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Impact of Replacement of Gelatin with Chitosan on the Physicochemical Properties of Ice-Milk

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ABSTRACT

This study has aimed to investigate effect of replacement gelatin with chitosan at rate 0, 20, 40, 60, 80 and 100%. Significantly ($p \leq 0.05$), the results show that total solids, fat, total protein, pH-values and specific gravity of mixes had not significant differences. Freezing point and ash content significantly increased with increasing of the replacement rate. On the contrary, replacement of gelatin with chitosan significantly decreased weight per gallon and melting resistance up to replacing ratio 60% then has increased. The overrun and viscosity of the ice-milk increased with the increasing of the replacement rate up to 60% then decreased. organoleptically, Ice-milk containing chitosan at rate 0, 20, 40 and 60% were rated acceptable by panelists and gained higher scores. It is recommended that chitosan can be used to replace up to 60% of gelatin to give ice milk of high quality and better nutritive value.

Key words: Ice-milk, chitosan, replacement of gelatin

INTRODUCTION

Ice cream is a frozen dairy product that is widely consumed around the world. Ice cream is formed of a mixture of air, water, milk fat, Milk Solids Non-Fat (MSNF), sweeteners, stabilizer, emulsifiers and flavors. Mix formulation are defined as percentage of the constituent, e.g., percentage of milk fat, MSNF, sugar egg yolk solid, stabilizer/emulsifier and total solids. They can be combined in varying proportions within acceptable ranges. Furthermore, a wide variety of ingredients can be chosen to supply these constituents and both the percentage and the source of constituent can affect the quality of mix (Arbuckle, 1986).

About 45% of processed seafood consists of shrimp, the waste of which is composed of exoskeleton and cephalothoraxes (Ibrahim *et al*., 1999; Venugopal *et al*., 1995). The latter has become a problem for the environment. This waste represents 50-70% of the weight of the raw material; however it contains valuable components such as protein and chitin (Roberts, 1992). A yearly production of approximately 1010-1012 t (Roberts, 1992).

Chitosan a linear polysaccharide consisting of (1→4)-linked 2-amino-deoxy-b-d-glucan, is a deacetylated derivative of chitin which is the second most abundant polysaccharide found in nature after cellulose. Chitosan has been found to be non-toxic, biodegradable, biofunctional, biocompatible and was reported by several researchers to have strong antimicrobial and antifungal activities (Kurita, 2001). Chitosan films have been successfully used as a packaging material for the quality preservation of a variety of foods.

Biopolymers such as proteins and polysaccharides are commonly used as natural hydrocolloids in the food industry. Proteins are employed primarily as emulsion forming

and stabilizing agents due to their high surface activity, while polysaccharides are mainly used as thickening or gelling agents (Neirynck *et al*., 2007).

Chitosan is a natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp and crayfish. During the past several decades, chitosan has been received increased attention for its commercial applications in biomedical, food and chemical industries (Sandford and Hutchings, 1987). Chitosan is now widely produced commercially from crab and shrimp shells wastes.

Chitosan has a water-holding property that is exploited in many fields. Water uptake of chitin, microcrystalline chitin and chitosan range from 325-440% (w/w) and the difference is possibly due to differences in the amount of salt-forming groups and differences in the protein contents of the materials (Jiang *et al*., 2010).

Water Binding (WBC) and Fat Binding Capacities (FBC) of commercial chitosan are lower than the extracted chitosan. Water and fat binding capacities of different commercial chitosan were reported as 458-805% and 314-535%, respectively, by Cho *et al*. (1998). WBC and FBC of six commercial chitosan products observed by No *et al*. (2000) were in the range of 355-611% and 217-477%, respectively.

The application of chitosan in dairy products such as kareish cheese improves the mycological quality and extends the shelf-life which could an alternative to chemical protective additives (El-Diasty *et al*., 2012).

They display a wide range of applications in different fields, e.g., in cosmetics, agriculture, food, pharmacy, biomedical, industry and also as absorbent materials for wastewater treatment (Bautista-Banos *et al*., 2006; Rashidova *et al*., 2004; Sashiwa and Aiba, 2004). Chitin and chitosan are easily processed into gels (Nagahama *et al*., 2008).

This study has aimed to utilize of shrimp shell chitosan as stabilizer during manufacturing of ice milk and study the effect of chitosan on the physicochemical properties of ice-milk.

MATERIALS AND METHODS

Materials: Fresh buffalo's milk, was obtained from Food Technology Research Institute, Agriculture Research Center, Giza, Egypt having 14.5% total solids, 4.0% fat, 4.5% lactose, 4.0% protein and 0.95% ash. Low heat skim milk powder (imported from USA) was used. Food grade gelatin was obtained from Arabic laboratory Equipment Company, Egypt. Commercial grade crystalline sucrose was obtained from the local market.

Shell of green shrimp Caridinababaulti was purchased from AbouGhalli Company for trading and exporting, Al-Obour market, Egypt. The shell were manually scraped (free of loose tissue), collected and brought to the laboratory in the same day. Whenever, the shell was brought to the laboratory it was freezed immediately (at -18°C) and stored for further analysis.

Chemicals and reagents: Hydrochloric acid, glacial acetic acid, sodium hydroxide, phenolphthalein, methyl orange, were purchased from El-Nasr Pharmaceutical Chemicals, El-Ameriea, Cairo, Egypt. Aniline blue was purchased from ROTH Bestellensiezum (Nulltarif, Germany), sulphoric acid was obtained from Merck, Darmstadt, Germany.

Preparation of shrimp shells: The shells are first cleaned several times with tap water and rinsed several time with distilled water. The rinsed shells were dehydrated in an electric draft oven at 45°C till drying. The dried shells were grounded in a grinder (Braun Biotech International GMBH. D.34212 Melsungen, Germany) to pass through a 1.6 mm sieves then demineralized using

1 M HCl (1/15 w/v) at 45°C for 2 h then deproteinized using 2 M NaOH at 75°C for 4 h (1/15 w/v) and finally deacetylated by steeping in 40% NaOH at 90°C for 2 h (1/15 w/v). The resulting chitosan was collected, washed as mentioned above and dried at 60°C for 4 h in a forced-air oven.

Manufacture of ice-milk: Ice-milk mixes were prepared according to Khader *et al.* (1992). Vanilla ice-milk base mix was planned to containing 6% fat, 12% MSNF, 14% sugar and the total solids reached 37% by using cream, sugar and skim milk powder, respectively. The 0.5% stabilizer "gelatin" was used to control treatment (c) and 5th treatments replacing gelatin with chitosan at 20, 40, 60, 80 and 100%. The mixes were heated at 85°C for 10 min then rapidly cooled to 5°C and aged at 5°C overnight. Vanilla was added prior to freezing and ice-milk mix was frozen in a batch type freezer (Taylor Model, 103, Italy). The product was packaged in 120 mL cups and hardened in cabined at -18°C.

Method of analysis: Moisture, fat, fiber, protein, total ash and total solids were determined according to AOAC (2005). Total carbohydrate content was calculated by difference. The pH was measured using pH meter HANNA 213 Italy. The specific gravity, weight per gallon, overrun and melting resistance of ice-milk were determined according to Arbuckle (1986), freezing point (FAO., 1977). The viscosity was determined using a viscometer L model D V-111, Rhea test, German.

Solubility, Water Binding Capacity (WBC) and Fat Binding Capacity (FBC) of chitosan solubility: Chitosan (0.1 g) was placed into a centrifuge tube (known weight) then dissolved with 10 mL of 1% acetic acid for 30 min using an incubator shaker operating at 240 rpm and 25°C. The solution was then immersed in a boiling water bath for 10 min, cooled to room temperature (25°C) and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted. The residue particles were washed with distilled water (25 mL) then centrifuged at 10,000 rpm. The supernatant was removed and the residue dried at 60°C for 24 h. Finally, weighed the dried residue and the percentage of solubility was calculated as followed:

$$\text{Solubility of chitosan } (\%) = \frac{\text{(Initial weight of tube+chitosan)-(Final weight of tube+chitosan)}}{\text{(Initial weight of tube+chitosan)-(Initial weight of tube)}} \times 100$$

Water and fat binding capacity: Water Binding Capacity (WBC) and Fat Binding Capacity (FBC) of chitosan were measured using the method of No *et al.* (2000). Briefly, the procedure was carried out by weighing a centrifuge tube containing 0.5 g sample, adding 10 mL of water or corn oil and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 sec, every 10 min and centrifuged at 3200 rpm for 25 min. The supernatant was decanted and the tube was weighed again. WBC and FBC were calculated using the following equation:

$$\text{WBC } (\%) = \frac{\text{Water bound (g)}}{\text{Sample weight (g)}} \times 100$$

$$\text{FBC } (\%) = \frac{\text{Fat bound (g)}}{\text{Sample weight (g)}} \times 100$$

Statistical analysis: Statistical analysis was done using analysis of variance (ANOVA) and Least Significant Difference (LSD) were obtained to compare the means of treatments, using Costat version 6.311 (Copyright 1998-2005, CoHort Software).

Sensory evaluation: The organoleptic properties of resultant ice+milk were assessed by 20 panelists from the staff members of Dairy Tech. Dept., Food Tech. Research Institute, Agriculture Research Center, Giza, Egypt, according to score sheet described by Salem (2001).

RESULTS AND DISCUSSION

Figure 1 illustrates the chemical composition of the produced shrimp shell chitosan which contained 8.91% moisture, 67.18% carbohydrates and 28.60 total fibers. Generally the chemical composition and the physical characteristic of chitosan depend on the method of preparation and deacetylation. Muzzarelli *et al.* (1994), Li *et al.* (1992) and Baxter *et al.* (1992) reported that among many characteristics, the degree of deacetylation is one of the most important chemical characteristics which influences the performance of chitosan in many of its applications. In addition, degree of deacetylation which reveals the content of free amino groups in the polysaccharide (Li *et al.*, 1992).

Fat binding capacity signifies how the chitosan can easily bind or absorb fat especially when used in the manufacture of dietary supplements. The trend recorded for water binding capacity was similarly observed for fat binding capacity. The water and fat binding capacity of chitosan were 532.12 and 422.15%, respectively with a solubility of 52.66% (Fig. 2). WBC and FBC of six commercial chitosan products observed by No *et al.* (2000) were in the range of 355-611% and 217-477%, respectively. The WBC (532.12%) and FBC (422.15%) of the prepared chitosan in the present study were compatible to those reported by Cho *et al.* (1998) and No *et al.* (2000).

Chemical composition and properties of ice-milk mixes: The chemical composition and some properties of ice-milk mixes made by replacement of gelatin with chitosan are presented in Table 1. The addition of chitosan had no effect on total solids, total protein, fat content, specific gravity and pH values of ice-milk mixes. Statistical analysis of these data indicated that the replacement gelatin with chitosan caused significant effect ($p \leq 0.05$) in weight per gallon of ice-milk

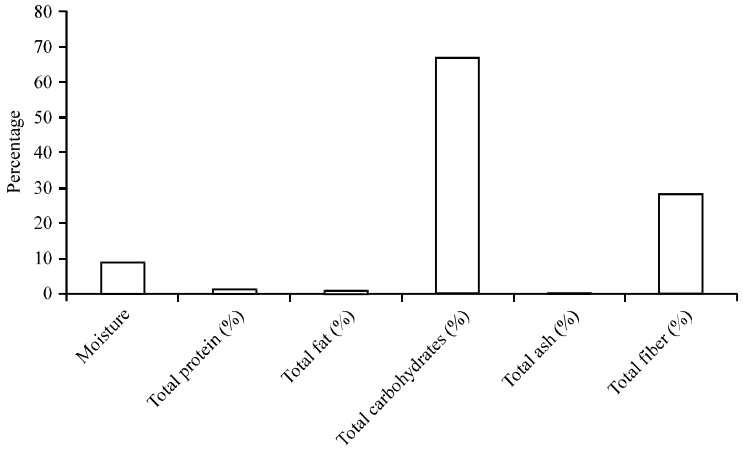

Fig. 1: Proximate composition (on dry weight) of shrimp shell chitosan

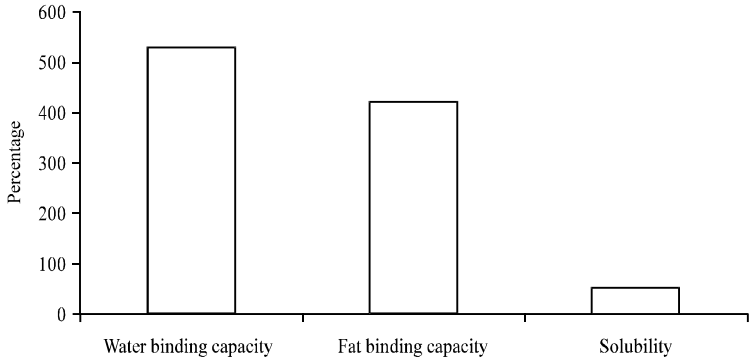

Fig. 2: Functional properties of shrimp shell chitosan

Table 1: Effect of replacing of gelatin with chitosan on physicochemical properties of ice-milk mixes

	Treatments					
Properties	C*	T1	T2	T3	T4	T5
Total solids (%)	37.82^{a}	37.84^{a}	37.80^{a}	37.78^{a}	37.83^{a}	37.84^{a}
Fat (%)	6.00^{a}	6.10^{a}	6.10^{a}	6.00^{a}	6.00^{a}	6.10^{a}
Total protein (%)	4.75^{a}	4.75^{a}	4.74^{a}	4.74^{a}	4.73^{a}	4.72^{a}
Ash (%)	1.07^{b}	1.09^{ab}	1.10^{ab}	1.11^{a}	1.11^{a}	1.12^{a}
ph value	6.70^{a}	6.71^{a}	6.71^{a}	6.72^{a}	6.72^{a}	6.73^{a}
Specific gravity (g cm^{-3})	1.073^{a}	1.073^{a}	1.074^{a}	1.075^{a}	1.075^{a}	1.076^{a}
Wight/gallon (kg)	5.833^{ab}	5.821^{b}	5.901^{ab}	5.848^{ab}	5.915^{a}	5.985^{a}
Viscosity (cp)	2.48^{ab}	2.50^{ab}	2.55^{a}	2.45^{b}	2.38^{c}	2.30^{d}
Freezing point (°C)	-2.16^{a}	-2.20^{ab}	-2.22^{ab}	-2.25^{b}	-2.26^{bc}	-2.30^{c}

C*: Control ice-milk made with 0.5% gelatin a source of stabilizer, T1, T2, T3, T4 and T5: Ice milk batches made by substitution gelatin with chitosan at the ratio of 20, 40, 60, 80 and 100%, respectively. Different letters in the same row (a, b, c, ...) means that multi comparison are different from each other, letter a is highest mean followed by b, c, ... etc., Significant at 0.05 level, Means with the same letter are not significantly different

mixes. Ash percentage increased as percentage of replacement gelatin with chitosan increased. This may be due to the effect of higher fiber content in chitosan. With regard to viscosity, it could be observed from Table 1 that the replacement gelatin with chitosan had significant effect ($p \leq 0.05$) in viscosity. It is known that the total solids content, amount of protein, fat, stabilizer, solute concentration and molecular weight change the magnitude of the viscosity ice-milk mix (Arbuckle, 1986; Marshall *et al*., 2003; Abd El-Ghany, 2008). The viscosity of ice-milk mix had increased significant effect ($p \leq 0.05$) with increase replacement gelatin with chitosan up to 60% then decreased (Table 1). The freezing point of ice-milk mixes was reduced below that of water to -2°C. Statistical analysis of freezing point depression of ice-milk mixes showed significant difference among mixes which can be attributed to the variable molecular weight of carbohydrate used (Marshall *et al*., 2003). These results are in agreement with that reported by Hammad *et al*. (1991), Abdou *et al*. (1996) and Mahran *et al*. (2001). Treatment No. 5 with increasing gelatin substitution level (100%) with chitosan had the lowest freezing point depression of -2.3°C.

Properties of the resultant ice-milk: It could be noticed from Table 2, that there were specific gravity and weight per gallon of the resultant ice-milk were closely related. These results indicated that the mean values of specific gravity of ice-milk were 0.694, 0.689, 0.665, 0.659, 0.701 and 0.720

Table 2: Effect of replacing of gelatin with chitosan on the resultant ice-milk properties

	Treatments					
Properties	C*	T1	T2	T3	T4	T5
Specific gravity (g cm^{-3})	0.694^{ab}	0.689^{ab}	0.665^{b}	0.659^{b}	0.701^{ab}	0.720^{a}
Wight/gallon (kg)	2.793^{b}	2.689^{bc}	2.502^{c}	2.481^{c}	2.770^{b}	2.920^{a}
Overrun (%)	48.000^{ab}	49.660^{ab}	51.880^{a}	52.550^{a}	48.560^{ab}	47.160^{b}
Melting resistance loss% after (min)						
30	6.5^{d}	5.5^{d}	5.0^{e}	7.0^{c}	8.0^{b}	9.5^{a}
60	14.0^{c}	12.5^{d}	10.6^{e}	15.5^{bc}	16.2^{b}	17.0^{a}
90	33.7^{c}	32.8^{cd}	27.5^{e}	28.8^{d}	45.0^{b}	50.2^{a}

C*: Control ice-milk made with 0.5% gelatin a source of stabilizer, T1, T2, T3, T4 and T5: Ice milk batches made by substitution gelatin with chitosan at the ratio of 20, 40, 60, 80 and 100%, respectively. Different letters in the same row (a, b, c, ...) means that multi comparison are different from each other, letter a is highest mean followed by b, c, ... etc., Significant at 0.05 level, Means with the same letter are not significantly different

Table 3: Effect of replacing of gelatin with chitosan on the organoleptic properties of ice-milk

	Treatments					
Properties	C*	T1	T2	T3	T4	T5
Flavor (50)	47^{a}	48^{a}	48^{a}	48^{a}	44^{b}	41^{b}
Body and texture (30)	27^{a}	26^{a}	27^{a}	27^{a}	24^{b}	23^{b}
Melting properties (10)	10^{a}	10^{a}	10^{a}	10^{a}	9^{b}	7^{c}
Color (10)	10^{a}	10^{a}	10^{a}	10^{a}	8^{b}	7^{c}
Total (100)	94^{a}	94^{a}	95^{a}	95^{a}	85^{b}	78^{c}

C*: Control ice-milk made with 0.5% gelatin a source of stabilizer, T1, T2, T3, T4 and T5: Ice milk batches made by substitution gelatin with chitosan at the ratio of 20, 40, 60, 80 and 100%, respectively. Different letters in the same row (a, b, c, ...) means that multi comparison are different from each other, letter a is highest mean followed by b, c, ... etc., Significant at 0.05 level, Means with the same letter are not significantly different

for treatments C, T1, T2, T3, T4 and T5, respectively. The corresponding values of weight per gallon were 2.793, 2.689, 2.502, 2.481, 2.770 and 2.920. Specific gravity and weight per gallon of ice-milk decreased with proportional increase of chitosan level in the mix up to 60%, this might be due to the increase of overrun. This high overrun could be due to the increase of viscosity of treatments C, T1, T2 and T3 which were made by substituting 0, 0.20, 0.40 and 0.60% of gelatin with chitosan. These results are in accordance with Salama and Azzam (2003). The effect of replacing gelatin with chitosan on the overrun of the resultant ice-milk was presented in Table 2. Replacement of gelatin with chitosan up to 60% caused an increase in the overrun of ice-milk (Table 2). Overrun values of ice-milk were 48.00, 49.66, 51.88 and 52.55 for treatments C, T1, T2 and T3, respectively. Increasing the replacement rate above 60% caused a reduction in overrun (Table 2). Melting resistance of the resultant ice-milk is determined as the loss weight percent of the initial weight. From the data present in Table 2, it could be seen that the melted portion after 30 min were 6.5, 5.5, 5.0, 7.0, 8.0 and 9.5% for treatments C, T1, T2, T3, T4 and T5, respectively. The corresponding portions were 14.0, 12.5, 10.5, 15.5, 16.2 and 17.0% after 60 min and 33.7, 32.8, 27.5, 38.0, 45.0 and 50.2% after 90 min. Also, the melting resistance of the resultant ice-milk was significantly affected by replacement gelatin with chitosan. Table 3 shows that there was positive correlation between the substitution of gelatin by chitosan and the total scores for organoleptic properties of ice-milk. Ice-milk containing the chitosan at the ratio of 0, 20, 40 and 60% for

treatments C, T1, T2 and T3, respectively, were rated acceptable by panelists and got the high score for organoleptic attributes. The chitosan imported an acceptable creamy color, sweet flavor and smooth body and texture.

The foregoing results indicated that possibility of using chitosan in ice-milk making in partial replacement gelatin up to 60% without remarkable effects on the physical and organoleptic properties of the product.

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