhonen and Pihlanto, 2003; Hartmann and Meisel, 2007and Plaisanciéa *et al.*, 2013). Since 1980s, the production of protein hydrolysate preparations with the specific taste profile has been developed. These preparations are frequently used as the source of protein substances in different kind of dietetic, nutritional, and medicinal specimen. They can be applied in: I) Food enrichment, especially fruit beverages. (II) Diets composed for elderly people with the deficiency of the stomach juice secretion.III) Diets for sportsmen – as the food being the source of available nitrogen provided as peptides and amino acids. IV) Specially composed diets for patients with the metabolic problems and digestive tract diseases such as: chronic pancreatitis, syndrome of loop intestine. V) Preparations necessary in parenteral and enteral feeding. VI) Food formulae for children and infants with the allergy for bovine milk proteins (Swiderski and Waszkiewicz-Robak 2000).Functional dairy beverages can be categorized into two basic groups: (i) fortified dairy beverages (including probiotics, prebiotics ⁄fibres, polyphenols, peptides, sterol ⁄ stanols, minerals, vitamins and fish oil) and (ii) whey-based beverages (both fruit juice-type and dairy-type) (Ö zer and Kirmaci., 2009). Milk-based beverages are liquid, processed milk products. They are mixtures of skim milk or skim milk powder with water, with for example colorants, flavors, acids, functional ingredients, fruit mixes juices, sugar and preservatives. The milk-based beverages market is still a niche market compared with the sales of yoghurt and milk. However, there have been many innovations and currently it is one of the fastest growing dairy segments. Yogurt does not fall under the category of drinks, because it is too firm and texturized. It can be processed (diluted with fruit juice, stirred), Therefore, in order to achieve a consistency suitable for pouring and drinking. Many of the current milk-based beverages are mixes of yogurt and fruit juice. The viscosity of milk-based beverages is usually higher than that of milk , and the pH is usually lower (3.8–4.4 vs. 6.7 for normal milk) (Mellema and Bot., 2009). Ortiz *et al*. (2009) found that the bitterness

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of casein hydrolysate evaluated by sensory tests, diminished when encapsulated with soy protein isolate as wall material. Microencapsulation technology has been widely used in food and pharmaceutical industry because it can mask or reduce the unpleasant flavor, increase the stability of ingredients and control the release of the encapsulated materials (Shahidi and Han, 1993). Recently, this technique has been used successfully to solve the problems limiting the application of some materials and additives in food as well. Spray drying is the most extensively used one due to its low processing costs, short contact time and good stability of final products (Reineccius, 1989). Leksrisompong *et al.* (2010) evaluated the taste of twenty-two whey protein hydrolysates (WPH) obtained from eight major global manufacturers by instrumental analysis and descriptive sensory analysis and suggested that bitter taste was crucial to limit the application of WPH in food systems. In the current context of functional foods, fruit beverages are often commercially supplemented with milk to provide bioactive components such as vitamin C, carotenoids and phenolic compounds (from the fruit), and to improve nutritional value derived from proteins and minerals such as calcium and phosphor (from the milk) (Cilla *et al,* 2010). García-Nebot *et al.* (2010) studied the Addition of milk or caseinophosphopeptides (CPPs) to fruit beverages to improve iron bioavailability. The addition of milk to fruit beverages exerted a positive effect on iron retention, transport and uptake versus fruit beverages, and this effect was greater than that of CPPs added to soluble fractions of fruit beverages. The addition of CPPs to soluble fractions of fruit beverages improved iron transport. Iron supplementation increased Fe retention, transport and uptake, the effect being more notable in samples with milk. Zinc supplementation did not affect Fe retention, transport or uptake.

The objective of current work is to utilize milk protein hydrolysate in special drinks as functional beverages and to study the acceptably of such drinks with fruits and flavor.

MATERIALS AND METHODS

2.1. Milk protein concentrate

Cow milk protein concentrate (MPC) was obtained from Fonterra Ltd, Auckland, New Zealand. The typical analysis of milk protein concentrate (Protein 69.89 %, Lactose 17 %, Minerals 7.2 %, Moisture 4.6 % and Milk fat 1.4 %).

2.2. Preparation of milk protein hydrolysates

Milk protein hydrolysate was prepared according to method of Otte *et al.* (2007). Protein solutions (2% w/w on protein basis) were made by dispersion of approximately 2.85 g of milk protein concentrate (according to the protein content) in 97.15 ml of distilled water to final weight 100 g protein solution, and stirring for 1 h at room temperature. Solutions were hydrated overnight at 5 °C, after that, the pH in first six samples were left without adjusting (pH ~ 6.8). However, in the second six samples, the pH was adjusted to 7.5 by 1 % NaOH solution. Trypsin enzyme solution (80 mg ml^{-1}) was made in distilled water immediately prior to use. For each hydrolysis experiment, 100 ml of protein solution was pre-incubated at 40 °C for 10 min. After withdrawal of 1ml sample (zero time sample), the hydrolysis process was started by addition of 0.5, 1.0, 1.5 and 2.0 ml of trypsin solution to the remaining 100 ml of protein solution, and vortex mixing for 30s. Enzyme to substrate ratios were in range of 2:100, 4:100, 8:100 (w/w) (2x, 4x, and 8x) respectively. The reaction mixture was incubated at 40 °C, and intervals samples (1 ml each) were withdrawn after 20 and 24 h of hydrolysis. Enzyme was inactivated by heating at 90 °C for 15 min, and then cooling for 20 min in ice bath and then centrifugation (Sigma centrifuge 113 VWR International, Germany) for 10 min at 10,000 xg, the supernatant was used for further analyses as described below.

2.3. Analysis of peptides by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC):

Separation of the peptide extracts was carried out using a HPLC system according to the method described by (Awad *et al.*, 1998). Solvent A was 0.1 % trifluoroacetic acid in water, and Solvent B was 0.1% trifluoroacetic acid in acetonitrile. WP 300 RP-18, 5 μm, 250 x 4.6 mm from Merck was used for analyses. Samples were filtered through a 0.2-μ membrane filter (Millipore Corp., Bedford, MA). 50 μl of peptides extract (3% peptides extract in water) were injected into the column.

RESULTS

Evaluations of the hydrolysis of milk protein concentrate

a. SDS-PAGE

Nowadays, polyacrylamie gel electrophoresis techniques are considered simple and accurate analytical methods and proper techniques and could be used for studing of the milk protein behavior. Thus in this study, gel electrophoretic techniques were applied in analysis of milk protein concentrate as well as in monitoring their proteolysis rate by trypsin. Figure (1) shows the electrophoretic (SDS-PAGE) patterns of trypsin treated-milk protein concentrate.

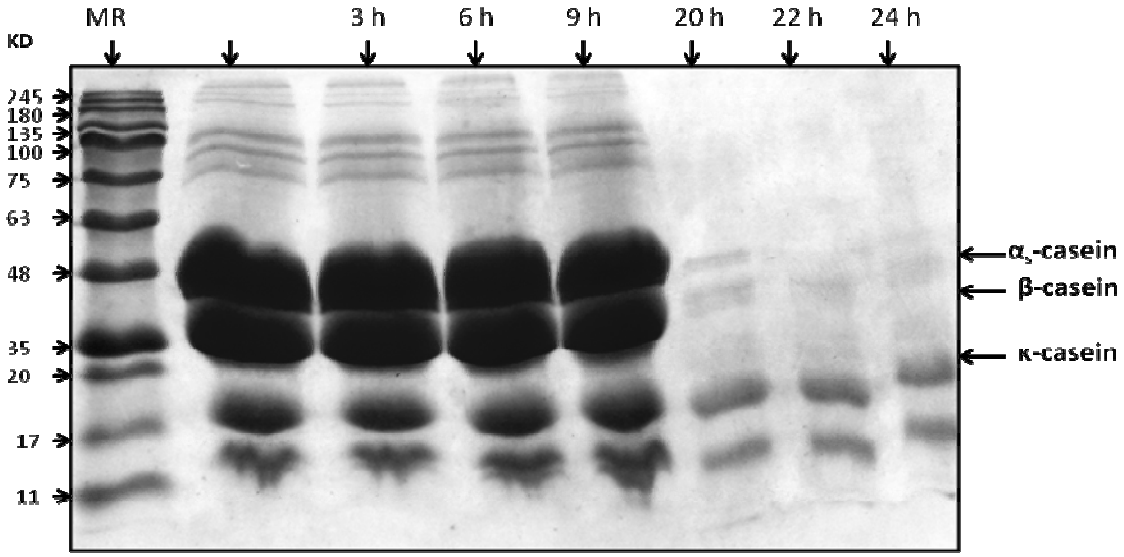

Fig. 1. SDS-PAGE (12.5%) of MPC treated with trypsin 8X for 3, 6, 9, 20,22and 24 hours.

The results indicated that caseins were fractionated to three fractions differ in their migration positions, intensities and molecular weights. Therefore it is clear that the action of trypsin enzyme on MPC was miner till nine hours, but there were intensive degradation of MPC at 20, 22, and 24 hours. Few and very small peptides with molecular weight (MW) less than 20 KD are appeared on the gel bottom. These results clearly showed that the action of enzyme on MPC was time depending to produces small molecular weight of protein hydrolysate.

b. RP-HPLC

The RP-HPLC chromatogram of the pH 4.6 soluble peptide extracts from milk protein concentrate hydrolysed by trypsin at pH 7.5 and 40° C for 20 h is illustrated in Figs. (2). A broad dominant peaks were seen at retention time (Rt.) of 36.8 to 54.2at 230 nm, while more peaks could be seen at 200 nm compared to that separated at 230 nm. In agreement with SDS-PAGE as the only low MW peptides were observed on the gel after 20 h of hydrolysis, there is a broad range of peptides separated by RP-HPLC which indicated that the MPC is degraded to small molecular weight peptides as a source of bioactive peptides.

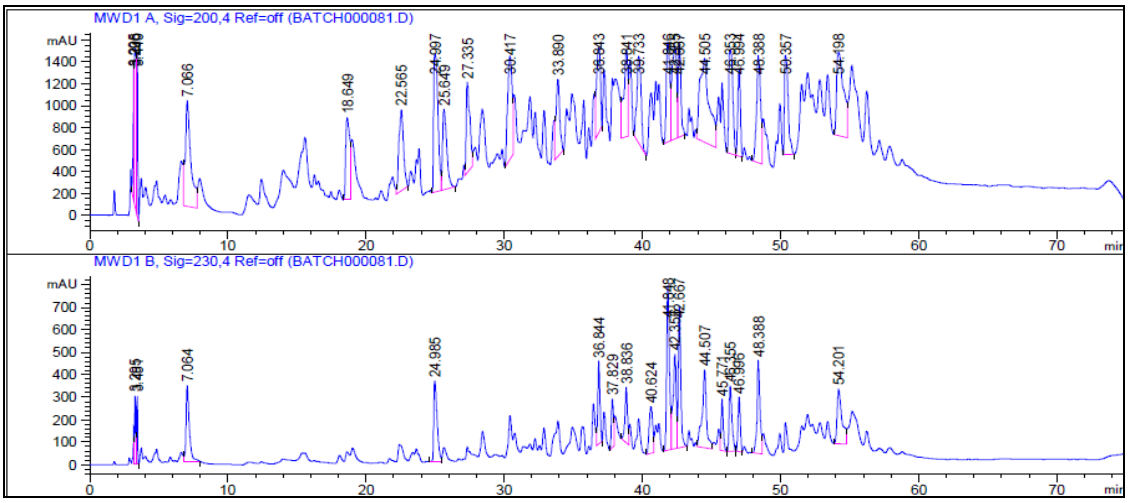

Fig. 2. RP-HPLC pattern of hydrolyzed product of MPC by trypsin.

Sensory Evaluation of functional beverages

Panelists sensed bitterness in MPCH by trypsin without addition of flavor or fresh fruits (Table 1 and Figure 3). Adding sucrose, fructose or sucralose to the milk protein hydrolysate almost eliminate the bitterness (Table 1 and Fig 3 and 4). There was no significant (P>0.05) difference in taste among samples sweetned by sucrose, fructose and sucralose (T18, 19, 20). Meanwhile, the results shows no significant (P>0.05) difference in color, odor, appearance and acceptability among all treated samples with sugars. The results of sensory evaluation of fresh and flavored strawberry juices received significant (P<0.05) higher scores in color, taste, odor, appearance and acceptability of T1 and T9 (stander samples) when compared to other treatments (T2, 3, 4 10, 11and 12) which contained MPCH (Table 2, Figure 3 and 4). No significant (P<0.05) difference were observed between T2, 3, 4, 10,11and12 in color scores. On the other hand, the fresh juices contained MPCH (T2, 3 and 4) received higher significant (P<0.05) scores in taste, odor, appearance and acceptability than other treatments (T10, 11and 12). Concerning to the effect of sweeteners on elimination of bitterness MPCH, the obtained results showed that the higher effects of sweeteners were with sucrose, fructose and sucralose in MPCH with fresh strawberry juices when compared to flavored strawberry juices. On the other hand, treatments of T2, 3 and 4 had a significant (P<0.05) higher total acceptability scores than the treatments of T10, 11 and 12. Moreover, there were no significantly (P>0.05) differences in taste among fresh juices sweetened with sucrose, fructose and sucralose (T2, 3 and 4),. There was no significantly (P>0.05) difference in taste between T10, 11 and 12.The obtained results revealed that the fresh strawberry juices were more acceptable than flavored juices containing the MPCH, and the addition of sweeteners (sucrose, fructose and sucralose) were highly reduced bitterness in fresh strawberry juices when compared to flavored juices. Same trend was found in mango juices, standard mango samples (T5 and 13) significantly (P<0.05) received higher scores in color, taste, odor, appearance and acceptability than all other treatments (T6, 7, 8, 14, 15 and 16) which containing MPCH (Table 3, Figure 3 and 4). There were no significant (P<0.05) differences between T6, 7, 8 14, 15 and 16 in color scores. On the other hand, the fresh mango juices containing MPCH (T6, 7and 8) received significantly (P<0.05) higher scores of taste, odor, appearance and acceptability than other treatments (T14, 15 and 16). Bitterness in MPH was eliminated by addition of sweeteners and the higher effects of sweeteners were in order sucrose> fructose > sucralose and the effects of sweetener in reduction bitterness of MPH was clearly observed in fresh mango juices than in flavor mango juices. In addition, fresh mango juices (T6, 7 and 8) were significantly (P<0.05) higher scores when compared to flavored juice treatments (T14, 15 and 16). Moreover, there were no significant (P>0.05) differences in taste between T2, 3 and 4 that sweetened by sucrose, fructose and sucralose respectively. At the same time there were no significant (P>0.05) differences in taste between T10, 11 and 12. The obtained results suggested that using MPCH in fresh strawberry and mango juices were more acceptable than the strawberry and Mango flavor juices. The effect of sweeteners (sucrose, fructose and sucralose) on reduction of bitterness was highly recognized in fresh strawberry juices than in flavored juices.

DISCUSSION

Enzymatic hydrolysis of proteins is widely used in the food industry to improve functional properties such as solubility, emulsification, gelation and taste, or to prepare extensively hydrolyzed proteins for hypoallergenic infant diets and nutritional therapy. More recently the pharmaceutical and food industries have shown increasing interest in peptides derived from protein hydrolysates as components of functional foods (Vanhoute *et al,* 2008). The most prominent enzymes are pepsin, trypsin and chymotrypsin that have been shown to release a number of antihypertensive peptides, calcium-binding phosphopeptides (CPPs), antibacterial, immunomodulatory and opioid peptides both from different casein (α-, β- and κ-casein) and whey proteins, e.g., α-lactalbumin (α-la), β-lactoglobulin (β-lg) and glycomacropeptide (GMP) (Meisel and FitzGerald., 2003; Yamamoto *et al*., 2003; FitzGerald *et al*., 2004; Gobbetti *et al*.,2004 and 2007). Our results are in agreement with previous studies in production of bioactive peptides from milk protein concentrate when treated by trypsin enzyme for 20, 22 and 24 h.

Bitterness associated with whey protein hydrolysate (WPH) and other hydrolyzed proteins are caused by generation of smaller peptide chains during the enzymatic hydrolysis processes (Maehashi and Huang 2009). Many methods can be applied on hydrolysates to reduce the bitterness of peptides. Removal of hydrophobic peptides, the key determinant of bitter taste, by enzymatic hydrolysis (Komai *et al.*2007) or processing methods reduce bitter taste in protein hydrolysates (Ziajka *et al.* 1994; Cheison *et al.* 2007). However, the majority of amino acids that are essential and contribute to bioactive properties are hydrophobic amino acids.

Table 1. Sensory evaluations of milk protein concentrate hydrolysate (MPCH)

Treatments	Color	Taste	Odor	Texture	Appearance	Acceptability	Total Score
T17	4.1±1.287^{cde}	4±1.247^{bcd}	3.9±1.59^{ed}	4.2±1.317^{def}	4.1±1.287^{bcd}	4±1.247^{bcd}	24.3±7.80^{cde}
T18	3.4±0.966^{fg}	2.7±1.059^{hij}	3±0.943^{f}	3.7±0.949^{fg}	3.4±0.843^{fgh}	2.9±0.994^{fgh}	19.1±4.84^{f}
T19	3.4±0.966^{fg}	2.2±1.135^{jk}	2.9±1.10 ^{f}	3.8±0.919^{efg}	3.2±0.789^{h}	2.3±1.06^{h}	17.8±5.07^{f}
T20	3±0.816^{g}	2.4±0.966^{ijk}	2.7±1.337^{f}	3.4±0.966^{g}	3.1±0.876^{gh}	2.6±1.07^{gh}	17.2±4.90^{f}

Treatment (17) water + 10%sucrose (control sample).
Treatment (18, 19 and 20) MPCH (1% protein) + 0.04 % sucralose, 13 % fructose, 15% sucrose respectively.
All values are expressed as means ± SE; n = ten for each treatment group
Mean values within a row not sharing a common superscript letters (a, b, c, d, e, f, g, h, I, j, k) weresignificantly different, $P<0.05$.

Table 2. Sensory evaluation of both natural and artificial strawberry juices containing and non-containing of MPCH.

Treatments	Color	Taste	Odor	Texture	Appearance	Acceptability	Total Score
T1	4.7±0.949^{abc}	4.7±0.675^{ab}	4.9±0.316^{a}	4.9±0.316^{ab}	4.9±0.316^{a}	4.9±0.316^{a}	29±2.16^{ab}
T2	3.7±1.252^{ef}	3.5±0.850^{cdefg}	4±1.054^{cd}	4.3±0.675^{cde}	4±0.667^{bcd}	3.9±0.738^{bcd}	23.4±4.52^{de}
T3	3.8±1.033^{ef}	3.6±0.843^{cdef}	4.1±0.738^{bcd}	4.5±0.527^{abcd}	4±0.667^{bcd}	4±0.667^{bcd}	24±3.71^{cde}
T4	4±1.054^{def}	3.4±1.174^{defgh}	4.3±0.675^{abcd}	4.3±0.823^{cde}	4±0.943^{cde}	3.7±0.949^{cde}	23.7±5.12^{cde}
T9	4.8±0.422^{ab}	4.2±0.789^{abc}	4.3±0.823^{abcd}	4.4±0.516^{bcd}	4.6±0.516^{ab}	4.5±0.527^{ab}	26.8±3.05^{abc}
T10	3.4±0.699^{fg}	2.2±0.919^{jk}	3.2±1.135^{ef}	3.3±0.675^{g}	3.2±0.632^{efg}	3.1±1.10^{efg}	18.4±3.92^{f}
T11	3.4±0.843^{fg}	1.8±0.632^{k}	3±1.054^{f}	3.3±0.675^{g}	3.2±0.632^{h}	2.4±1.174^{h}	17.1±3.66^{f}
T12	3.7±0.675^{ef}	2.1±0.994^{jk}	3±0.943^{f}	3.4±0.699^{g}	3.2±0.632^{h}	2.4±1.174^{h}	17.8±3.49f

Treatment (1) fresh mango juice + 10% sucrose (control sample) .
Treatment (2, 3 and 4) fresh mango juice + MPCH (1% protein) + 0.04 %sucralose,13 % fructose,15% sucrose, respectively.
Treatment (9) mango flavor and color +water + 10%sucrose (control sample).
Treatment (10, 11 and 12) mango flavor and color + MPCH (1% protein) +0.04 % sucralose,13 % fructose,15% sucrose, respectively.
All values are expressed as means ± SE; n = ten for each treatment group.
Mean values within a row not sharing a common superscript letters (a, b, c, d, e,f, g, h, I, j, k) weresignificantly different, $P<0.05$.

Table 3. Sensory evaluation of both natural and artificial mango juices containing and non-containing of MPCH.

Treatments	Color	Taste	Odor	Texture	Appearance	Acceptability	Total Score
T5	5±0.0^{a}	4.9±0.316^{a}	5±0.00^{a}	5±0.00^{a}	5±0.00^{a}	4.9±0.316^{a}	29.8±0.63^{a}
T6	4.8±0.422^{ab}	3.9±0.568^{cde}	4.5±0.527^{bcd}	4.7±0.483^{abcd}	4.7±0.483^{ab}	4.2±0.632^{bc}	26.8±2.1^{abc}
T7	4.7±0.675^{abc}	3.8±1.033^{cde}	4.1±1.10^{bcd}	4.8±0.422^{abc}	4.7±0.483^{ab}	4.4±0.516^{ab}	26.5±2.83^{bcd}
T8	4.7±0.675^{abc}	4±1.247^{bcd}	4.7±0.483^{abc}	4.9±0.316^{ab}	4.7±0.675^{ab}	4.5±0.707^{ab}	27.5±3.27^{ab}
T13	5±0.00^{a}	4.8±0.422^{a}	4.8±0.422^{ab}	5±0.00^{a}	5±0.00^{a}	5±0.00^{a}	29.6±0.84^{ab}
T14	4.3±0.483^{bcde}	3.2±0.919^{efgh}	4.1±0.994^{bcd}	4.3±0.675^{cde}	4.2±0.632^{bcd}	4±0.943^{bcd}	24.1±3.54^{cde}
T15	4.3±0.675^{bcde}	2.8±0.632^{ghij}	4±1.054^{cd}	4.3±0.675^{cde}	4.2±0.632^{def}	3.4±1.17^{def}	23±3.62^{e}
T16	4.6±0.516^{abcd}	3±1.054^{fghi}	4±0.943^{cd}	4.4±0.699^{bcd}	4.2±0.632^{def}	3.4±1.17^{def}	23.6±3.37^{de}

Treatment (5) fresh strawberry juice + 10%sucrose (control sample)
Treatment (6, 7 and 8) fresh strawberry juice + MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose, respectively.
Treatment (13) strawberry flavor and color +water + 10%sucrose (control sample).
Treatment (14, 15 and 16) strawberry flavor and color +MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose, respectively.
All values are expressed as means ± SE; n = ten for each treatment group.
Mean values within a row not sharing a common superscript letters (a, b, c, d, e,f, g, h, I, j, k) weresignificantly different, $P<0.05$.

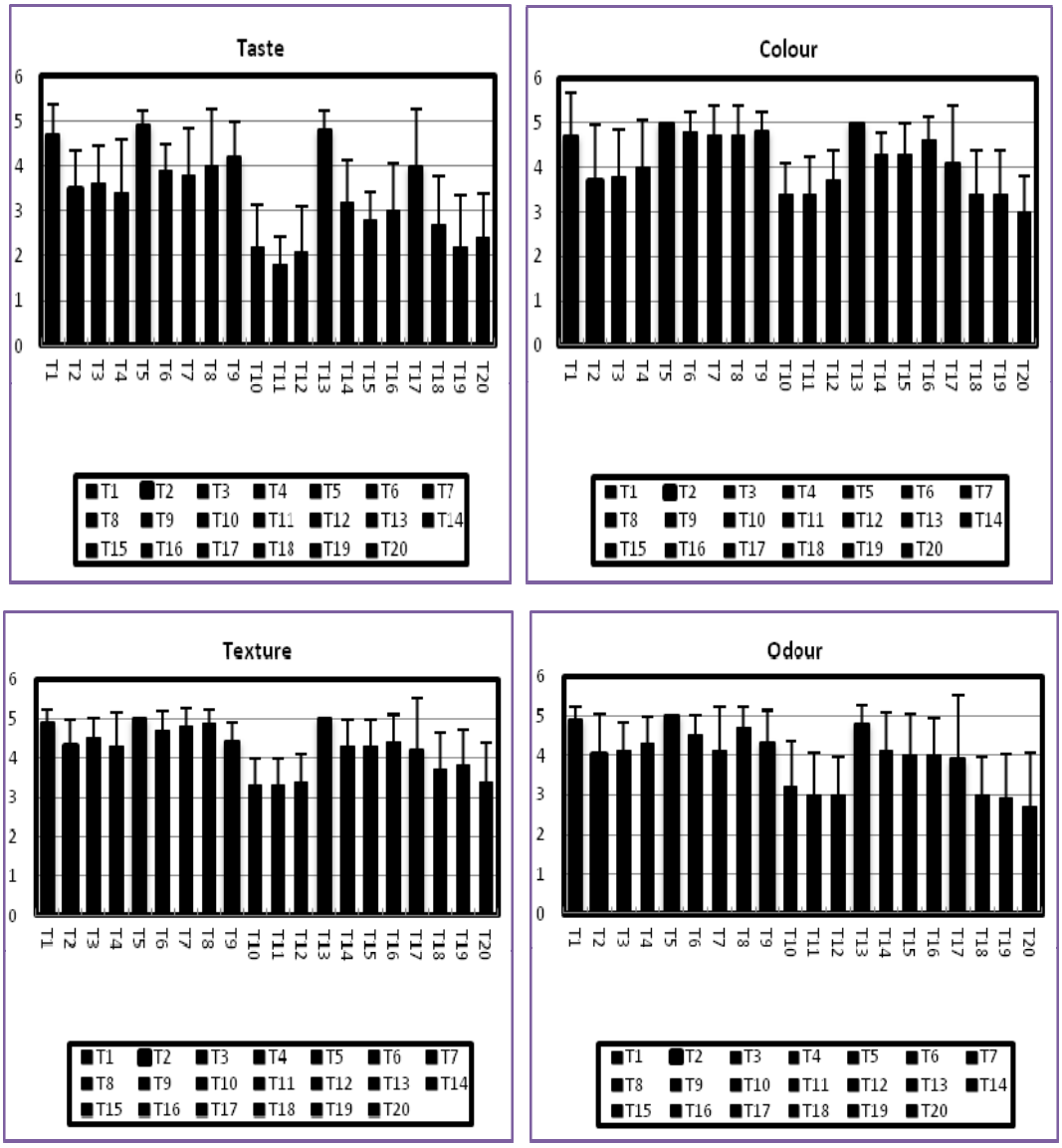

Fig. 3.Sensory evaluation of color, taste, odor and texture of juices based-MPCH and treated by sucrose 15%, fructose 13% and sucralose 2%.

Treatment (1) fresh mango juice + 10% sucrose (control sample) .
Treatment (2, 3 and 4) fresh mango juice + MPCH (1% protein) + 0.04 %sucralose, 13 % fructose,15% sucrose respectively.
Treatment (5) fresh strawberry juice + 10%sucrose (control sample)
Treatment (6, 7 and 8) fresh strawberry juice + MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose respectively.
Treatment (9) mango flavor and color +water + 10%sucrose (control sample).
Treatment (10, 11 and 12) mango flavor and color + MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose respectively.
Treatment (13) strawberry flavor and color +water + 10%sucrose (control sample).
Treatment (14, 15 and 16) strawberry flavor and color +MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose respectively.
Treatment (17) water + 10%sucrose (control sample).
Treatment (18, 19 and 20) MPCH (1% protein) + 0.04 %sucralose,13 % fructose,15% sucrose respectively.

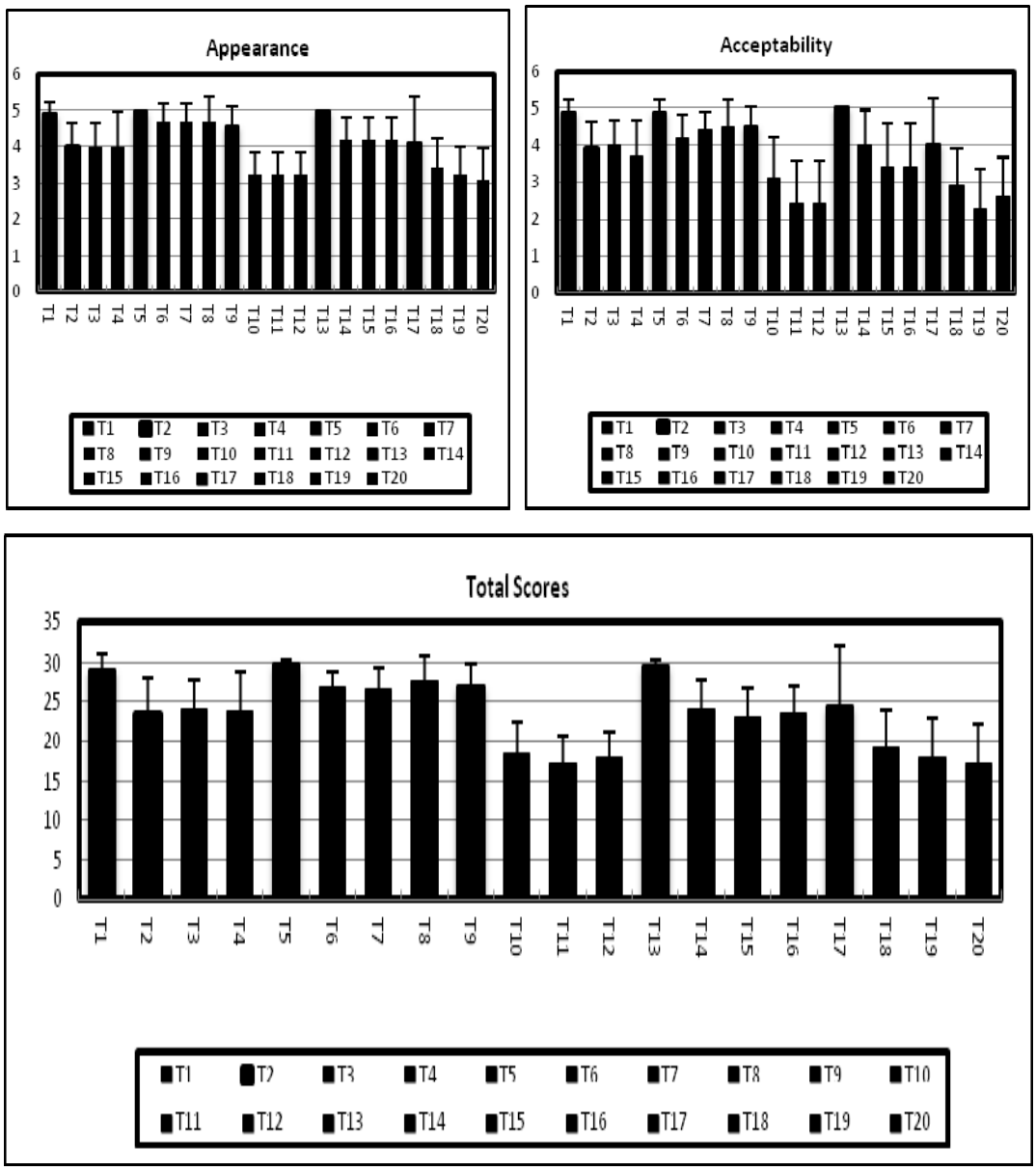

Fig. 4. Sensory evaluation of appearance, acceptability and total scores of juices based-MPCH and treated by sucrose 15%, fructose 13% and sucralose 0.04%.

Treatment (1) fresh mango juice + 10% sucrose (control sample) .
Treatment (2, 3 and 4) fresh mango juice + MPCH (1% protein) + 0.04 %sucralose,13 % fructose,15% sucrose respectively.
Treatment (5) fresh strawberry juice + 10%sucrose (control sample)
Treatment (6, 7 and 8) fresh strawberry juice + MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose respectively.
Treatment (9) mango flavor and color +water + 10%sucrose (control sample).
Treatment (10, 11 and 12) mango flavor and color + MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose respectively.
Treatment (13) strawberry flavor and color +water + 10%sucrose (control sample).
Treatment (14, 15 and 16) strawberry flavor and color +MPCH (1% protein) + 0.04 % sucralose,13 % fructose,15% sucrose respectively.
Treatment (17) water + 10%sucrose (control sample).
Treatment (18, 19 and 20) MPCH (1% protein) + 0.04 %sucralose,13 % fructose,15% sucrose respectively.

Thus, bioactivity of the hydrolysate may be compromised when debittering of peptides is accomplished by the removal of bitter hydrophobic side chains (Morato *et al.* 2000).

Our results are in agreement with that mentioned by Leksrisompong *et al,* (2012) who found that the salts and nucleotides decreased bitter taste intensity of WPH. However, they also potentiated their own flavors or muted other desired flavors(vanilla or chocolate flavors), or sweet taste. Sweeteners, especially fructose and sucralose, were more promising in suppressing bitter taste of WPH while enhancing desired flavors in chocolate and vanilla beverages. . More studies reported that salts, nucleotides, and amino acids efficiently inhibit the bitter taste of many bitter pharmaceutical agents peripherally (Keast *et al,*. 2001; Keast and Breslin 2002; Ogawa *et al.,* 2005). Meanwhile, Sweeteners and volatile compounds can also impact bitter taste cognitively (Miyanaga *et al.* 2003; Mukai *et al.* 2007). Ogawa *et al.* (2005) found that the bitter taste inhibition of quinine by amino acids might be due to the interaction at the taste receptor site as amino acids maycompete with quinine for the taste receptors, thus, closing the gate of the cation channel transportation and inhibiting the perception of bitterness.

CONCLUSION

Addition of MPCH to fresh strawberry and mango juices were more accepted than flavored juices and the effect of sweeteners (sucrose, fructose and sucralose) on reduction of bitterness were highly observed with fresh strawberry and mango juices compared to flavored juices. However, MPCH showed best sensory evaluation when treated by sucrose, fructose and sucralose without flavor compared to MPCH with strawberry flavor. The results also suggested that the utilization of MPCH in fresh mango juices sweetened by sucrose, fructose and sucralose received the highest acceptability scores and lowest bitterness compared to all other treatments.

REFERENCES

Awad, S., Lűthi-Peng, Q.Q., and Puhan, Z. (1998).Proteolytic Activities of Chymosin and Porcine Pepsin on Buffalo, Cow, and Goat Whole and β-Casein Fractions. J. Agric. Food Chem.46, 4997-5007.

Chen, L.Y., Remondetto, G.E and Subirade, M. (2006). Food protein based materials as nutraceutical delivery systems. Trends food sci tech 17:272–283.

Cheison, S.C,Wang Z., and Xu, S.Y. (2007). Preparation of whey protein hydrolysates using a single- and two-stage enzymatic membrane reactor and their immunological and antioxidant properties: characterization by multivariate data analysis. J Agric Food Chem 55:3896–904.

Cilla, A., Lagarda, M. J., Alegría, A., De Ancos, B., Cano, M. P., Sánchez-Moreno,C., Plaza, L., and Barberá, R.(2010). Effect of processing and food matrix on calcium and phosphorous bioavailability from milk-based fruit beverages in Caco-2 cells. Food Research International, 44: 3030–3038.

FitzGerald, R. J., Murray, B. A., and Walsh, D. J. (2004). Hypotensive peptides from milk proteins. Journal of Nutrition, 134: 980S–988S.

García-Nebot, M.J., Alegría, A., Barberá, R., Clemente, G., and Romero, F. (2010). Addition of milk or caseinophosphopeptides to fruit beverages to improve iron bioavailability?. Food Chemistry, 119: 141–148.

Gobbetti, M., Minervini, F., and Rizzello, C. G. (2004). Angiotensin I-converting enzyme-inhibitory and antimicrobial bioactive peptides. International Journal of Dairy Technology, 57: 172–188.

Gobbetti, M., Minervini, F., and Rizzello, C. G. (2007).Bioactive peptides in dairy products. In Y. H. Hui (Edt.), Handbook of food products manufacturing (pp. 489–517). Hoboken, New Jersey: John Wiley and Sons, Inc.

Hames, B.D. and Rickwood, D. (1990). In: Gel electrophoresis of proteins. A practical approach.TRL, London, England. Puplishing Co.pp.34, 36, 37, 44, 45 and 48.

Hartmann, R., Meisel, H.(2007). Food-derived peptides with biological activity: from research to food applications. CurrOpinBiotechnol; 18:163–9.

Keast, R.S.J and Breslin, P.A.S. (2002). Modifying the bitterness of selected oral pharmaceuticals with cation and anion series of salts. Pharm Res 19:1019–26.

Keast, R.S.J., Breslin, P.A.S and Beauchamp, G.K. (2001). Suppression of bitterness using sodium salts. Chimia 55:441–7.

Komai, T., Kawabata, C., Tojo, H., Gocho, S., and Ichishima, E. (2007). Purification of serine carboxypeptidase from the hepatopancreas of Japanese common squid *Todarodes pacificus* and its application for elimination of bitterness from bitter peptides. Fisheries Sci 73:404–11.

Korhonen.H. (2009). Milk-derived bioactive peptides: From science to applications. Journal of Functional Foods, 1:177 –187.

Korhonen, H., and Pihlanto, A. (2003). Food-derived bioactive peptides opportunities for designing future foods. Current Pharmaceutical Design, 9: 1297–1308.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 277: 680-685.

Leksrisompong, P., Gerard, P., Lopetcharat, K., and Drake, M.A. (2012).Bitter taste inhibiting agents for whey protein hydrolysate and whey protein hydrolysate beverages. Journal of Food Science, s282-s287.

Leksrisompong, P.P., Miracle, R.E and Drake, M. (2010). Characterization of flavor of whey protein hydrolysates. J Agric Food Chem 58:6318–6327.

Lin, S.B., Nelles, L.P., Cordle, C.T and Thomas, R.L. (1997) . Debittering casein hydrolysates with octadecyl-siloxane (C18) columns. J Food Sci, 62:665–670.

Maehashia, K., Huang, L. (2009) Bitter peptides and bitter taste receptors. Cell Mol Life Sci 66:1661–1671.

Meisel, H., & FitzGerald, R. J. (2003). Biofunctional peptides from milk proteins: Mineral binding and cytomodulatory effects. Current Pharmaceutical Design, 9: 1289–1295.

Mellema,M and Bot, A. (2009). Milk-based functional beverages. In Functional and speciality beverage technology Edit by Paul Paquin. Woodhead Publishing Limited, Oxford ,Cambridge, and New Delhi.

Miyanaga, Y., Inoue, M., Ohnishi, A., Fujisawa, E. Y., amaguchi, M and Uchida, T. (2003). Quantitative prediction of the bitterness suppression of elemental diets by various flavors using a taste sensor. Pharm Res 20:1932–8.

Morato, A.F., Carreira, R.L., Junqueira, R.G., and Silvestre, M.P.C. (2000). Optimization of casein hydrolysis for obtaining high contents of small peptides: use of subtilisin and trypsin. J Food Compos Anal 13:843–57.

Mukai, J., Tokuyama, E., Ishizaka, T., Okada, S and Uchida, T. (2007). Inhibitory effect of aroma on the bitterness of branched-chain amino acid solutions. Chem Pharm Bull 55:1581–4.

Ogawa, T., Hoshina, K., Haginaka, J., Honda, C., Tanimoto, T and Uchida T. (2005). Screening of bitterness-suppressing agents for quinine: the use of molecularly imprinted polymers. J Pharm Sci 94:353–62.

Ortiz, S. E. M., Mauri, A., Monterrey-Quintero, E. S., Trindade, M. A., Santana, A. S and Favaro-Trindade, C. S. (2009). Production and properties of casein hydrolysate microencapsulated by spray drying with soybean protein isolate. LWT– Food Science and Technology 42, 919–923.

Otte, J., Shalaby, S. M., Zakora, M., Pripp, A. H., & El-Shabrawy, S. A. (2007). Angiotensin-converting enzyme inhibitory activity of milk protein hydrolysates: Effect of substrate, enzyme and time of hydrolysis. International Dairy Journal, 17, 488–503.

Ö zer, B. H and Kirmaci, H. A. (2009). Functional milks and dairy beverages. International Journal of Dairy Technology. Vol 63, No 1: 1-15.

Plaisanciéa, P., Claustrec, J., Estiennea, M., Henryd, G., Boutroud, R., Paqueta, A., and Léonild, J. (2013) . A novel bioactive peptide from yoghurts modulates expression of the gel-forming MUC2 mucin as well as population of goblet cells and Paneth cells along the small intestin. Journal of Nutritional Biochemistry 24 213–221.

Reineccius, G.A. (1989). Flavor encapsulation. Food Rev Int 5:147–176.

Shahidi, F and Han, X.Q. (1993). Encapsulation of food ingredients. Crit Rev Food Sci 33:501–547

Swiderski, F., Waszkiewicz-Robak, B. (2000). Peptides and proteins as bioactive components of functional food.Przem.Spo. 11, 41-43.

Vanhoute, M., Froidevaux, R., Pierlot, C., Krier, F., Aubry, J.M., and Guillochon, D. (2008).Advancement of foam separation of bioactive peptides using an aeration column with a bubbling-draining method. Separation and Purification Technology, 63:460–465.

Yamamoto, N., Ejiri, M., and Mizuno, S. (2003).Biogenic peptides and their potential use. Current Pharmaceutical Design, 9: 1345–1355.

Ziajka, S., Dzwolak, W, and Zubel J. (1994). The effect of processing variables on some properties of whey protein hydrolysates. Milchwissenschaft 49:382–4.

الملخص العربي

إستخدام متحال بروتين االلبن في المشروبات الوظيفية

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الهدف من هذه الدراسة هو إستخدام متحللات بروتينات التي تنتج من معاملة بروتينات اللبن بالإنزيمات الهاضمة في انتاج منتجات لها خواص وظيفية وصحية كبيرة ولذلك فقد قمنا بإستخدام هذه المتحللات البروتينية (١% بروتين) الناتجة من تحال مركز بروتينات اللبن بواسطة إنزيم التربسين لمدة ٢٤ ساعة علي درجة حرارة ٤٠ درجة مئوية وقيمة رقم حموضة ٧.٥في انتاج مشروبات صحية عن طريق إضافتها الي العصائر الطبيعية مثل عصير المانجو أو الفراولة وكذلك إضافتها الي مشروبات مصنعة لها نكهة المانجو والفراولة.ونظراً لأن هذه المتحللات البروتينية تكون عادة ذات طعم مر والذي يكون راجعاً لوجود الببتيدات الكارهة للماء(الهيدروفوبية) فقد بحثنا في كيفية التخلص من هذا الطعم المر باستخدام السكروز بنسبة ١٥% أو الفركتوز بنسبة ١٣% أو السوكرالوز بنسبة ٠.٠٤% وقد وجد أن هذه السكريات قد إختزلت إلي حد كبير الطعم المر من هذه المشروبات وكان أعلي معدل إختزال للطعم المر مع المشروبات الطبيعية ومع جميع السكريات بنفس المعدل تقريبا.

أما عن التقييم الحسي للمشروبات المنتجة باضافة هذه المتحللات البروتينية اللبنية فقد وجد أن أفضل درجات اللون والطعم والرائحة والقوام والمظهر العام والتقبل العام قد لوحظت مع عصير المانجو الطبيعي المضاف إليه المتحللات البروتينية يليه عصير الفراوله الطبيعي والعصير المصنع بنكهة المانجو ثم العصير المصنع بنكهة الفراولة فقد كان أقلهم درجة في التقييم الحسي.