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β_V SEEDING AS AN ALTERNATIVE PRE-CRYSTALLIZATION TECHNIQUE IN SYNBIOTIC MILK CHOCOLATE PRODUCTION

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ABSTRACT

In the present study, synbiotic milk chocolate including *Lactobacillus acidophilus* and inulin was prepared using β_v seeds as an alternative to conventional tempering method. For this aim, different concentrations of β_v seeds (0.5-1.5%) were used in the chocolate production. The effect of β_v seed concentration on water activity, moisture content, hardness value, color and rheological properties of the chocolate was examined and compared with those of the sample produced by conventional tempering method. Water activity and moisture content values of the samples were found to be very close to each other. Hardness value was significantly affected by β_v seed concentration. Yield stress and plastic viscosity values decreased with increasing seed concentration. All of the quality parameters highlighted that β_v seed can be used as a pre-crystallization technique without negatively affecting quality characteristics, providing economic gain and fast production when compared with classical one.

Key words: Chocolate, inulin, prebiotic, probiotic, tempering, seeding, synbiotic

β_V TOHUMLAMA TEKNİĞİNİN ALTERNATİF PRE-KRİSTALİZASYON YÖNTEMİ OLARAK SİNBİYOTİK SÜTLÜ ÇİKOLATA ÜRETİMİNDE KULLANIMI

ÖZ

Bu çalışmada, inulin ve *Lactobacillus acidophilus* içeren sinbiyotik sütlü çikolata, konvensiyonel temperlemeye alternative olarak β_v tohumlama tekniği kullanılarak üretilmiştir. Bu amaçla, çikolata üretiminde β_v tohumları farklı oranlarda (0.5-1.50%) kullanılmıştır. β_v tohum konsantrasyonunun çikolata örneklerinin su aktivitesine, nem miktarına, sertlik değerine, renk ve reolojik özelliklerine etkisi araştırılmış ve konvansiyonel temperleme ile üretilen örneğin kalite özellikleri ile karşılaştırılmıştır. Çikolata örneklerinin su aktivitesi ve nem içerikleri birbirlerine oldukça yakın bulunmuştur. Sertlik değeri, β_v tohum konsantrasyonundan istatistiksel olarak önemli şekilde etkilenmiştir. Eriyik çikolata örneklerine ait akma sınırı ve plastic viskozite değeri, tohum konsatrasyonu arttıkça azalmıştır. Örneklerin kalite parametreleri, β_v tohumlamanın alternatif pre-kristalizasyon tekniği olarak ürünlerin kalite özelliklerine zarar vermeden kullanılabileceğini göstermiştir. Bu şekilde çikolata üretiminde ekonomik kar ve hızlı üretim sağlanmış olacaktır.

Anahtar kelimeler: Çikolata, inulin, prebiyotik, probiyotik, temperleme, tohumlama, sinbiyotik

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INTRODUCTION

Chocolate is defined as a suspension of solid particles derived from ingredients such as; sugar, and cocoa solids dispersed within a continuous cocoa butter fat phase and milk fat depending on the chocolate type (Fernandes et al., 2013), which is enjoyed by people of all ages worldwide. Primary chocolate categories are dark, milk and white. Dark chocolate formulation consists mainly of cocoa liquor, sugar and cocoa butter. Milk chocolate is made up of sugar, cocoa butter, milk solids and cocoa liquor and white chocolate contains sugar, cocoa butter and milk solids. Sugar, cocoa and milk (depending on chocolate type), make approximately 70% of the chocolate formulation (Afoakwa, 2010). The type and amount of each ingredient in chocolate formulation plays an important role in obtaining a high quality product.

The chocolate recipe and manufacturing processes are important for obtaining high-quality product. Chocolate manufacturing processes generally consists of 6 steps; i.) mixing, ii.) pre-refining, iii.) refining, iv.) conching, v.) tempering and vi.) molding. Refining of chocolate is an important process for obtaining desired particle size of 25-30 μm normally using a combination of two- and five-roll refiners. The conching process contributes to development of viscosity and final texture and aroma (Afoakwa et al., 2007). Conching is usually carried out by agitating chocolate at temperatures above 50°C for few hours. In the first hours, moisture is reduced and some undesirable flavor-active volatiles such as acetic acid are removed, and subsequently color is changed due to emulsification and oxidation of tannins (Afoakwa et al., 2007). Tempering process refers to a controlled melting and cooling of chocolate to achieve form V which is the best crystalline structure of cocoa butter among the six existing polymorphic forms (Schenk and Peschar, 2004). A poorly tempered chocolate will induce fat bloom in product with serious quality defects on texture, color and surface gloss. Moreover the chocolate will be soft and not effectively demolded (Afoakwa et al., 2007). Tempering is an important step in chocolate production. A well-tempered chocolate will have a good gloss and snap with a long shelf-life.

The conventional tempering process is summarized in Figure 1. As can be seen, the tempering has four key steps; complete melting (at 50°C), cooling to point of crystallization (at 32°C), crystallization (at 27°C) and conversion of any unstable crystals (at 29-31°C) (Afoakwa et al., 2008).

Figure 1

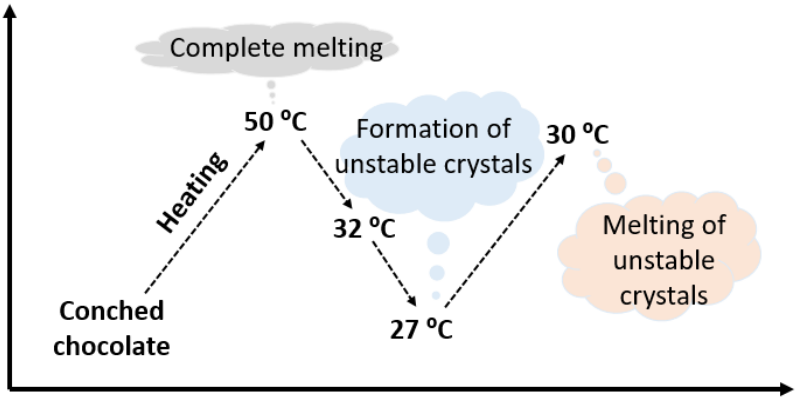

Figure 1. Conventional tempering method

As an alternative to the conventional tempering method, the seeding a pre-crystallization process is one of the techniques used in chocolate production in recent years. In the this technique (seeding), the most stable crystals of cocoa butter (β_v) is used in conched chocolate at 32-34°C which results in a large number of small well-defined crystal nuclei. Pre-crystallization with seeding may ensure some advantages such as, faster crystallization, lower energy consumption,

less equipment and specialized personnel needs and also cost reduction (Kinta and Hartel, 2010). This technique is beneficial for food industry manufacturers, because it can increase efficiency of productivity in terms of fastening process and decreasing costs. In addition pre-crystallization have considerable effects on chocolate quality, such as, fat blooming reduction (Zeng et al., 2002), shelf life improvement (Riberio et al., 2013) and thermodynamic stability and melting profile (Kinta and Hartel, 2010).

In recent years, important progress has been made in terms of scientific knowledge about the interactions between diet and health that resulted in the development of the concept of functional foods. Chocolate is high in sugar (30-50%) and fat (25%) and is classified as a high calorie food. Of the different ingredients in chocolate, the fat phase has the major influence on its quality. It affects the rheological properties of chocolate, melting properties, release from the mold, prevention of bloom, and flavor release. Consumers are concerned about the high sugar levels and calories in confectionery products, hence increasing popularity of 'sugar-free' products. Therefore sugar substitutes have been used most frequently for manufacturing functional chocolates.

High-intensity sweeteners and sugar alcohols are commonly used as sugar substitutes in chocolate production. High intensity sweeteners are many times sweeter than sucrose, therefore smaller amounts of high-intensity sweeteners are needed to achieve the same level of sweetness as sugar in food. Replacement of sugar with high-intensity sweeteners poses a serious challenge in chocolate production, because sucrose fulfills both a structural and sweetening function in this product. Therefore, sugar alcohols along with high-intensity sweeteners are widely used in food industry. Sugar alcohols as well as bulking agents such as polydextrose, maltodextrin and inulin has great potential for the successful manufacture of sugar-free chocolate products (Aidoo et al., 2013). Since maltitol has organoleptic and technological properties close to those of sucrose (Portmann and Kilcast, 1996), therefore in the current project, it has been used as a sugar substitute in white chocolate manufacturing.

In recent years, a worldwide consumer's tendencies towards functional foods that possess certain health properties have been noted. Prebiotic and probiotics are among such functional ingredients mostly used in the food industry. In the present study, milk chocolate was enriched with prebiotic and probiotic and quality characteristics of the synbiotic product were investigated.

MATERIALS AND METHODS

Materials

Maltitol (Roquette Frenes, Lestres, France), cocoa butter, cocoa mass (Altinmarka, Istanbul, Turkey), whole and skimmed milk powder (Besel, Konya, Turkey), soy lecithin (Brenntag Chemistry, Istanbul, Turkey), polyglycerol polyricinolate (PGPR) (Palsgaard, Zierikzee, the Netherlands), cocoa butter originated β_V seed crystals (SEED100, Uelzena, Uelzen, Germany) and lyophilised *L. acidophilus* (LOT No 41127003932) (Danisco, Niebüll, Germany) were used in the formulation of the dark chocolates.

Sample Preparation and Probiotic Inoculation

Maltitol (34%), cocoa butter (18.5%), cocoa mass (18%), whole milk powder (13.5%), skimmed milk powder (6.52%), soy lecithin (0.30%), PGPR (0.15%), vanilla flavour (0.03%) and inulin (9.0%) were used in the chocolate formulation. Each sample group was prepared in lots of 10 kg. For this purpose, the melted fat components (comprising 20% of the total cocoa butter present in the formulation) and the dry powders were mixed until being homogeneous and heated to 40°C. At the end of the mixing and warming, the chocolate mass was first pre-refined on a pilot-scale 3-roll refiner (Lehmann, Aaelen, Germany) and then mixed again and warmed to 50°C. To achieve a mean particle size of approximately 20-25 μm, the gap size between the rollers of the 3-roll refiner and pressure applied was adjusted and d_{90} values were measured using a micrometer (Mitutoyo, Manufacturing Co. Ltd., Japan, 0.001 mm accuracy). After reaching the desired particle size mentioned above, dry conching process was

performed for 80 min, and the remaining cocoa butter (80% of the total), soy lecithin and PGPR were then added and then the wet conching process was employed. The whole conching process lasted 480 min at 70°C.

After conching, probiotics, *L. acidophilus* (9 log CFU/25 g) were added to the prepared chocolate mass at 35°C. Then the mass was mixed nearly 5 min. Addition of probiotics was followed by a three-stage tempering process (33-35, 24-25 and 25-26°C) for control samples. The achievement of tempering process was checked by temper index value measured using temper meter (Chocometer, Aasted Farum, Denmark). Temper index values of the produced samples ranged between 5.5 and 6.0. For seeded samples, different concentrations of β_V seeds (0.50, 0.75, 1.00 and 1.50 g seed/100 g chocolate) were added at 32°C and mixed for 10 minutes. Subsequently, the molding and vibration process (Aasted Farum, Denmark) was conducted at 27-30°C for all samples. After 20 min of cooling (Aasted Farum, Denmark) at 5°C, the process was completed. Samples were stored at temperatures between 13 and 15°C, and subsequently prevented from light and heat prior to analysis.

Water activity and Moisture Content

The water activity of the chocolates was measured using a Lab-Master a_w (Novasina, Switzerland) according to the method described by Konar (2013). A_w values of each sample were measured in triplicate at after a follow-up day of sample preparation. Moisture content of the samples was determined using the method described by Lonchampt and Hartel (2006).

Texture Analysis

As textural parameters, hardness values of the chocolates were determined using TA-TX plus Texture Analyser (Stable Micro Systems, UK). During analyses, load cell with 5 kg and 3 point bend ring were used to obtain displacement versus force curve. By using this curve force at peak, necessary for breaking the sample, was calculated. Pre-test, test and post-test speeds applied during textural measurement were 1 mm/sec, 1 mm/sec and 10 mm/sec, respectively. Hardness values of each sample were measured 7 times.

Particle size determination

D_{90} value calculated from particle size distribution analysis representing the size of the larger particles is very close to value read by micrometer (Beckett, 2009). Therefore, the particle size of the produced chocolates was measured using micrometer (Mitutoyo, Manufacturing Co. Ltd., Japan, 0.001 mm accuracy).

Viability of probiotic bacteria

20 g of chocolate was weighed and it was put into 180 mL 0.1% peptone solution prepared by distilled water (0.1%). After that the prepared mixture was maintained at 40°C for melting of the chocolates. Prepared dilutions (200 µL) were plated on specific media suitable for viable cell counts. The plates were incubated at 37.5°C for 48 h, which was followed by counting of LAB on De Man, Rogosa, and Sharpe Agar (Oxoid CM 361). The LAB counts were conducted at 0^{th}, 30^{th}, 60^{th} and 90^{th} days of the storage. Three replications were performed to all samples.

Rheological Analysis

Steady shear rheological characteristics of the melted chocolate samples were determined at 40°C by rheometer (Anton Paar, MCR 302, Austria) which can be controlled considering stress or strain parameters. The temperature of the system was adjusted by Peltier system and water bath. CC27 probe was used for the analysis. After melting of the chocolate samples at 40°C, shear rate versus shear stress data were measured using steady shear rheological properties of chocolate samples melted at 40°C prior to the method of International Confectionery Association (ICA) which includes the four steps as mentioned following.

1^{st} Step: Shearing at 5 s^{-1} for 500 s for homogenizing the sample and equilibrating the material temperature to set value.

2^{nd} Step: Shear rate ranged between 2 s^{-1} to 50 s^{-1} (upward) was applied within180 s

3^{rd} Step: Shearing the samples at 50 s^{-1} for 60 s

4^{th} Step: Applying the shear rates ranged from 50 s^{-1} to 2 s^{-1} (downward) within 180 s

Shear stress at 5 s^{-1} shear rate was accepted as yield point almost at rest and viscosity at high shear rate is equal to apparent viscosity at shear rate 40 s^{-1}. By using obtained experimental data, these parameters were acquired.

Colour measurement

Colour parameters (*L**: brigthness, *a**: ±red-green and *b**: ±yellow-blue) of the compounds produced were measured by colorimeter (Chroma Meter CR-400, Konica Minolta, Japan) and chroma (*C**), hue (*h°*) and whiteness index (*WI*) values were calculated by using these parameters according to the following equations (Periche et al., 2015);

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^\circ = \arctan\ (b^*/a^*) \quad (2)$$

$$WI = \sqrt{(100 - L)^2 + a^{*2} + b^{*2}} \quad (3)$$

Statistical Analysis

The quantitative data were expressed as the mean ± standard deviation in the tables. The results were analysed using analysis of variance (ANOVA). Tukey's test was applied to determine if the differences between quality parameters of the samples were significant. The statistical analyses were performed using the MINITAB-Express (Minitab Inc., State College, PA, USA) and MSTAT statistical packages (Michigan State University, East Lansing, MI, USA). Differences with *P* values less than 0.05 were considered as statistically significance effect of the corresponding factor on the quality parameter.

RESULTS AND DISCUSSION

Water activity

The effect of β_v seed concentrations on the water activity of milk chocolates are presented in Table 1. The a_w values of chocolate treatments were found to be in the range of 0.238-0.253. The water activity of milk chocolate is often below 0.4. According to the statistical analysis results, no significant differences was generally observed between water activity of the samples (P>0.05).

Table 1. Water activity, moisture content and hardness value of milk chocolates samples

β_V concentration (%)	a_w	Moisture (g/100 g)	Hardness (g)	*L. acidophilus* (log CFU/25 g)	*Rheological parameters*	
					Yield stress (Pa)	Viscosity (Pa.s)
0.00	0.241 ± 0.002^{a}	1.13 ± 0.06^{bc}	8991 ± 279^{b}	8.085 ± 0.013^{a}	134 ± 17^{b}	14.4 ± 0.1^{b}
0.50	0.248 ± 0.001^{a}	1.29 ± 0.01^{a}	11043 ± 127^{a}	7.838 ± 0.043^{b}	222 ± 21^{a}	23.9 ± 0.2^{a}
0.75	0.253 ± 0.003^{a}	1.28 ± 0.03^{ab}	8817 ± 373^{bc}	7.573 ± 0.083^{c}	85 ± 10^{c}	9.2 ± 0.1^{c}
1.00	0.238 ± 0.009^{a}	1.25 ± 0.01^{ab}	8172 ± 301^{cd}	7.913 ± 0.042^{b}	44 ± 5^{d}	4.7 ± 0.0^{e}
1.50	0.239 ± 0.007^{a}	1.02 ± 0.11^{c}	7602 ± 246^{d}	7.952 ± 0.085^{ab}	62 ± 5^{cd}	6.6 ± 0.1^{d}

The findings showed that pre-crystallization technique did not change water activity of the milk chocolate samples significantly (P>0.05). Osmotic agents such as maltitol have a definitive role in reducing water activity (Shadwell et al., 2013). Maltitol used in this study, had low hygroscopicity therefore, this property is effective in gaining low water activity of the chocolate samples (Table 1). Therefore, it will not absorb water in humid conditions. There is a direct relationship between hygroscopic capacity and water absorption. Besides, sugar alcohols disperse uniformly in continuous oil phase (cocoa butter). Thus sugar alcohols could affect the water activity and fat phase and the processing conditions and the quality of the treatments. It seems that investigating the effects of sugar alcohols on some chocolate properties seems necessary (Sokmen and Gunes, 2006).

Higher solid content of chocolate alters the diffusion rate of moisture migration, therefore leads for low water activity in the product. Higher proportion of solid phase changes the texture and

sensory characteristics of chocolate by reducing the a_w of liquid phase (Beckett, 2008).

Moisture content

Measured moisture content values were in the range between 1.02-1.29 for the milk chocolate samples (Table 1), which is within the acceptable limit (<1.5%). Regarding the effect of seeding, for milk chocolate samples, seeding concentration at 0.5 g/100g showed higher moisture content value than the other concentrations and control treatment. β_v seed amount at low concentrations added significantly affected the moisture content of the samples (P<0.05).

Textural properties

The hardness value of the conventionally tempered and β_V seeded pre-crystallized milk chocolates samples are presented in Table 1. Hardness value of sugar-free chocolates is found to be between 7602-11043 g. Hardness values of milk chocolates containing 0.75 g/100 g seed were very close to control treatment. As can be seen in Table 1, milk chocolate samples tempered by using 0.5 g/100 g β_V seeds had the highest hardness among the other β_V seeded and control samples. The result in Table 1 clearly demonstrated that the samples having seed concentration of 0.5 g/100g required significantly stronger forces compared to the other samples (P<0.05). Lower hardness value of other samples pre-crystallized with seeds at concentration higher than 0.5 g/100g might have resulted from agglomeration of seed particles. Hence, high concentration of β_V seed might result in lower mechanical strength (Glicerina et al., 2016). The samples produced by tempering at 0.75 g/100g seeds were more similar to control sample in terms of textural properties.

These results indicated that variation in crystallization behavior in sugar-free chocolate during tempering can influence the degree of crystalinity of β_V seeded products at different levels (Glicerina et al., 2013). It can be concluded that the seeds had a homogeneous dispersion in the cocoa butter and crystal network was stronger. Therefore, pre-crystallization process produced adequate amounts of crystal nuclei (Glicerina et al., 2016). Several parameters such as; chocolate composition, tempering, seeding rate and activity determine the hardness of chocolate (Afoakwa et al., 2007).

Proper tempered chocolate has closer packing and higher thermodynamic stability (Aidoo et al., 2013). Under tempered samples will be softer due to its weaker fat crystal structure. Therefore, hardness is a helpful indicator to understand the level of tempering index (Afoakwa et al., 2007).

Particle size (D_{90})

D_{90} parameters of chocolate samples were found to be 21 μm. Particle size of the treatments were similar to each other (P>0.05). This result was expected since the formulation and production process (refining) of the chocolates were same except for β_V seeds used.

The findings indicated that particle size was not affected by seeding technique and particle size of the milk chocolates was similar to each other. It could be finalized that there was no adverse effect expected in terms of coarseness, textural character and desirable flow properties as these parameters had a key role in chocolate manufacture (Beckett, 1999).

Viability of probiotics

The obtained results showed that the survival of probiotic strain *L. acidophilus* in milk chocolate samples was very good (Table 1). The results illustrated that no considerable differences (P>0.05) was observed between the chocolates samples produced by β_v seeding technique at 1.5 g/100g seed concentrations compared to conventional tempering method. Considering a threshold of about 6 log CFU/g of viable probiotic bacteria, the results demonstrated an acceptable amount of the probiotic strains assayed in milk chocolates during a storage period of 90 d. The minimum count of probiotic bacteria at the time of intake should be $\geq 10^6$ CFU/g for gaining therapeutic effects (Doleyres and Lacroix, 2005; Nag and Das, 2013).

Water activity, temperature, oxygen exposure, sugar concentration, osmotic tension and mechanical shearing are the critical factors

affecting the viability of probiotic cells in confectionery (Crittenden, 2009; Saarela et al., 2000). Low water activity and high concentration of sugar and fat in chocolate actually prevents the possible growth of microorganisms and ensures the maintenance of probiotic strains in an inactive state. Also, the packaging with aluminum foil limits penetration of oxygen and protects the chocolates from humidity and other damages during storage period. In fact, oxygen may negatively affect the survival of probiotics, especially those on the surface of chocolates (Lalicic-Petronijevic et al., 2015; Żyżelewicz et al., 2010).

In the study of Lalicic-Petronijevic *et al.* (2015), *L. acidophilus* and *Bifidobacterium lactis* were incorporated in milk and dark chocolates. The results illustrated that the number of viable cells decreased gradually within the storage period. Nebesny *et al.* (2007) stated that the total number of *L. casei* and *L. paracasei* cells during the storage for 12 months at 4°C was almost unchanged. Also keeping the chocolate at ambient temperature (18°C) maintained the number of live cells at the functional level.

The population level was maintained at the functional level of 10^7 CFU/g during 90 day storage period. It can be consider chocolate as a suitable food for the delivery of probiotic strains with excellent viability levels. The result was satisfactory. The final population was sufficient to classify the chocolate as a probiotic food.

Rheological properties

Yield stress (τ_0) and plastic viscosity (η_{pl}) parameters of sucrose-free chocolates were found between 43.66 and 222.11 Pa and 4.7 and 23.9 Pa.s, respectively (Table 1). The results indicated that using β_V seeds at different concentrations influenced the rheological properties. The differences might have resulted from formulation. Rheological characteristics are important for transportation, pumping and mixing processes; therefore, β_V level should be also considered. However, no direct proportion was considered between β_V concentration seeds and value of rheological parameters.

In traditional tempering method, it should be very careful with the cooling-heating-cooling steps in the tempering process. However, in the seeding technique, the crystallization process is suitably controlled. This result is proved in the viscosity of seed-crystallized chocolate. Koyano et al. (1990) studied the viscosity changes of dark chocolate made by the normal tempering and by the β_2 seeding. They reported that viscosity of the tempered chocolate started to increase instantly after the completion of tempering. However, viscosity of the β_2 seeded chocolate slowly increased after an induction period of about 50 minutes. This is a clear advantage for the chocolate manufacturer because it permits easier plant operation. The researchers finalized that seeding technique gives rise to an accelerated crystallization of form V of cocoa butter and moderates changes in viscosity.

Campbell and Keeney (1968) added chocolate powders to molten chocolate and measured the viscosity changes to evaluate the level of seed crystal formation during the tempering process. They concluded that the optimal amount of seed crystals to be added in the seeding techniques is 0.5%. Hachiya et al. (1988) studied the changes in viscosity during crystallization process of cocoa butter and chocolate. They used a rotational viscometer to measure the viscosity of the samples. Varying amounts of the cocoa butter seed crystals of Form VI were added and the viscosity was recorded to make a calibration curve between the CB crystal concentration and the viscosity changes. The results demonstrated that the amount of the seed crystals crystallized in the tempering process was 0.2%.

Chocolate viscosity during the solidification process is critically important for workability, which is significantly influenced by crystallization. As seen, β_V seed addition at concentrations more than 0.5 g/100g reduced rheological parameters of the milk chocolates, thus it is an advantages for manufacturers in terms of using of β_V seeds as an alternative for conventional tempering process in the production of chocolates without altering production process steps.

Colour Properties

Table 2 exhibits the colour properties (lightness, hue angle, chroma and whiteness index) of the milk chocolates tempered with conventional method and addition of β_V seeds at different concentrations. Considering the colour parameters it was determined that brightness, *C** and hue angle was affected significantly by the pre-crystallization technique ($P<0.05$). The results indicated that hue angle was not affected significantly by using different concentrations of β_V seeds ($P>0.05$). The hue angle value of milk chocolate samples changed between 0.75-0.81. Moreover, when comparing the lightness and chroma values of the chocolate samples, it can be concluded that higher values was observed for samples containing high concentrations of β_V seeds ($P<0.05$) (Table 2). As a general no significant differences was observed between brightness and chroma values of samples containing high levels of β_V seeds ($P<0.05$). The differences observed regarding the effect of different concentrations of β_v seeds and conventional tempering technique on brightness values and chroma of the chocolate samples was not substantial.

Table 2. Colour parameters of milk chocolate samples

β_V seed concentration (g/100 g chocolate)	*L**	*C**	*h°*	*WI*
0.00 g/100g (Control)	29.7 ± 0.3^{c}	9.7 ± 0.1^{c}	0.75 ± 0.01^{a}	70.9 ± 0.4^{a}
0.50 g/100g	30.7 ± 0.1^{bc}	11.1 ± 0.1^{a}	0.81 ± 0.01^{a}	70.2 ± 0.1^{ab}
0.75 g/100g	30.9 ± 0.5^{ab}	10.7 ± 0.1^{b}	0.80 ± 0.01^{a}	69.9 ± 0.5^{ab}
1.00 g/100g	31.3 ± 0.2^{ab}	11.5 ± 0.1^{a}	0.81 ± 0.00^{a}	69.7 ± 0.2^{b}
1.50 g/100g	31.7 ± 0.6^{a}	11.2 ± 0.2^{a}	0.80 ± 0.00^{a}	69.3 ± 0.6^{b}

Lindecrantz (2014) stated that tempering protocols has a great impact on the colour, surface brightness and molding properties of chocolate. A well-tempered chocolate has glossy surface, good snap, appropriate colour and molding properties. It is also more heat resistant and it has longer shelf-life and the melting properties are better compared to a chocolate that is not well-tempered (Afoakwa et al., 2007).

White index values of milk chocolates are shown in Table 2. The whiteness index values for chocolates samples ranged between 69.3-70.9. Control samples implied higher whiteness index. However no remarkable differences ($P>0.05$) was observed for samples tempered with different levels of β_v seeds crystals. The whiteness index is determined by crystal network and the polymorphic state of the TAGs crystals. WI is used as an indicator for fat bloom development. Fat blooming of chocolate is influenced by various factors such as; ingredients compositions, storage conditions and temper regimes.

Hartel (2001) reported that the re-crystallization of fats (fat blooming) plays critical role in the final structure, mechanical properties, appearance, quality and marketability of the chocolate products. Beckett (2000) has attributed whitening index to fat migration, basically induced by insufficient formation of stable polymorphs in cocoa butter during tempering. Improper storage conditions, changes in temperature, or poor tempering and the polymorphic transformation from β_v to β_{v1} develop an increase in the whiteness. The whiteness index could be used as one of the parameters of the defining of polymorphic changes in chocolate matrix (Rousseau and Sonwai, 2008).

Bloom on chocolate with different levels of cocoa butter seed addition was investigated by Kinta and Hartel (2010). The results indicated that when inadequate cocoa butter seed crystals were added to give proper temper, the chocolate developed bloom as dark brown spheres in lighter color areas, similar to that seen in bloom on un-tempered chocolate. However by increasing the seed amount, the dark colored spheres overlapped and the lighter color areas disappeared. The relationship between seed

amount and bloom showed that over 270 ppm seeds (fat basis) were needed to accomplish good tempering.

The results demonstrated that the mechanism of bloom formation on poorly tempered chocolate (insufficient seeds) is due to enough time and space for phase (particles and fat) separation as the stable polymorphs grow.

CONCLUSIONS

Consumer behaviour has headed towards functional foods due to consumer awareness between diet and health. For this aim, synbiotic milk chocolate was produced using inulin as a prebiotic and *L. acidophilus* as a probiotic. Instead of conventional tempering, seeding technique was employed for a pre-crystallization using β_V seeds at different concentrations ranged between 0.5 and 1.5%. Water activity and moisture content of the chocolate samples tempered with β_V seeds were very close to those of the control sample. Hardness value of the synbiotic milk chocolate was significantly affected by β_V addition. Yield stress and plastic viscosity values of the melted chocolate decreased with increasing seed level. The findings of the present study indicated that pre-crystallization can be achieved by seeding method instead of conventional one.

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REFERENCES

Afoakwa, E.O., Paterson, A., Fowler, M. (2007). Factors influencing rheological and textural qualities in chocolate-a review. *Trends Food Sci Technol*, 18(6): 290–298.

Afoakwa, E., Paterson, A., Fowler, M., Vieira, J. (2008). Particle size distribution and compositional effects on textural properties and appearance of dark chocolates. *J Food Eng*, 87(2): 181–190.

Afoakwa, E.O. (2010). Chocolate science and technology. Oxford, UK: Wiley- Blackwell.

Aidoo, R.P., DeClerq, N., Afoakwa, E.O., Dewettinck, K. (2013). Industrial manufacture of sugar free chocolates—applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development. *Trends Food Sci Technol*, 32: 84–96.

Beckett, S.T. (1999). Industrial chocolate manufacture and use (3rd ed.) Blackwell Science, Oxford pp. 153–181, 201–230, 405–428, 460–465.

Beckett, S.T. (2000). The Science of Chocolate, London, Royal Society of Chemistry Paperbacks.

Beckett, S.T. (2008). The science of chocolate (2nd Ed.) London, Cambridge, UK: Royal Society of Chemistry Campbell LB, Keeney PG (1968). Temper level effects on fat bloom formation on dark chocolate coatings. Food Technology, 22: 1150.

Beckett, S.T. (2009). Industrial Chocolate Manufacture and Use. Wiley-Blacwell Publication, 732 s, United Kingdom.

Campbell, L.B., Keeney, P.G. (1968). Temper level effects on fat bloom formation on dark chocolate coatings. *Food Technol*, 22: 1150.

Crittenden, R. (2009). Incorporating probiotics into foods. In Y. K. Lee & S. Salminen (Eds.), Handbook of probiotics and prebiotics (pp. 58–75). Hoboken, NJ: JohnWiley & Sons, Inc.

Doleyres, Y., Lacroix, C. (2005). Technologies with free and immobilised cells for probiotic bifidobacteria production and protection. *Int Dairy J,* 15(10): 973–988.

Fernandes, V.A., Müller, A.J., Sandoval, A. J. (2013). Thermal, structural and rheological characteristics of dark chocolate with different compositons. *J Food Eng*, 116(1): 1226–1231.

Glicerina, V., Balestra, F., Rosa, M.D., Romani, S. (2013). Rheological, textural and calorimetric modifications of dark chocolate during process. *J Food Eng,* 119(1): 173–179.

Glicerina, V., Balestra, F., Rosa, M.D., Romani, S. (2016). Microstructural and rheological characteristics of dark, milk and white chocolate: a comparative study. *J Food Eng*, 169: 165–171.

Hachiya, I., Koyano, T., Sato, K. (1988). Changes in viscosity during crystallization processes of cocoa butter and chocolate. J Jpn Oil Chem Soc TI: 613–617.

Hartel, R.W. (2001). Crystallization in Food, Gaithersburg, USA, Aspen Publishers Inc.

Kinta, Y., Hartel, R.W. (2010). Bloom formation on poorly-tempered chocolate and effects of seed addition. *J Am Oil Chem Soc*, 87: 19–27.

Konar, N. (2013). Influence of conching temperature and some bulk sweeteners on physical and rheological properties of prebiotic milk chocolate containing inulin. *Eur Food Res Technol,* 23: 135–143.

Koyano, T. Hachiya, I. Sato, K. (1990). Fat polymorphism and crystal seeding effects on fat bloom stability of dark chocolate. *Food Struct*, 9: 231–240.

Lalicic-Petronijevic, J., Popov-Raljic, J., Obradovic, D., Radulovic, Z., Paunovic, D., Petrusic, M., Pezo, L. (2015). Viability of probiotic strains *Lactobacillus acidophilus* NCFM® and Bifidobacterium lactis HN019 and their impact on sensory and rheological properties of milk and dark chocolates during storage for 190 days. *J Func Foods*, 15: 541–550.

Lindecrantz, A. (2014). Investigation of seedpowder technology for pre-crystallization processing for dark chocolate- Effect on fat crystal structure and storage stability. Master of Science Thesis in the Master Degree Program Biotechnology.

Lonchampt, P., and Hartel, R.W. (2006). Surface bloom on improperly tempered chocolate. *Europ J Lipid Sci Technol,* 108(2): 159–168.

Nag, A., Das, S. (2013). Improving ambient temperature stability of probiotics with stress adaptation and fluidized bed drying. *J Func Foods*, 5: 170–177.

Nebesny, E., Zyz elewicz, D., Motyl, I., Libudzisz, Z. (2007). Dark chocolates supplemented with *Lactobacillus* strains. *Eur Food Res Technol,* 225, 33–42.

Periche, A., Heredia, A., Escriche, I., Andrés, A., Castelló, M.L. (2015). Potential use of isomaltulose to produce healthier marshmallows. *LWT - Food Sci Technol,* 62: 605–612.

Portmann, M.O., Birch, G.G. (1995). Sweet taste and solution properties of α,α-trehalose. *J Sci Food Agr*, 69: 275–281.

Ribeiro, A.P.B., Basso, R.C., Kieckbusch, T.G. (2013). Effect of the addition of hardfats on the physical properties of cocoa butter. *Eur J Lip Sci Technol*, 115: 301–312.

Rousseau, D., Sonwai, S. (2008). Influence of the dispersed particulate in chocolate on cocoa butter microstructure and fat crystal growth during storage. *Food Biophys*, 3: 273–278.

Saarela, M., Mogensen, G., Fonden, R., Matto, J., Mattila- Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *J Biotech*, 84: 197–215.

Schenk, H., Peschar, R. (2004). Understanding the structure of chocolate. *Radiation Phys Chem*, 71: 829–835.

Shadwell, N., Villalobos, F., Kern, M., Hong, M.Y. (2013). Blooming reduces the antioxidant capacity of dark chocolate in rats without lowering its capacity to improve lipid profiles. *Nutrition Res*, 33: 414–421.

Sokmen, A., Gunes, G. (2006). Influence of some bulk sweeteners on rheological properties of chocolate. *LWT-Food Sci Technol*, 39: 1053–1058.

Zeng, Y., Braun, P., Windhab, E.J. (2002). Tempering—continuous precrystallization of chocolate with seed cocoa butter crystal suspension. *Manuf Confect*, 82(4): 71–80.

Żyżelewicz, D., Nebesny, E., Motyl, I., Libudzisz, Z. (2010). Effect of Milk Chocolate Supplementation with Lyophilised Lactobacillus Cells on its Attributes. *Czech J Food Sci* 28(5): 392–406.