ICE MILK QUALITY ATTRIBUTES AS AFFECTED BY TRYPSINIZED WHEY PROTEINS

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ABSTRACT: *Two types of trypsin (0.4%) modified whey protein concentrates were prepared, one from whey protein precipitated by heat treatment of salted sweet whey (mSWPC) and the another one by ultrafilteration of acid whey (mFWPC). Nine treatments of vanilla ice milk were made to study the effect of replacing non-fat dry milk with enzyme modified WPC on the quality of ice milk. Replacement of non-fat dry milk with trypsin modified WPC caused a significant increase in moisture, total protein, non protein, nitrogen, viscosity, specific gravity and weight per gallon of ice milk mixes and melting resistance and pH values of ice milk and this increase was proportional to the rate of replacement. Ice milk mixes made by adding mFWPC were not significantly different from those made by adding mSWPC. Substitution of non-fat dry milk with trypsin modified whey protein concentrates up to the rate of 50% increased the overrun, while increasing the substitution rate above that, caused a significant decrease in the overrun of ice milk. Specific gravity and weight per gallon of ice milk decreased by replacing non-fat dry milk with trypsin MWPC up to the rate of 50% then increased by increasing the replacing rate above that. Replacing up to 50% increased the scores of organoleptic evaluation, while increasing the replacing rate to 75 or 100% decreased the scores of organoleptic evaluation of ice milk. Scores of organoleptic properties did not changed significantly during storage, while pH values decreased during the first four weeks of storage, then were unchanged.*

Key words: *Ice milk, modified whey proteins, trypsin, salted whey, acid whey.*

INTRODUCTION

Consumption of frozen desserts has increased in Egypt. It has been estimated that the annual amount of whey and milk permeate more than one million ton (Mahmoud, 2000). This amount is disposed in the sewage system that might cause environmental pollution. Utilization of whey products has been increased during the lost decade, particularly in dairy food system (Morr, 1989; Morr and Foegeding, 1990, Morr and Ha, 1991). Although whey products have been used in the manufacture of ice cream, the deterioration of the resultant ice cream quality limits the amount of added whey products and their use. The quality of ice cream depends not only on the amount of added whey products, but also on the type of whey product (Khader *et al.*, 1992; Lee and White, 1991; Vulink, 1995). Using enzyme modified whey protein concentrates in the manufacture of ice cream would presents an economically attractive alternative for non-fat dry milk required to frozen desserts industry.

The objective of this study was to investigate the effect of using the trypsin modified whey protein concentrates to replace some of non-fat dry milk in the manufacture of ice milk.

MATERIALS AND METHODS

Ingredients:

Two types of whey protein concentrates were prepared, one of them was precipitated by heat treatment from salted whey (SWPC) and the other was prepared by ultrafiltration (FWPC) of acid whey. Both were modified by trypsin (Sigma Chemical Com., St. Louis, MO, USA) as described by Kebary (2008). Buffalo's milk (Faculty of Agric., Minufiya University, Shibin El-Kom), non-fat dry milk (Ecoval N. V., Paris, France), Sucrose (local market), stabilizer (Palsgarrd 5936, Palsgarrd Industries, Juelsmind, Denmark), cream was obtained by separating buffalo's milk, Vanilla (local market).

Manufacture of ice milk:

Nine ice milk mixes were prepared with the following composition, 4% fat, 13% solid not fat, 15% sugar, 3% cocoa and 0.5% stabilizer. Non fat dry milk was used to supply the milk solid not fat in the control mix, while in the other eight ice milk patches 25, 50, 75 and 100% of the amount of non-fat dry milk used in the control mix were substituted with hydrolyzed either SWPC or FWPC, respectively. Vanilla ice milk was made according to Khader *et al.* (1992). The resultant ice milk treatments were packed in plastic cups and kept for 24 hr at -18°C for hardening. Ice milk batches were stored at -25 ± 2°C for 8 weeks. The experiment was performed in triplicate and two determinations were conducted per replicate.

Physical and chemical analysis:

The prepared mixes were analyzed before freezing for viscosity (Tracy and Tucky, 1938), specific gravity (Winton, 1958), weight per gallon (Burke, 1947), moisture and total nitrogen content (Ling, 1963), ash (AOAC, 1975) and non protein nitrogen (Patel *et al.*, 1990). The resultant ice milk were tested for specific gravity (Winton, 1958), weight per gallon (Burke, 1947) overrun (Arbuckle, 1986), melting resistance (Reid and Painter, 1933) and pH values was determined during storage (Ling, 1963).

Sensory evaluation:

Fifteen panelists of the staff members of Dept. of Dairy Science and Tech., Minufiya University Shibin El-Kom Egypt evaluated the organoleptic properties of each patch of ice milk at zero day and every two weeks up to the end of storage period according to Khader *et al.* (2001).

Statistical analysis:

Factorial design 2 factors × 3 replicates and the completely randomized design were used to analyze all the data, and student Newman Keuls test was followed to make the multiple comparisons (Steel and Torrie, 1980) using COSTATE program. Significant differences were determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Gross composition of ice milk is presented in Table (1). Substitution of non fat dry milk with trypsin modified whey protein concentrates (mFWPC, mSWPC) caused a significant ($P \leq 0.05$) increase in moisture content and this increase was proportional to the rate of replacement. This result may be due to the increase of water absorption by whey protein so that increasing the moisture with the increase of the rate of replacement. This result is in agreement with that reported by Khader *et al.* (2001).

Moisture contents of ice milk made with addition trypsin modified whey protein concentrates were not significantly different ($P > 0.05$) from those of the corresponding batches made with addition of modified salted whey protein concentrates (Tables 1, 6). Control ice cream contained the lowest moisture content, while mF_4 and mS_4 made with modified whey protein contained the highest moisture contents. Total protein and non protein nitrogen showed almost similar trends were substitution of non-fat dry milk with modified whey protein concentrates (mFWPC and mSWPC) caused a significant ($P \leq 0.05$) increase in total protein and non protein nitrogen. This increase was proportional to the rate of replacement. These results might be due to that modified whey protein contained higher total protein (62.91% for mFWPC and 70.09% for mSWPC). Ice milk treatments made with modified ultrafiltered whey protein concentrates (mFWPC) were not significantly ($P > 0.05$) different from corresponding ice milk treatment made with modified salted whey protein concentrates (mSWPC). Similar trends were reported by Khader *et al.* (2001).

Ash content of ice milk treatments made with adding mFWPC were not significantly ($P > 0.05$) different from those of corresponding treatments made with adding mSWPC. Replacement of non-fat dry milk with hydrolyzed whey protein contents did not have significant ($P > 0.05$) effect on ash content (Tables 1, 6)

Table (1). Effect of adding trypsin modified whey protein concentrates on chemical composition of ice milk.

Treatments	Moisture %*	Total protein %	NPN %*	Total ash %
C	63.13	5.37	0.33	0.716
mS_1*	64.18	5.88	0.40	0.719
mS_2	64.94	6.77	0.53	0.722
mS_3	65.27	7.15	0.65	0.726
mS_4	65.54	7.83	0.77	0.728
mF_1**	64.12	5.92	0.43	0.713
mF_2	64.78	6.81	0.51	0.719
mF_3	65.13	7.19	0.69	0.723
mF_4	65.63	7.86	0.81	0.726

C : Ice cream milk made with non-fat dry milk.

* mS_1, mS_2, mS_3 and mS_4: Dried modified salted whey protein were substituted for 25, 50, 75 and 100% of non-fat dry milk respectively.

** mF_1, mF_2, mF_3 and mF_4: Dried modified ultrafiltrated whey protein were substituted for 25, 50, 75 and 100% of non-fat dry milk, respectively.

Table (2). Effect of adding trypsin modified whey protein concentrates on some properties of ice milk mixes.

Treatments	Viscosity (cp)	Specific gravity	Weight / gal. (kg)
C	361.52	1.071	4.055
mS_1*	533.16	1.078	4.081
mS_2	624.45	1.082	4.096
mS_3	836.18	1.088	4.119
mS_4	968.29	1.094	4.142
mF_1**	472.39	1079	4.085
mF_2	584.64	1.083	4.100
mF_3	695.12	1.089	4.123
mF_4	792.86	1.095	4.145

C, *, ** see Table (1).

Table (3). Effect of adding trypsin modified whey protein concentrates on some properties of fresh ice milk.

Treatments	Overrun %	Specific gravity	Weight / gal (kg)	Melting resistance (loss % at 30°C)		
				First 60 min.	Next 30 min.	Last 30 min.
C	52.23	0.687	2.601	32.40	36.80	28.70
mS_1*	55.27	0.683	2.578	31.60	34.60	30.30
mS_2	57.53	0.681	2.457	29.40	32.30	31.20
mS_3	46.01	0.756	2.870	28.30	30.40	32.60
mS_4	35.90	0.801	3.032	27.50	29.90	35.80
mF_1**	56.45	0.649	2.586	30.20	33.60	29.60
mF_2	66.49	0.652	2.468	28.10	31.20	301.0
mF_3	49.93	0.758	2.862	26.20	28.80	31.30
mF_4	38.73	0.798	3.021	26.10	27.90	34.50

C, *, ** see Table (1).

Table (4). Effect of adding trypsin modified whey protein concentrates on pH values.

Treatments	Fresh	2 weeks	4 weeks	6 weeks	8 weeks
C	6.04	5.78	5.36	5.29	5.28
mS_1*	6.06	5.85	5.60	5.54	5.52
mS_2	6.09	5.88	5.70	5.61	5.60
mS_3	6.28	6.11	5.90	5.82	5.79
mS_4	6.33	6.17	5.95	5.90	5.87
mF_1**	6.11	5.82	5.57	5.51	5.49
mF_2	6.13	5.95	5.63	5.52	5.98
mF_3	6.30	6.18	5.82	5.79	5.76
mF_4	6.36	6.24	5.90	5.85	5.82

C, *, ** see Table (1).

Table (5). Scores of organoleptic properties of ice milk made with modified whey protein concentrates.

Treatment	Flavour (50)					Body and texture (40)					Melting quality (10)					Total scores (100)				
	Storage period (weeks)					Storage period (weeks)					Storage period (weeks)					Storage period (weeks)				
	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8
C	43	42	42	44	42	36	35	35	34	33	7	7	6	7	7	88	84	83	85	82
mS_1*	43	43	44	43	41	35	37	37	36	36	8	8	8	8	8	88	88	89	89	85
mS_2	43	42	43	42	42	36	36	38	36	37	8	8	9	9	8	88	89	90	88	87
mS_3	38	40	38	35	32	34	33	32	32	33	6	7	6	7	9	79	79	76	74	74
mS_4	36	38	37	37	33	29	29	28	28	24	6	6	7	7	8	71	73	72	72	70
mF_1**	41	43	44	42	41	37	35	37	37	37	8	7	8	8	7	88	87	87	87	86
mF_2	42	42	44	44	42	37	38	38	38	36	8	8	9	8	9	89	88	91	90	87
mF_3	42	42	41	42	41	36	36	37	36	36	7	7	8	8	8	85	81	87	82	81
mF_4	38	35	38	37	31	31	31	33	36	31	6	7	7	8	8	75	80	76	80	79

C, *, ** see Table (1).

Table (6). Statistical analysis of ice milk properties.

Ice milk properties	Means square	Effect of treatments									Means squares	Effect of storage (weeks) Multiple comparison				
		C	mS_1	mS_2	mS_3	mS_4	mF_5	mF_6	mF_7	mF_8		0	2	4	6	8
Moisture	1.3196833*	E	D	C	B	A	D	C	B	A						
Protein	1.98870000*	E	D	C	B	A	D	C	B	A						
N.P.N	0.1016203*	E	D	C	B	A	D	C	B	A						
Ash	0.09983358	A	A	A	A	A	A	A	A	A						
Sp. gravity (mix)	1.7550001	E	D	C	B	A	D	C	B	A						
Viscosity (mix)	109395.0635**	I	G	E	B	A	H	F	D	C						
Weight/gal (mix.)	0.440851333*	E	D	C	B	A	D	C	B	A						
Overrun	280.6893*	E	D	B	F	H	C	A	G	I						
Sp. gravity (ice milk)	8.5470833*	C	D	F	B	A	D	E	B	A						
Weight/gal (ice milk)	0.13318283*	C	D	F	B	A	D	E	B	A						
Melting resistance:																
First 60 min.	12.8400001*	H	F	D	B	G	F	D	C	A						
Next 30 min.	28.110000*	I	G	E	C	H	F	D	B	A						
Last 30 min.	10.3133333*	B	D	F	H	A	C	E	G	I						
pH	2.97923333*	E	D	C	B	A	D	C	B	A	0.32553666*	A	B	C	D	D
Falvour	154.76666	A	A	A	C	E	A	A	B	C	36.899999*	A	A	A	A	A
Body texture	165.216666	F	C	B	D	E	B	A	C	D	10.1666666*	A	A	A	A	A
Melting quality	5.999999	C	B	A	D	E	AB	A	B	D	3.7333333*	A	A	A	A	A
Total scores	635.616666	C	B	A	E	F	B	A	D	E	22.98333316*	A	A	A	A	A

* Significant at 0.05 level

For each effect the different letters (the same raw) means the multiple comparison are different from each other letter A is the highest mean followed by B, c.... etc.

Viscosity of ice milk mixes increased markedly ($P \leq 0.05$) by replacing non-fat dry milk with modified whey protein concentrates (mSWPC, mFWPC) (Table 2, 6). There was positive correlation between viscosity and the rate of replacing. This increase in viscosity might be attributed to the higher protein content of modified whey protein concentrates than that of non-fat dry milk. Similar trends were obtained by Khader *et al.* (1992); Magdoub *et al.* (1992) and Goff *et al.* (1989). Ice milk mixes made with mSWPC had higher viscosity than those of corresponding mixes made with mFWPC (Tables 2, 6).

Specific gravity and weight per gallon of all ice milk mixes increased significantly ($P \leq 0.05$) by replacement of non fat dry milk with modified whey protein concentrates (mFWPC and mSWPC) (Table 2). The increase in specific gravity and weight per gallon were proportional to the amount of modified whey protein concentrates substituted for non-fat dry milk. The specific gravity and weight per gallon of ice milk mixes made with mFWPC were not significantly different from those of corresponding ice milk mixes made with mSWPC. These results agree with those reported by Khader *et al.* (1992), Magdoub *et al.* (1992) and Mahmoud (2000).

Replacement of non fat dry milk with modified either mFWPC or mSWPC up to 50% increased ($P \leq 0.05$) the overrun of resultant ice milk compared with the control ice milk while increasing the replacement rate above 50% caused a pronounced ($P \leq 0.05$) reduction in overrun (Tables 3, 6). These results agree with those reported by Khader *et al.* (1992) and Magdoub *et al.* (1992). mF_2 treatment that made by replacing 50% of non-fat dry milk with mFWPC had the highest overrun (66.49%), followed by mSWPC treatment that made by replacing 50% with mSWPC (57.45%), while the overrun of control ice milk was (52.23%). This increase in overrun might be due to the better functional properties of mFWPC and mSWPC compared with non-fat dry milk. Decreasing the overrun by increasing the replacement ratio to 75 and 100% could be attributed to increase in mix viscosity. On the other hand, the overrun of ice milk treatments made with adding ultrafiltrated modified filtered whey protein concentrates (mFWPC) were higher ($P \leq 0.05$) than those of corresponding ice milk treatments made with mSWPC, which might be due to the better functional properties of mFWPC (Table 3).

Specific gravity and weight per gallon of ice milk decreased ($P \leq 0.05$) as the replacing rate of non-fat dry milk with modified whey protein concentrates (mFWPC and mSWPC) increased up to 50% which might be due to the increase of overrun. On the other hand, increasing the replacing rate above 50% up to 100% increased ($P \leq 0.05$) the specific gravity and weight per galleon, which could be attributed to the decrease of overrun. mF_1, mS_1, mF_2, mS_2 ice milk treatments which made with replacing 25 and 50% of non-fat dry milk with mFWPC and mSWPC respectively, showed lower ($P \leq 0.05$) specific gravity and weight per gallon than that of control ice milk. Treatments of mF_3,

mS_3, mF_4, mS_4 that made by replacing 75 and 100% of non-fat dry milk with mFWPC and mSWPC respectively, had higher ($P \leq 0.05$) specific gravity and weight per gallon than that of control ice milk (Tables 3, 6). These results are in agreement with those reported by Khader *et al.* (1992) and Magdoub *et al.* (1992).

Replacement of non-fat dry milk with modified whey protein concentrates (mFWPC and mSWPC) caused an obvious ($P \leq 0.05$) decrease in the rate of melting ice milk after 60 min. and the next 30 min., which means that increasing the melting resistance of the resulting ice milk. The increase of melting resistance increased as the rate of replacing was increased (Tables 3, 6). These results are in agreement with those reported by Magdoub *et al.* (1992) and Hofi (1989) Melting resistance of ice milk treatments made with adding MFWPC after 60 min. and the next 30 min. were higher than those of corresponding ice milk treatments made with MSWCP. The melting resistance of all ice milk treatments after the last 30 min. had contradictory trend of those of the first 60 min. and the next 30 min. (Tables 3, 6).

pH values of ice milk increased significantly ($P \leq 0.05$) by substituting non-fat dry milk with modified whey protein concentrates (Tables 4, 6). The increase of pH was proportional to the rate of replacing non-fat dry milk with modified whey protein concentrates, which means there was positive correlation between pH value of ice milk and the rates of replacement. The increase of pH value by increasing the rates of replacement may be due to the increasing of proteins by adding modified whey protein concentrates compared by adding non-fat dry milk and subsequently increasing the buffering capacity. These results agree with those of Khader *et al.* (1992).

Scores of flavour, body and texture, melting quality and the total scores of organoleptic evaluation of all ice treatments are presented in Tables (5, 6). Control ice milk and treatments made with replacement 25 and 50% of non-fat dry milk with mFWPC and mSWPC had the highest scores of flavour and total scores, while increasing the rate of replacement to 75 and 100% decreased the scores compared with control ice milk. On the other hand, replacing of 25 and 50% of non-fat dry milk with modified whey protein concentrates (mFWPC and mSWPC) improved significantly ($P \leq 0.05$) the boy and texture of the resultant ice milk (Tables 5, 6). But at 75 and 100% replacing ratio, the scores of body and texture decreased. Body and texture of all ice milk treatments made with mFWPC had higher scores than those of corresponding ice milk treatments made with mSWPC.

Storage of all ice milk treatments for 8 weeks did not affected significantly ($P > 0.05$) the scores of organoleptic properties of ice milk (Tables 5, 6).

Finally, it could be concluded that substitution of non-fat dry milk up to 50% with trypsin modified whey protein concentrates (mFWPC and mSWPC) increased the overrun, melting resistance and improved the acceptability of the resultant ice milk, while increasing the replacing rates to 75% and 100% decreased the overrun, melting resistance and scores of organoleptic properties of the resultant ice milk.

Therefore it could be recommended that it is possible to replace of non-fat dry milk used in the manufacture ice milk with modified whey protein concentrates (mFWPC and mSWPC) up to 50% without any determintal effects on ice milk quality.

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جودة المثلوج اللبنى المصنع باستبدال اللبن الفرز المجفف بواسطة بروتينات الشرش المعاملة بإنزيم التربسين

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الملخص العربى

فى هذا البحث تم التعديل الإنزيمى بواسطة التربسين بتركيز ٠,٤% لنوعين من بروتينات الشرش أحدهما تم تحضيره من الشرش المملح بالترسيب الحرارى والآخر من الشرش الحامضى بواسطة الترشيح الفائق ولقد استخدمت هذه البروتينات المجفدة فى تصنيع ٩ معاملات من المثلوج اللبنى حيث تم استبدال اللبن الفرز المجفف المستخدم فى صناعة المثلوج اللبنى بنسب ٠ ، ٢٥ ، ٥٠ ، ٧٥ ، ١٠٠% من كلا نوعين بروتينات الشرش . ولقد أوضحت النتائج المتحصل عليها بعد تحليلها ما يلى :

- أدى استبدال اللبن الفرز المجفف بواسطة بروتينات الشرش المعاملة بإنزيم التربسين لزيادة نسب كلٍ من الرطوبة ، البروتين الكلى ، النيتروجين الغير بروتينى وكذلك اللزوجة والوزن النوعى والوزن بالجالون لمخاليط المثلوج اللبنى ، ولم تختلف المعاملات المتماثلة المصنعة بكلا نوعى بروتينات الشرش وكذلك ازدادت المقاومة للانصهار للمثلوج اللبنى الناتج وهذه الزيادة كانت أكثر خصوصاً بزيادة نسبة الاستبدال.
- ازداد الريع باستبدال اللبن الفرز المجفف بواسطة بروتينات الشرش المعاملة بإنزيم التربسين حتى نسبة ٥٠% بينما انخفض الريع بزيادة نسبة الاستبدال عن ذلك .
- أدى استبدال اللبن الفرز المجفف بواسطة بروتينات الشرش المعاملة بإنزيم التربسين لزيادة قيم الـ pH ولقد انخفض الـ pH فى الأسابيع الأربعة الأولى من التخزين ثم ثبت بعد ذلك حتى نهاية فترة التخزين.
- أدى استبدال اللبن الفرز المجفف بواسطة بروتينات الشرش المعاملة بإنزيم التربسين حتى نسبة ٥٠% لزيادة درجات التحكيم للمثلوج اللبنى بينما أدى زيادة نسبة الاستبدال أعلى من ذلك لخفض درجات التحكيم .

ومن هنا يُمكن القول أنه يُمكن استبدال حتى ٥٠% من اللبن الفرز المجفف بواسطة بروتينات الشرش المعاملة بإنزيم التربسين إنزيمياً دون أن تتأثر خواص المثلوج اللبنى .