EFFECT OF REPLACING NON-FAT DRY MILK WITH ALGAE PROTEIN ISOLATES ON THE QUALITY OF ICE MILK

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ABSTRACT: *Effect of replacing non-fat dry milk with algae protein isolate on the quality of ice milk was studied. Control ice milk containing 4% fat, 13% milk solid not fat, 17% sucrose, 0.7% stabilizer and 3.0% cocoa powder was prepared and 10, 20 and 30% of non-fat dry milk were replaced by algae protein isolate. Replacement of non-fat dry milk with algae protein isolate caused a significant increase in specific gravity, weight per gallon, freezing point, viscosity and flow time of ice milk mixes, while overrun was decreased. On the other hand, replacing of non-fat dry milk with algae protein did not significantly affect the ash or titratable acidity values of ice milk. Ice milk treatment made by replacing 10% of non-fat dry milk with algae protein isolate was not significantly different from control ice milk, while increasing the level of replacement above that caused a significant reduction in organoleptic properties.*

Key words: *Algae protein isolate, non-fat dry milk, ice milk.*

INTRODUCTION

Non-fat dry milk usually used to provide the solids in the manufacture of ice milk. However, the fluctuating prices and availability of non-fat dry milk have encouraged researcher to explore the feasibility of using other ingredient for full or parital replacement of non-fat dry milk.

Marine algae has long been used as food and medicine in Asian countries such as Japan, China and Korea. Most of the brown marine algae intake in the daily diet is derived of Undaria (Wakame) and Laminaria (Kombu) algae species (Yubin and Guangmei, 1998 and Hoppe *et al.,* 1979).

The algal protein contains all essential amino acids (EAA) which are available throughout the year although seasonal variations in their concentrations are known to occur (Galland-Irmouli *et al.,* 1999).

Algal protein has many potential applications in new product fortification and hence offers an exciting alternative protein source for various food products. Functional properties like foaming, viscosity and emulsification of this algal protein have the potential to find use in meat, ice cream bakery food, pharmaceuticals and baby food formulations (Mahajan *et al.,* 2010).

The objective of this study was to evaluate the possibility of replacing non-fat dry milk with algal protein isolate in the manufacture of ice milk and its effect on composition and quality attributes of ice milk.

MATERIALS AND METHODS

Materials:

Fresh skim milk and cream (39% fat) were prepared from buffaloe's milk, which was obtained from the herd of Faculty of Agric., Menoufia University, Shibin El-Kom. Non-fat dry milk (NFDM) produced by Fresno, California, USA. Sucrose and Cocoa were obtained from local market. Stabilizers was obtained from Mifad, Co. Algae protein isolate with the following composition (5.10% Moisture, 64.0% protein, 0.90% Lipids, 9.00% Ash, 1.26% crude fibers and 19.54 carbohydrate) was extracted from *Taonia atomaira* algae according to Chronakis and Sanchez (1998).

Preparation of ice milk:

Cocoa ice milk mix containing 4% fat, 13% MSNF, 17% sugar, 0.7% stabilizer and 3% cocoa was prepared. Milk powder that was used to supply the milk solid not fat in the control mix was replaced by 10, 20 and 30% with algal protein isolate. In each treatment ingredients were mixed together, then sifted slowly to the standardized milk at 45 to 60°C under vigorous agitation to prevent lumping according to the method of Khader *et al.* (1992). All mixes were heated at 85°C for 10 min, then rapidly cooled to 4°C over night. Ice milk mixes were frozen in a batch-type freezer (Cattabriga, Bolongia, Italy). The frozen ice milk was packaged in platic cups, hardened at for 24 hr and stored at -20 $\pm$ 2°C for 10 weeks. All ice milk treatments were analyzed when fresh (0 time) and every five weeks for chemical and every two weeks for sensory evaluation. Each experiment was carried out in triplicate. The gross composition of row materials used in ice milk formulation is shown in Table (1).

Methods of physical and chemical analysis:

Determination of total solids, protein of algal protein isolate (total nitrogen × 6.25) lipid, ash and fiber contents were estimated according to AOAC (2007). Fat content in milk, titratable acidity and total protein were determined according to Ling (1963). Minerals contents were determined according to Shaole *et al.* (1997) using Atomic Absorption (Perken Elemer Emission Spectrophotometer, USA). Free amino acids were determined according to Block *et al.* (1958), using Automatic Amino Acid Analyzer (AAA 400, 1 ngos Ltd.). The prepared mixes were analyzed before freezing to Specific gravity (Winton, 1958). Weight per gallon (Burke, 1947). Freezing point (FAO, 1977), using an electronic thermometer (Wheatson 650, Type-K, Chromel-Alvmel.). Apparent viscosity was determined at 20 $\pm$ 2°C using Brookfield digital rheometer (HADV III ultra, Brookfield Engineering Laboratories INC) using spindle No. 7 set at 10 rpm. Flow time of the mixes was measured as the time in seconds required to discharging a 50 ml pipette at 5°C under atomospheric pressure (Arbuckle, 1986).

The resultant ice milk were tested for specific gravity (Winton, 1958), weight per gallon (Burke, 1947). The overrun and melting resistance were determined according to Sommer (1951) and Reid and Painter (1933), respectively. Titratable acidity and pH values were determined according to Ling (1963).

Organoliptic properties:

Sensory evaluation of the produced ice milk was carried out by 15 panelists at the Dairy Sci. & Tech. Dept, Faculty of Agriculture, Menoufia Univ., for flavor (50 points), body and texture (35 points), melting quality (10 points) and colour (5 points) as mentioned by according to Kebary and Hussein (1997).

Statistical analysis:

Factorial design and completely randomized block design were used to analyze all the data and Newman Keuls test was followed to make the multiple comparisons (Steel and Torri, 1980) using Costat program. Significant differences were calculated at ($p \leq 0.05$).

Table (1). Gross composition of raw ingradients used in ice milk formulation.

Ingredient	T.S%	Fat%	T.P	Ash	Lactose	Crude fiber
Buffalo's milk	16.39	6.60	4.12	0.89	4.78	-
Cream	46.27	39.00	2.79	0.63	3.85	-
Fresh skim milk	9.12	0.10	3.67	0.80	4.55	-
Non-fat dry milk	97.25	1.25	35.00	8.00	53.00	-
Algae protein isolates	93.89	0.09	64.00	9.00	19.54*	1.26

* Calculated carbohydrate.

RESULTS AND DISCUSSION

1. Ice milk mix properties:

Specific gravity and weight per gallon of ice milk mix were significantly increased ($p \leq 0.05$) as the percentage of algal protein isolates increased (Table 2). The results are in agreement with those of Salama and Azam (2003) and Mohamed *et al.* (2012).

Viscosity of ice milk mix significantly increased by replacing NFDM with algal protein isolate (Table 2). This may be due to the high protein and fiber contents of algal protein isolate and high water hydration. These results are in accordance with those given by Salama and Azzam (2003).

The freezing point of ice milk mixes increased significantly by using algal protein isolate (Table 2). These results agree with those of Omar (1983), Who mentioned that the freezing point of ice milk mix was lowered by increasing the percentage of milk solid not-fat which contained lactose and minerals. The freezing point of ice cream is dependent on soluble constituents in mix (Arbuckle, 1986).

The flow time of ice milk mixes significantly increased by replacing of NFDM with algal protein isolate (Table 2). Algal protein isolate contains high amount of protein that may increase the viscosity and flow time under processing condition of ice milk mix. The results are in agreement with those reported by Awad and Metwally (2000).

Acidity values of ice milk mixes showed slight but not significantly increased while pH values decrease significantly (Table 2) by replacing MSNF with algae protein. These changes in acidity and pH values may be a scribed to the increase of protein and ash contents due to the use of algae protein isolate instead of non-fat dry milk as MSNF source. These results are in accordance with those reported by Shenana *et al.* (2007), El-Sonbaty *et al.* (2011) and Mohamed *et al.* (2012).

Resultant ice milk properties:

It is clear from Table (3) that specific gravity and weight per gallon of resultant ice milk increased with the proportional increase of algal protein isolate level. These results might be due to the decrease in overrun of ice milk, which could be attributed to the the higher value of ice milk mix viscosity. The results are in accordance with those reported by Salama and Azzam (2003). Mahran *et al.* (1984) and Farag *et al.* (2010) stated that the specific gravity of ice cream is inversely related to the changes in overrun.

Table (2). Effect of replacing non-fat dry milk with algal protein isolate on ice milk mixes properties.

Properties	Treatments*			
	C	T_1	T_2	T_3
Specific gravity	1.171^c	1.190^b	1.193^b	1.195^a
Weight / gallon (kg)	4.433^c	4.505^b	4.516^b	4.524^a
Viscosity (Cp)	575.55^d	706.66^c	928.88^b	977.17^a
Freezing point (°C)	-2.36^d	-2.30^c	-2.03^{ab}	-2.00^a
Flow time (Sec.)	120^d	135^c	192^b	205^a
Acidity	0.17^b	0.18^{ab}	0.19^{ab}	0.20^a
pH value	6.66^a	6.64^a	6.61^{ab}	6.55^b

C* = Control ice milk made with 4% non-fat dry milk (NFDM) as a source of milk solids not fat (MSNF).
T_1, T_2, T_3 = Ice milk samples made by substituting non-fat dry milk (NFDM) with algae protein isolates at the ratio of 10, 20 and 30%, respectively.
Cp = Centiposie.
a, b, c: Means with different superscript letters in the same row are significantly different ($p \leq 0.05$).

Table (3). Effect of replacing non-fat dry milk with algal protein isolate on some properties of resultant ice milk.

Properties	Treatments*			
	C	T_1	T_2	T_3
Specific gravity	0.730^{d}	0.742^{c}	0.749^{b}	0.757^{a}
Weight / gallon (kg)	2.768^{d}	2.809^{c}	2.836^{b}	2.866^{a}
Overrun %	64.83^{a}	62.56^{ab}	60.72^{bc}	59.63^{c}
Loss % after:				
At 30 °C after 15 min	10.33^{d}	8.88^{c}	7.10^{b}	6.78^{a}
30 min	20.44^{d}	16.66^{c}	14.77^{b}	11.78^{a}
45 min	25.11^{d}	23.33^{c}	20.67^{b}	18.56^{a}
60 min	32.55^{d}	30.11^{c}	28.44^{b}	26.33^{a}

* See Table (2).

The overrun percentage of resultant ice milk decreased by replacement of non-fat dry milk with algal protein isolate up to 30. The decrease in overrun could be attributed to the increase of mix viscosity (Table 2).

The increase in melting resistance of the resultant ice milk was proportional to the amount of algal protein isolate used (Table 3). This increase could be attributed to higher water hydration of algae protein isolate (Table 3). Control ice milk showed lower melting resistance than those of other ice milk treatments made with replacement of non-fat dry milk. The results are in line with Arbuckle (1986), who found that using low lactose products in ice cream making caused some influence on the rate of melting.

The total solids (TS), fat and ash contents of ice milk from all treatment were not significantly different ($p > 0.05$) (Tables 4, 9) which means that replacement of non-fat dry milk with algal protein isolate did not affect significantly ($p > 0.05$) these constituents in the resultant ice milk. These results could attributed to the similar composition of both non-fat dry milk and algal protein isolate. The TS, fat and ash content of all ice milks were not changed significantly ($p > 0.05$) during storage period (Tables 4, 9). These results agree with those reported by Kebary (1996) and Badawi *et al.* (2010).

Total protein of ice milk increased significantly ($p \leq 0.05$) by replacing non-fat dry milk with algal protein isolate (Tables 4, 9), which might be due to the higher protein content of algal protein isolate than the non-fat dry milk. Total protein content of all ice milk samples did not change significantly ($p > 0.05$) during storage period (Tables 4, 9).

Titratable acidity of ice milk samples were not significantly ($p > 0.05$) different from each other which means that replacement of non-fat dry milk with algae protein isolates did not affect significantly ($p > 0.05$) the titratable acidity of the resultant ice milk at any times of storage period (Tables 5, 10). Similar results were reported by Mahran *et al.* (1976). Titratable acidity of all ice milk samples increased slightly significantly ($p > 0.05$) during storage period (Tables 5, 10).

There were no significant ($p > 0.05$) differences in pH values of ice milk samples made from non-fat dry milk with algal protein isolate (Tables 5, 10). pH value of all ice milk sample did not change significantly ($p > 0.05$) as storage period advanced.

Data presented in Table (6) show the effect of substituting 10% of NFDM milk with algal protein isolate on the essential and non-essential amino acids. It is clear from the data that ice milk made with algal protein isolate had higher content of both essential and non-essential amino acids compared to control ice milk made without algae protein isolates. These results are in accordance with those reported by Matz (1991).

Table 4

Table 5

Table (6). Effect of replacing non-fat dry milk with algal protein isolate on amino acids contents of ice milk.

Amino acids*	Control	T_1
Essential amino acids:		
Leucine	0.8115	0.9946
Isoleucine	2.8601	3.0294

Lysine	0.5330	0.5952
Phenylalanine	0.6454	0.7026
Threonine	0.2553	0.3692
Valine	0.5326	0.6366
Total	5.6379	6.3276
Non essential amino acids:		
Alanine	0.4350	0.5809
Aspartic acid	0.6568	0.8880
Glutamic acid	1.3649	1.535
Glycine	0.1009	0.1310
Histidine	0.3620	0.3810
Proline	1.2369	1.4718
Serine	0.3529	0.4708
Tyrosine	0.4130	0.5472
Arginine	0.4922	0.5714
Total	5.4146	6.5771

* g/100 g of ice milk "on dry weight basis".
T_1 = Ice milk made with 10% substitution of non-fat dry milk with algae protein isolate.

Table (7). Crude fibers (%) and minerals concentration (mg / 100 g) of ice milk "on dry weight basis".

Constituents	Treatments	
	Control	T_1
Fibers	–	0.085 ± 0.0021
Mg	408.043 ± 0.00506	414.601 ± 0.2165
Na	1069.554 ± 0.0046	1087.128 ± 0.0575
K	1428.460 ± 0.0202	1511.764 ± 0.3123
Fe	593.927 ± 0.0049	690.050 ± 0.2266
Ca	1370.695 ± 0.5990	1463.580 ± 0.0708
Zn	110.619 ± 0.4542	113.445 ± 0.0459

T_1 = Ice milk made with 10% substitution of non-fat dry milk with algal protein isolate.

Table (8). Effect of replacing non-fat dry milk with algae proteins isolate on the organoleptic properties of ice milk stored for 10 weeks at -20°C ± 2.

Treatments*	Percent of NFDM replacement by API	Flavour (50)	Body and texture (35)	Melting properties (10)	Colour (5)	Total (100)

	Ice milk stored for (0.0 week)					
C	0.0	42	33	8	9	92
T_1	10	41	32	9	9	91
T_2	20	38	31	8	8	85
T_3	30	37	30	8	8	83
	Ice milk stored for (2 weeks)					
C	0.0	42	33	8	9	92
T_1	10	41	32	8	8	89
T_2	20	38	31	8	8	85
T_3	30	37	31	8	8	84
	Ice milk stored for (4 weeks)					
C*	0.0	42	33	8	9	92
T_1	10	41	31	8	8	88
T_2	20	39	30	8	7	84
T_3	30	37	30	8	7	82
	Ice milk stored for (6 weeks)					
C	0.0	43	33	8	8	92
T_1	10	43	31	8	8	90
T_2	20	41	30	7	7	85
T_3	30	38	30	7	7	82
	Ice milk stored for (8 weeks)					
C	0.0	44	32	7	8	91
T_1	10	44	30	7	8	89
T_2	20	42	29	7	7	85
T_3	30	40	29	7	7	83
	Ice milk stored for (10 weeks)					
C	0.0	44	32	7	8	91
T_1	10	44	30	7	7	88
T_2	20	43	29	6	7	85
T_3	30	41	29	6	7	83

* See Table (2).

Table 9

Table 10

The results in Table (7) indicate a remarkable increase in fibers and mineral contents in ice milk made with 10% substitution of NFDM with algal protein isolate.

Organoleptic scores of resultant ice milk as affected by NFDM replacement with different level of algal protein isolates are illustrated in Tables (8, 10). Ice milk made by replacing 10% of non-fat dry milk with algal protein isolate gained higher score and was not significantly different than that of control ice milk. Increasing the ratio of replacement above 10% decreased the flavor score of ice milk. This might be due to the trace of pleasant seafood aroma. Flavor of ice milk treatments improved after 6 week of storage and then after up to the end of storage period (Tables 8, 10). Increasing the algae protein isolates up to 10% does not affect the body and texture of ice milk. These results are in agreement with those observed by Hofi (1989), Farag *et al.* (1993), Shenana *et al.* (2007), El-Sonbaty (2011) and Awad *et al.* (2012).

Ice milk treatments made by replacing NFDM with algal protein isolate exhibited darker color than control. The color intensity increased with increasing algal protein isolate level. These results might be due to the presence of natural pigment protein components, in algal protein isolate. There were no significant differences among ice milk treatment in melting properties (Tables 8, 10).

These results are in agreement with those observed by Hofi (1989), Shenana *et al.* (2007) and Farag *et al.* (2010).

From the obtained results it could be concluded that it is possible to replace of non-fat dry milk that used in making ice milk with algal protein isolate up to 10% without detrimental adverse effect on ice milk quality.

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تأثير استبدال اللبن الفرز المجفف بواسطة بروتين الطحالب المعزول على خواص المثلوج اللبنى

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الملخص العربى

يهدف هذا البحث دراسة إمكانية استبدال اللبن المجفف بواسطة البروتين المستخلص من الطحالب البحرية لذلك فقد تم إعداد 3 معاملات من المثلوج اللبنى بالإضافة إلى معاملة للمقارنة تحتوى على 4% دهن ، 13% جوامد لبنية صلبة ، 17% سكر ، 0.7% مثبت و 3% كاكاو ، أما المعاملات الثلاث الأخرى قد تم تحضيرها بحيث استبدل اللبن المجفف المستخدم كمصدر للجوامد الصلبة اللادهنية ببروتين الطحالب المعزولة بنسب 10 ، 20 ، 30% على الترتيب ولقد أوضحت النتائج المتحصل عليها بعد تحليلها إحصائياً ما يلى :

- أدى استبدال اللبن المجفف بواسطة بروتين الطحالب المعزول إلى زيادة كل من الوزن النوعي والوزن

بالجالون ونقطة التجمد والزوجة وزمن السريان لمخاليط المثلوج اللبنى وكذلك الوزن النوعي والوزن بالجالون والمقاومة للانصهار للمثلوج اللبنى فى حين انخفضت نسبة الريع .

- لم يُؤثر استبدال اللبن الفرز المجفف بواسطة بروتين الطحالب المعزول على نسب كل من الجوامد الصلبة الكلية والرماد والدهن ونسبة الحموضة فى حين ازدادت نسبة البروتين الكلى .
- أظهرت نتائج التحكيم الحسى أن العينة المصنعة باستبدال 10% من اللبن المجفف بواسطة بروتين الطحالب المعزول لم تختلف إحصائياً عن عينة المقارنة فى حين أن زيادة نسبة الاستبدال أعلى من 10% أدت إلى خفض درجات التحكيم .
- لم تتغير نسب الجوامد الصلبة الكلية والبروتين الكلى والدهن والرماد بتقدم فترات التخزين فى حين زادت نسب الحموضة قليلاً .
- لوحظ زيادة محتوى المثلوجات اللبنية المحتوية على 10% من بروتين الطحالب المعزول من الأحماض الأمينية الأساسية والغير أساسية وكذلك الألياف وبعض الأملاح المعدنية وهى المغنسيوم – الصوديوم – البوتاسيوم – الحديد – الكالسيوم والزنك مما سبق يتضح أنه يُمكن إنتاج مثلوج لبنى ذو خواص وظيفية وحسية جيدة ومرتفع القيمة الغذائية والصحية باستخدام بروتين الطحالب المعزول بنسبة 10% استبدال بدلاً من اللبن الفرز المجفف بدون تأثير غير مرغوب على النكهة أو خواص التركيب والقوام .

Table (4). Effect of replacing non-fat dry milk with algal protein isolate on total solids, total protein, fat and ash contents (%) of ice cream.

Ice milk treatment*	Total solids content (%)			Total protein content (%)			Fat content (%)			Ash content (%)		
	Storage period (weeks)			Storage period (weeks)			Storage period (weeks)			Storage period (weeks)		
	Fresh	5 weeks	10 weeks	Fresh	5 weeks	10 weeks	Fresh	5 weeks	10 weeks	Fresh	5 weeks	10 weeks
C	37.62	37.60	37.63	6.22	6.23	6.22	4.0	4.1	4.0	1.210	1.209	1.211
T_1	37.68	37.72	37.74	6.34	6.32	6.34	4.1	4.1	4.1	1.214	1.212	1.213
T_2	37.74	37.75	37.76	6.46	6.47	6.45	4.2	4.2	4.1	1.217	1.215	1.217
T_3	37.83	37.85	37.86	6.57	6.56	6.58	4.3	4.3	4.2	1.219	1.218	1.217

* See Table (2).

Table (5). Effect of replacing non-fat dry milk with algal protein isolate on titratable acidity (%) and pH value of ice milk.

Ice milk treatments*	Titratable acidity (%)						pH value					
	Storage period (weeks)						Storage period (weeks)					
	Fresh	2	4	6	8	10	Fresh	2	4	6	8	10
C	0.180	0.180	0.182	0.182	0.184	0.186	6.65	6.63	6.59	6.59	6.56	6.52
T_1	0.190	0.191	0.191	0.192	0.193	0.194	6.64	6.61	6.56	6.56	6.54	6.52
T_2	0.200	0.201	0.203	0.203	0.204	0.205	6.61	6.58	6.54	6.52	6.52	6.50
T_3	0.210	0.210	0.212	0.214	0.215	0.215	6.60	6.58	6.54	6.54	6.51	6.50

* See Table (2).

Table (9). Statistical analysis of chemical composition of ice milk.

Ice milk properties	Effect of treatments					Effect of storage period (weeks)			
	Multiple comparisons●					Multiple comparisons●			
	Mean squares	C♦	T_1	T_2	T_3	Mean squares	0	5	10
Total solids (%)	11.348	A	A	A	A	0.8113	A	A	A
Protein (%)	0.1431*	D	C	B	A	0.8218	A	A	A
Fat (%)	0.0909	A	A	A	A	0.0209	A	A	A
Ash (%)	0.0055	A	A	A	A	0.0237	A	A	A

♦ See Table (2).

● For each effect the different letters in the same row means the multiple comparisons are different from each other, letter A is the highest mean followed by B, C, … etc.

* Significant at 0.05 level ($p \leq 0.05$).

Table (10). Statistical analysis of ice milk properties.

Ice milk properties	Effect of treatments					Effect of storage period (weeks)						
	Multiple comparisons●					Multiple comparisons●						
	Mean squares	C♦	T_1	T_2	T_3	Mean squares	0	2	4	6	8	10
Titratable acidity (%)	0.0216	A	A	A	A	0.063*	C	BC	BC	B	B	A
pH value	0.5382	A	A	A	A	0.1024	A	A	A	A	A	A
Organoleptic properties												
Flavour	77.50*	A	AB	C	D	29.60*	D	D	D	C	B	A
Body & texture	30.458*	A	AB	C	C	6.525*	A	A	AB	AB	AB	B
Melting properties	49.667	A	A	A	A	48.570	A	A	A	A	A	A
Appearance	5.792*	A	B	C	C	2.825*	A	A	B	B	B	B
Total score	290.792*	A	AB	C	D	2.625	A	A	A	A	A	A

♦ See Table (2).
● For each effect the different letters in the same row means the multiple comparisons are different from each other, letter A is the highest mean followed by B, C, … etc.
* Significant at 0.05 level ($p \leq 0.05$).