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THE EFFECT OF ENZYME TRANSGLUTAMINASE ON SOME PHYSICO-CHEMICAL PROPERTIES OF PREBIOTIC LOW-FAT TRADITIONAL ICE CREAM

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ABSTRACT

Ice cream is one of the highest consumption desserts in Iran and the world; so, the type and quantity of its constituents could be important in terms of health care for many consumers. In recent years much attention has been made in reducing the amount of fat and producing diet products such as low-fat ice cream. However, the type and amount of fat replacer used in the composition of ice cream have an important effect on the texture and sensory acceptance of the final product. In this research, the effect of fat replacement via microbial transglutaminase (0, 0.3, 0.4, 0.5 and 0.6g/L) and inulin (0, 2 and 4%) addition on physicochemical characteristics of traditional prebiotic low-fat ice cream was investigated. Meanwhile, the sample containing 0g/L enzyme and 0% inulin was considered as control treatment. The results showed that increasing the amount of transglutaminase enzyme and inulin led to an increase in the melting resistance and viscosity of ice cream samples. Also, the increasing inulin to 4% led to be provided the increasing overrun in significance level ($p<0.05$). No difference was observed in pH of the samples. Samples containing transglutaminase enzyme in comparison with control sample had higher protein while there was no change in the protein content with the addition of inulin in levels 2 and 4%.

Keywords: Low-fat traditional ice cream, Fat replacer, Transglutaminase enzyme, Inulin.

INTRODUCTION

Ice cream is a complex-colloidal system that is contained ice crystals, air bubbles, fat globules, sugars, proteins, salts, polysaccharides and water (Goff, 2002; Marshell *et al.*, 2003). The decreasing fat content affect viscosity, ice crystallization, hardness, melting rate and flavor of ice cream (El-Nagar *et al.*, 2002). In recent years, consumers tend to use low-fat food products because the use of these products associated with a reduced risk of cardiovascular disease. The motivation for incorporating inulin into dairy products included replacement of milk fat in reduced-fat dairy products. Transglutaminase enzyme and inulin are considered as fat substitutes. The microbial transglutaminase (TG; EC 2.3.2.13) has the capacity to modify protein properties through acyl transfer reactions between the g-carboxyamide group of peptides bound to glutamine residues and a variety of primary amines, including the e-amino group of lysine residues in certain proteins. Hence, it can improve solubility, heat stability, and the gelation of foods, as well as, emulsifying and rheological characteristics (Hinz *et al*., 2007). This specification leads to be replaced part of the fat in the food by microbial transglutaminase enzyme. Inulin is a carbohydrate built up from β (2-1)-linked fructosyl residues mostly ending with a glucose residue and it is present as storage carbohydrate in a large number of plants (van Loo *et al*., 1995). It has a degree of polymerization (DP) of 2 to 60. The fat substituting property of inulin is based on its ability to stabilize the structure of the aqueous phase, which creates an improved creaminess mouth feel (EL-Nagar *et al.*,2002).

Inulin and transglutaminase enzyme have been developed and used in the ice cream industry with success (Rossa *et al.*, 2012; Akalm *et al.*, 2008; Metwally, 2007; El-Nagar *et al.*, 2002); however, until now, there is no report in relation to the simultaneously use of inulin and transglutaminase enzyme in low-fat traditional ice cream. So, The aim of the present research was to use different concentrations of inulin and transglutaminase enzyme in the production of reduced-fat traditional ice cream; then, determine the influence of various concentration of inulin and transglutaminase enzyme on selected physicochemical and sensory characteristics of final products; and compare the properties of experimental ice cream samples to those of control.

MATERIALS AND METHODS

Additives

The following additives are involved in the production of prebiotic low-fat traditional ice-cream samples:

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- Cow's skim milk [fat, 0/8% (w/w); SNF, 8% (w/w); pH 6.8] (Sara Dairy, Iran).
- Transglutaminase enzyme (Ajinomoto, France).
- Inulin Frutafit (Powdery, SENSUS, China).
- Stabilizer (Powdery, JELITA, Iran).
- Sucrose (Karaj suger factory, Iran).
- Cream (Behtak Dairy, Iran).
- Flavors (Vanilla, Polar Bear, China).
- Color (Saffron, Iran).

Prebiotic low-fat traditional ice-cream samples preparation

The following steps were involved in the production of prebiotic low-fat traditional ice-cream.

- Standardization of the milk fat, to 3.5 and 12% (according to Table 1).
- Heat treatment at 80°C for 15 minutes.
- Cooling to 55°C.
- Addition of TGase (according to Table 1).
- Incubation at 55°C for 1.30 hours.
- Preparation of ice cream mixes (flavors, 1%; colors, 0.5%; stabilizer, 1%; and sucrose,18%).
- Addition of inulin (according to Table 1).
- Pasteurization at 68°C for 30 minutes.
- Homogenization (two stages, 3.4/13.8 MPa).
- Aging at 4°C for 24 hours.
- Hardening at -18°C for 8 hours.

Physicochemical analyses

pH

The pH of the samples was measured at room temperature using a pH meter (Metrohm, Germany).

Viscosity

Viscosity of ice cream was measured at 4.0 ± 0.1°C using a Brookfield DV-II+Pro viscometer (Brookfield Engineering Laboratories, USA). The Viscometer was operated at 25 rpm with spindle number LV3 after 20s (Rossa *et al*., 2012).

Protein

Protein was measured by Kjeldahl method reported by AOAC (2005).

Overrun

The overrun was evaluated as (Wt. of mix _ Wt. of same vol. of ice cream)/Wt. of same vol. of ice cream) _ 100% (Rossa *et al*., 2012).

Melting rate

Melting resistance was determined by placing 100 g of ice-cream on a 10-mesh wire screen fitted in a funnel that drained into a graduated cylinder. The sample was allowed to melt in a controlled temperature room at 30 ± 0.5 °C. Weight of drainage was determined at 10min intervals and the percentage of melted ice cream was then calculated as a function of time (Metwally, 2007).

Statistical analysis

Each experiment was independently replicated three times in a completely randomized design. Analysis of data extracted from experimental design was a randomized complete block. The statistical significance of the data was determined using Duncan test. *P*-value <0.05 was considered sufficient to reject the null hypothesis. Statistical analysis was performed by running the SAS 9.1 software.

RESULTS AND DISCUSSION

Physicochemical properties of prebiotic low-fat traditional ice Cream samples

pH

The mean values of pH of ice cream samples produced are shown in Fig 1. There was no significant difference in pH value between samples ($p > 0.05$). The addition of inulin slightly decreased the pH of the ice cream samples. This decrease can be explained by the low pH of inulin used in this study. However, this decrease of pH is not

statistically significant. This result disagrees with that reported by Hager *et al.* (2011), who demonstrated that inulin increased the pH of reduced fat cake.

Table 1. Treatments used in the study

Treatment	Fat(%)	Transglutaminase enzyme (g/l)	Inulin(%)
TG0-I0 (Control)	**12**	**0**	**0**
TG1-I0	**3.5**	**0.3**	**0**
TG1-I1	**3.5**	**0.3**	**2**
TG1-I2	**3.5**	**0.3**	**4**
TG2-I0	**3.5**	**0.4**	**0**
TG2-I1	**3.5**	**0.4**	**2**
TG2-I2	**3.5**	**0.4**	**4**
TG3-I0	**3.5**	**0.5**	**0**
TG3-I1	**3.5**	**0.5**	**2**
TG3-I2	**3.5**	**0.5**	**4**
TG4-I0	**3.5**	**0.6**	**0**
TG4-I1	**3.5**	**0.6**	**2**
TG4-I2	**3.5**	**0.6**	**4**

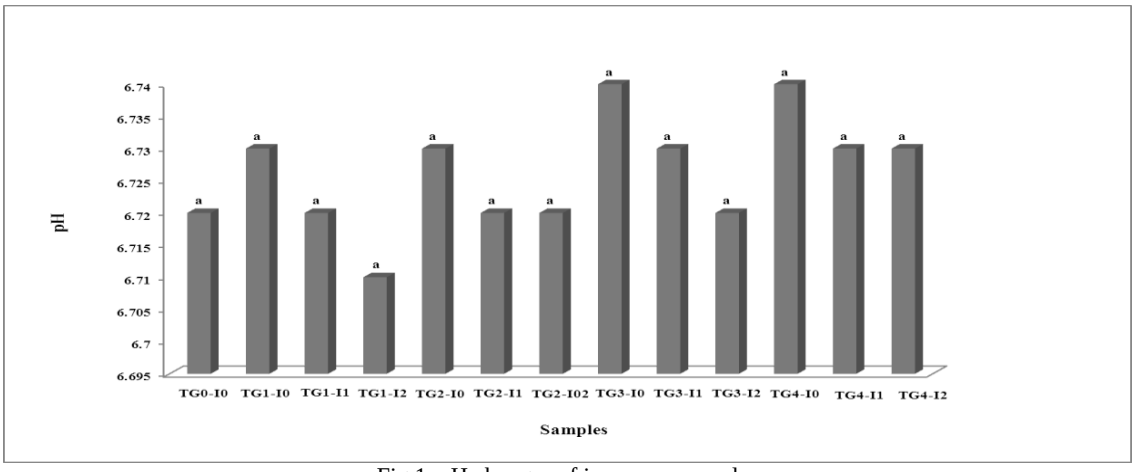

Fig 1. pH changes of ice cream samples
I: Inulin (%); TG: Transglutaminase enzyme (g/L)
TG0-I0: I=0;TG=0. TG1-I0: I=0;TG=0.3. TG1-I1: I=2;TG=0.3.
TG1-I2: I=4;TG=0.3. TG2-I0: I=0;TG=0.4.TG2-I1: I=2;TG=0.4.
TG2-I2: I=4;TG=0.4. TG3-I0: I=0;TG=0.5.TG3-I1: I=2;TG=0.5.
TG3-I2: I=4;TG=0.5. TG4-I0: I=0;TG=0.6. TG4-I1: I=2;TG=0.6.TG4-I2: I=4;TG=0.6.

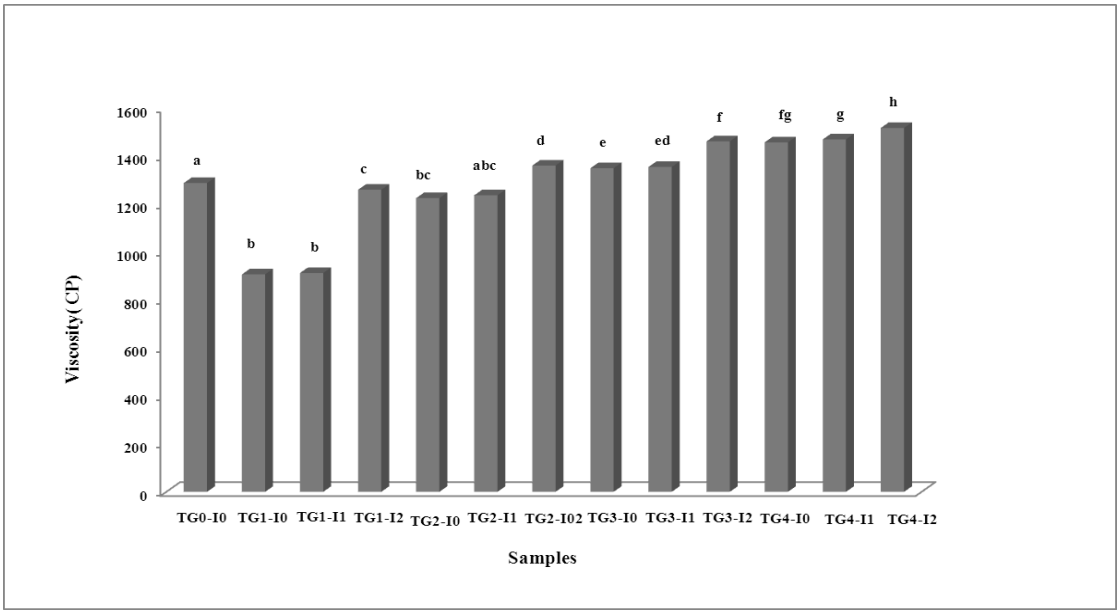

Fig 2. Viscosity changes of ice cream samples
I: Inulin (%); TG: Transglutaminase enzyme (g/L)
TG0-I0: I=0;TG=0. TG1-I0: I=0;TG=0.3. TG1-I1: I=2;TG=0.3.
TG1-I2: I=4;TG=0.3. TG2-I0: I=0;TG=0.4.TG2-I1: I=2;TG=0.4.
TG2-I2: I=4;TG=0.4. TG3-I0: I=0;TG=0.5.TG3-I1: I=2;TG=0.5.
TG3-I2: I=4;TG=0.5. TG4-I0: I=0;TG=0.6. TG4-I1: I=2;TG=0.6.TG4-I2: I=4;TG=0.6.

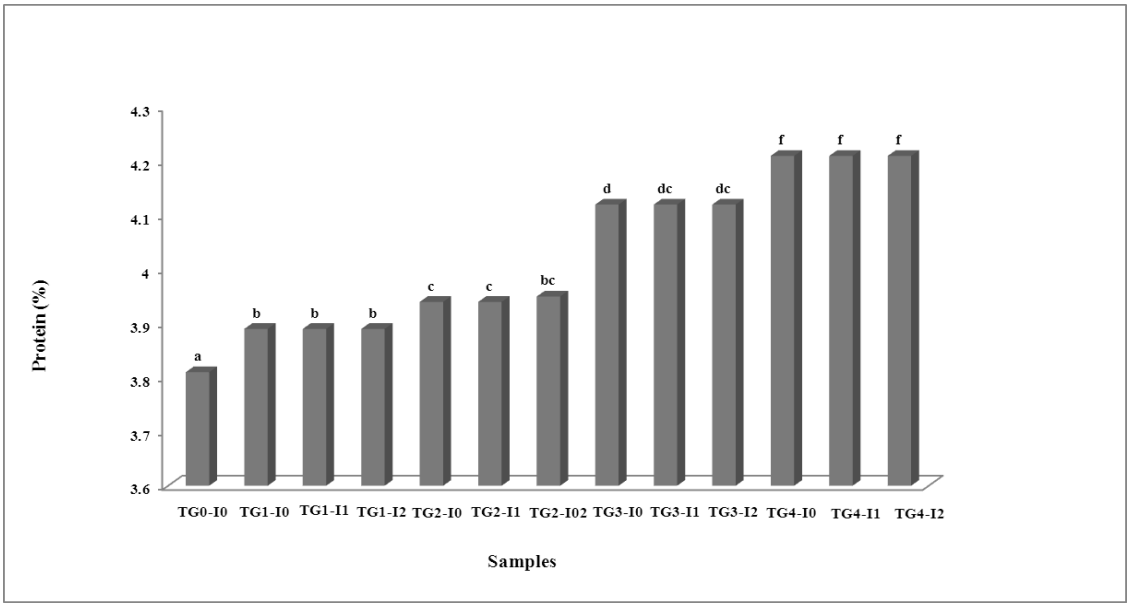

Fig 3. Protein changes of ice cream samples
I: Inulin (%); TG: Transglutaminase enzyme (g/L)
TG0-I0: I=0;TG=0. TG1-I0: I=0;TG=0.3. TG1-I1: I=2;TG=0.3.
TG1-I2: I=4;TG=0.3. TG2-I0: I=0;TG=0.4.TG2-I1: I=2;TG=0.4.
TG2-I2: I=4;TG=0.4. TG3-I0: I=0;TG=0.5.TG3-I1: I=2;TG=0.5.
TG3-I2: I=4;TG=0.5. TG4-I0: I=0;TG=0.6. TG4-I1: I=2;TG=0.6.TG4-I2: I=4;TG=0.6.

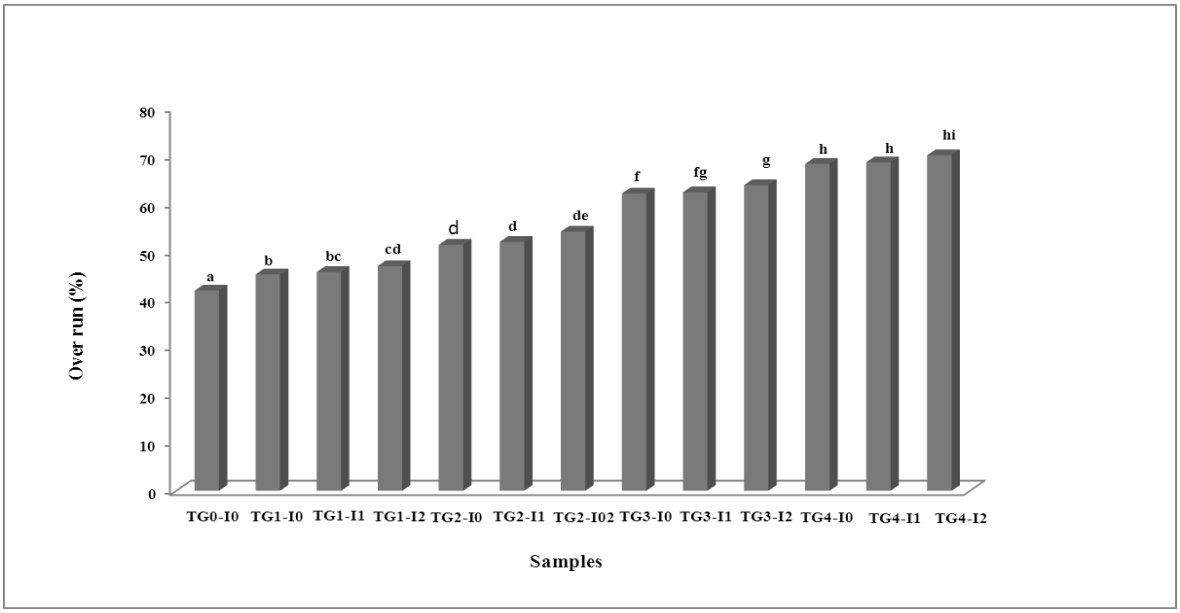

Fig 4. Over run changes of ice cream samples
I: Inulin (%); TG: Transglutaminase enzyme (g/L)
TG0-I0: I=0;TG=0. TG1-I0: I=0;TG=0.3. TG1-I1: I=2;TG=0.3.
TG1-I2: I=4;TG=0.3. TG2-I0: I=0;TG=0.4.TG2-I1: I=2;TG=0.4.
TG2-I2: I=4;TG=0.4. TG3-I0: I=0;TG=0.5.TG3-I1: I=2;TG=0.5.
TG3-I2: I=4;TG=0.5. TG4-I0: I=0;TG=0.6. TG4-I1: I=2;TG=0.6.TG4-I2: I=4;TG=0.6.

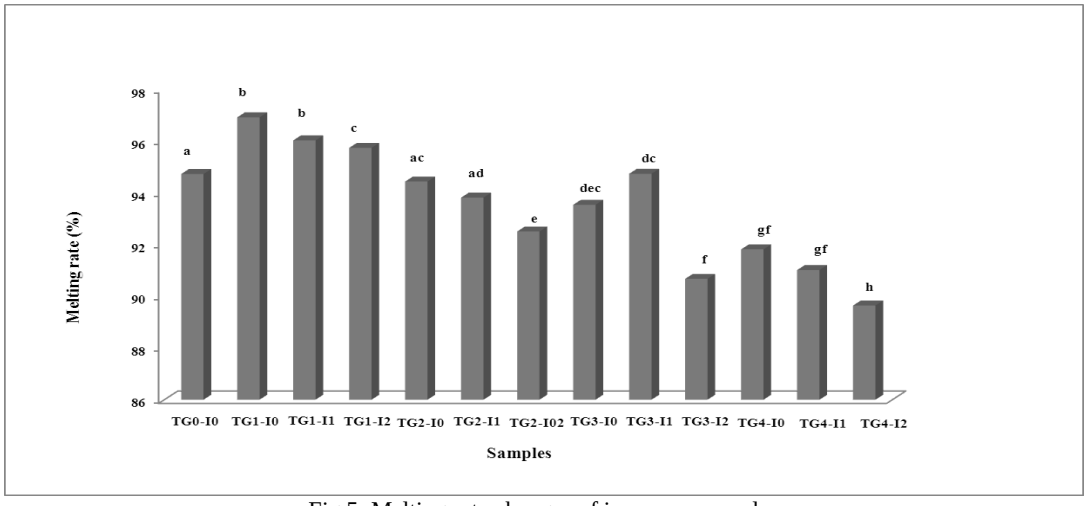

Fig 5. Melting rate changes of ice cream samples
I: Inulin (%); TG: Transglutaminase enzyme (g/L)
TG0-I0: I=0;TG=0. TG1-I0: I=0;TG=0.3. TG1-I1: I=2;TG=0.3.
TG1-I2: I=4;TG=0.3. TG2-I0: I=0;TG=0.4.TG2-I1: I=2;TG=0.4.
TG2-I2: I=4;TG=0.4. TG3-I0: I=0;TG=0.5.TG3-I1: I=2;TG=0.5.
TG3-I2: I=4;TG=0.5. TG4-I0: I=0;TG=0.6. TG4-I1: I=2;TG=0.6.TG4-I2: I=4;TG=0.6.

Viscosity

All samples containing TG and inulin had significantly a higher apparent viscosity compared to control sample (Fig.2). It was noted that according to our finding an increase in the concentration of inulin and TG induced a significant increment in viscosity content; so that the sample TG4-I2 showed the highest viscosity ($P < 0.05$). Covalent bonds created by TG have unique effects on gel formation capacity, thermal stability and water-holding capacity of the proteins (Kuraishi *et al*., 2001). The polymerization of the milk proteins by the TG during the

reaction, and thus, the formation of proteins, which their molecular weight is high, lead to modification in the viscosity of the milk (Hinz *et al*., 2007). Our results are similar to those reported by Lorenzen and Schlimme (1998), who confirmed the effect of this enzyme on gel strength and increased viscosity of dairy products.

One of the most important strategies for using fat replacers such as inulin (a mainly soluble dietary fiber) is because of the increase of water binding capacity of ice cream matrix. It has been suggested that water can bind directly to inulin. The results show that the viscosity was influenced strongly by inulin. The high molecular weight of inulin was reflected in the high viscosity values for the ice cream mix; this is in agreement with results obtained by Akalm *et al*. (2008). So, the results revealed a relationship between inulin and viscosity, which confirmed the report of El-Nagar *et al.* (2002) and Akın (2005) for yog-ice cream and probiotic-fermented ice cream, respectively.

Protein

The proteins obtained for ice cream samples are given in Fig.3. The protein of the samples with added TG shows significant increase ($p<0.05$). Increased protein in the reduced-fat samples containing TG can be explained, according to Rossa *et al*. (2012), by the protein nature of enzyme. There is no change in the protein content of the samples with the addition of inulin in levels 2 and 4%., because of carbohydrate nature of the dietary fiber.

Over run

The addition of TG and inulin increased the over run of the treatments in comparison to the control ($P < 0.05$). Over run is a characteristic which indicates an increment in the volume of an ice cream product during processing (Cruz *et al*., 2009). The highest over run was observed for the sample TG4-I2 (Fig.4). This observation established that polymerization of caseins by TG increases over run and air bubble stabilization in the system Faergemand *et al.* (1999). Inverse relationship between fat content and overrun has been observed by Adapa *et al*. (2000). As inulin content increased to 4%, the over run was incremented ($P < 0.05$); but there was no significant difference among the samples containing 0% and 2% inulin ($P > 0.05$). The highest over run value was also obtained in ice cream mix containing inulin ($P < 0.05$), indicating inulin responsibility for the increased air incorporation (Akalm *et al*., 2008).

Melting rate

All treatments showed decreasing in melting rate as inulin and TG increased (Fig. 5). The sample TG4-I2 showed the lowest melting rate; meanwhile, this sample had the highest over run. Ice cream containing a high amount of air (high over run) tends to melt slowly. The finding of Cruz *et al.*, (2009) revealed a relationship between the melting time of ice cream and its stability after over run. It is worthwhile to note that the melting time increased linearly with increasing coalesced fat (Metwally, 2007). The polymerization of the whey protein and casein present in the fat globules of the the ice cream samples containing TG may be associated with increased coalesced fat (Rossa *et al*., 2012); so, as mentioned before, the melting time of ice cream and its stability after over run are increased in relation to increasing coalesced fat. The samples containing inulin exhibited a lower melting rate value in comparison to the samples without it; the lowest melting rate was observed in the sample containing inulin at a level of 4% (w/w) ($P < 0.05$). This finding is agreement with El-Nagar *et al.* (2002), who reported the beneficial effect of inulin on the melting rate and firmness of yog-ice cream. As Inulin acts as a stabilizer, water molecules bind; so, water molecules are immobile and unable to move freely between other molecules in the blend of ice cream. As a result, by the increasing the concentration of inulin from 2 to 4% (w/w), the melting rate of ice cream decreased.

Conclusion

The microbial transglutaminase can form Glutamine -Lysine bonds in many food proteins, and this leads to the development of novel foods and processing methodologies. The present results demonstrated that TG can improve the functional properties of low fat ice cream. The ability of inulin as a good fat replacer is not only related to the modification of rheological behaviour of the product but also to changes of other mouthfeel attributes as creaminess. It seems that, in general, to obtain low-fat products with texture quality close to that of full-fat products, the simultaneously use of inulin and transglutaminase enzyme is desired.

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