Cumhuriyet Science Journal
CSJ

e-ISSN: 2587-246X
ISSN: 2587-2680

Cumhuriyet Sci. J., Vol.40-1(2019) 233-244

Usage of β_V Seeds as an Alternative Pre-crystallization Technique in Production of Synbiotic Sugar-free Dark Chocolate: Effect of Quality Characteristics

Ömer Said TOKER[1*], Derya GENÇ POLAT[2]

[1]*Yildiz Technical University, Chemical and Metallurgical Engineering Faculty, Food Engineering Department, Istanbul TURKEY*

[2]*Tayaş Gıda A.Ş., Gebze, Kocaeli, TURKEY*

Received: 25.12.2017; Accepted: 25.12.2018 *http://dx.doi.org/10.17776/csj.370613*

Abstract. Pre-crystallization process in chocolate production requires high investment cost and it is employed with multistage thermal processes. Improper tempering process results in fat bloom main quality defect of the chocolate products. Seeding is an alternative tempering process and it is performed by addition of β_V and/or β_{VI} seeds, extracted by chocolate or cocoa butter, to chocolate or chocolate products. In the present study, synbiotic dark chocolate was produced by inulin (DP<10) used as a prebiotic fiber at concentarion of 9.00 g / 100 g and probiotic *L. acidophilus*. Effect of different β_V concentrations (0.50-1.50 g/100 g) on the quality parameters and viability of probiotics was observed. Probotic enrichment (9.0 log cfu/25 g) was performed after conching process. During production process, 0.50-1.50 log cfu/25 g vitality loss was observed and application of seeding technique caused to decrease in probiotic level (P<0.05). The other quality parameters, namely, water activity, water content, colour properties (L^*, C^*, $h°$, WI), hardness, rheological parameters (yield stress and plastic viscosity) and sensory properties were not negatively affected by seeding technique. Higher vitality level can be obtained by optimization of seeding process such as shear rate.

***Keywords*:** Functional, Prebiotic, Probiotic, Tempering.

Sinbiyotik Şekersiz Bitter Çikolata Üretiminde Alternatif Pre-Kristalizasyon Tekniği Olarak β_V Tohum Kristali Kullanımı: Kalite Parametrelerine Etkisi

Özet. Çikolata üretiminde pre-kristalizasyon prosesi, konvansiyonel olarak yüksek sabit yatırım maliyeti gerektiren ekipmanlar kullanılarak çok aşamalı ısıl işlemler ile yürütülmektedir. Uygun olmayan temperleme işlemi başlıca çikolata kalite kusurlarından olan yağ çiçeklenmesi ile sonuçlanmaktadır. Tohumlama adı verilen alternatif temperleme işlemi ise, çikolata ve/veya kakao yağından elde edilen β_V ve/veya β_{VI} kristal tohumların çikolataya ilave edilmesi sonucu gerçekleştirilebilir. Bu çalışmada, prebiyotik lif olarak 9.00 g/100 g düzeyinde inulin (DP<10) ve probiyotik aktiviteye sahip *L. acidophilus* içeren bitter çikolatalarda farklı düzeylerde (0,50-1.50 g/100 g) tohum kristal (β_V) kullanımının başlıca kalite parametreleri ve probiyotik canlılıktaki proses stabilitesi üzerine etkisi incelenmiştir. Konçlama prosesi sonrası 9.00 log CFU/25 g düzeyinde probiyotik ilavesi gerçekleştirilmiştir. Probiyotik canlılık düzeyinde proses kaynaklı yaklaşık 0.50 – 1.50 log cfu/25 g aralığında kayıp olduğu, prekristalizasyon tekniği olarak tohumlama tekniği uygulamasının, kullanılan tohum kristal miktarından bağımsız olarak probiyotik canlılık düzeyi üzerinde olumsuz etkisi belirlenmiştir (P<0.05).

* Corresponding author. *Email address: stoker@yildiz.edu.tr; os.toker85@gmail.com*

Ancak çalışma kapsamında yeralan başlıca çikolata kalite parametrelerinden su aktivitesi, nem, renk (L^*, C^*, $h°$, *WI*), tekstür (sertlik), reolojik özellikler (akma basıncı (yield stress) ve plastik viskozite) ve duyusal profil için tohumlama tekniği uygulamasının avantaj unsuru olabileceği belirtilmiştir. Probiyotik ve/veya sinbiyotik bitter çikolata geliştirilmesinde tohum kristal ilavesi aşamasında karıştırma süresi optimizasyonu ile daha yüksek probiyotik canlılık düzeyine ulaşılması mümkün olabilir.

***Anahtar Kelimeler*:** Fonksiyonel, Prebiyotik, Probiyotik, Temperleme.

1. INTRODUCTION

Chocolate is a product which is widely consumed throughout the world by people of all ages. The change in consumer preferences observed in recent years is also seen in chocolate or chocolate products, high sugar and fat composition of chocolate products gives people worry due to health conscious of them. Therefore, production of sugar-free products by substituting sugar with alternative compounds is major efforts of the food industry in order to achieve consumer desire.

However, different quality defects can be observed when alternative compounds, especially bulk sweeteners, are used in chocolate products; indicating that process and formulation optimization studies are required to obtain the products with desired quality level. For example, substituting of sucrose with bulk sweeteners influenced the rheological parameters; thereby quality of the products [1]. Isomalt, maltitol, xylitol [2-6], lactitol [7], sorbitol and mannitol [8] are major ingredients used in development of sugar-free chocolate products.

General production of chocolate is generally composed of mixing, refining, conching and tempering steps. Pre-crystallization process is conventionally performed by multi-stage heat processes [9, 10]. This process is reqired for obtaining the most stable polymorph of cacao butter (β_V) [11]. Cacao butter can be crystallised into six different polymorhic forms; which are named as form I-VI or form sub-α, α, β_2', β_1', β_2, β and this gradation is done according to increasing melting point and stability [12]. Tempering process is employed for the formation of β_V crystals, the most stable form among six polymorphs, by controlled heating and cooling cycle [13].

There are four important stages of tempering process: complete melting occured at 50°C, cooling to crystallization temperature (32°C), crystallization (27°C) and transformation of unstable-crystals (29-31°C) [14]. Conventional pre-crystallization is performed by pumping of melted chocolate through different tempering zones [15]. Conventional tempering method perfomed by adjusting temperature requires significant energy and production time. Therefore, alternative tempering methods were investigated to overcome disadvantages of the conventional process. Alternative tempering process is achived by addition/inoculation of form β_V and/or β_{VI} crystal seeds, extracted from cacao butter and/or solid chocolate, to melted chocolate for formation of stable crystals in chocolate. This process is known as “seeding” and crystal seeds are designated as “seed”.

Enrichment of chocolates with several bioactive compounds as well as reducing sugar level or substituting the sugar completely is also ranked among consumer expectations. Probiotics and prebiotic fibers are of capital importance and food products including both of them are defined as synbiotics [16]. Previous studies were conducted to enrich dark chocolate products with probiotics for improving functionality of the products [17-22]. However, in these studies, dark chocolate samples were produced with conventional formulations and production processes. In the study carried out by Konar at al. [23], the chocolate products were manufactured by traditional tempering technique and inulin with different polymerisation degrees (DP>23 and DP<10), *Lactobacillus paracasei* and *L. acidophilus* probiotics were incorporated to chocolate products prepared by sucrose or maltitol and some quality parameters of chocolates and vitality level of probiotics were investigated. Findings of the research showed that usage of inulin with DP lower than 10 and *L. acidophilus* was suggested for both sweetener types considering vitality of probiotic level.

In the present study, the effect of usage of β_V crystal seeds at different concentations on the main quality characterictics of dark chocolate sapmles including inulin with DP lower than 10 as a prebiotic fiber and *L. acidophilus* as a probiotic was investigated.

2. MATERIAL AND METHOD

2.1. Materials

Regarding production of dark chocolate samples, maltitol (Roquette Frenes, Lestres, Fransa), cacao butter, natural and alkalized cacao liquor (Altinmarka, Istanbul, Turkey), inulin (Beneo-Orafti, Belgium), soy lecithin (Brenntag Chemistry, Istanbul, Turkey), polyglycerol polyricinoleate (PGPR) (Palsgaard, Zierikzee, Holland), cacao butter-based β_V seed crystal (SEED100, Uelzena, Uelzen, Germany) and lyophilised *L. acidophilus* (LOT No 41127003932) (Danisco, Niebüll, Germany) were used.

2.2. Production of Dark Chocolates and Inocculation of Probiotics

Maltitol (32.5 g), cacao butter (13.0 g), natural cacao liquor (28.0 g), alkalized cacao liquor (17.0 g), soy lecithin (0.35 g), PGPR (0.15 g) and inulin (DP<10, 9 g) were present in formulation of dark chocolate with 100 g. Batches of 10 kg were prepared for each sample group. For this aim, melted cacao butter (20% of the total cacao butter in the formulation) and maltitol, natural cacao liquor and alkalized cacao liquor were heated to 40°C and mixed until uniformity was obtained. At the end of the mixing and heating process, chocolate mass was pre-refined by three roll refiner (Lehmann, Aaelen, Almanya) and it was heated to 50°C by mixing. Gap of three rolls and pressure leveles were adjusted to achieve desired particle size ranged between 20-25 µm. D_{90} values were controlled by measusing with micrometer (Mitutoyo, Manufacturing Co. Ltd., Japan, 0.001 mm accuracy). After reaching desired particle size, dry-conching process was performed at 70°C for 80 min. After that the residual cacao butter (80% of the total cacao butter), soy lecithin and PGPR were added and wet conching process was conducted. Total conching period lasted 480 min.

Following the conching process, *Lactobacillus acidophilus* (9 log cfu/25 g) was inoculated to chocolate samples cooled to 35°C. After inoculation, mixing process was performed approximately 5 min. For production of control sample, conventional tempering process with 3 stages was employed (33-35°C, 24-25°C and 25-26°C). Suitability of tempering process was checked by temper index values (TI) measured using temper meter (Chocometer, Aasted Farum, Denmark). Tempering process was terminated when TI value of the sample reached to between 5.5-6.0. Regarding pre-crystallization process as an alternative to conventional tempering, seeding technique was performed at 32°C. For this aim, several levels of β_V seeds (0.50, 0.75, 1.00 and 1.50 g seed/100 g chocolate) were added and mixing process was applied for 10 min. Afterwards, moulding and vibration processes (Aasted Farum, Denmark) were carried out at 27-30°C for all sample groups. Later, cooling step was employed at temperature level ranged between 12-14°C for 20 min in cooling tunnel (Aasted Farum, Denmark). The prepared samples were packaged with aluminium foil and stored at 20±2°C until analyses.

Particle size of the chocolate samples produced was measured using micrometer (Mitutoyo, Manufacturing Co. Ltd., Japan, 0.001 mm accuracy). Acquired value represents the largest particle size present in the samples [24]. Particle size of the produced chocolate samples was found to be 20.33 ± 0.58 µm.

2.3. Determination of Vitality of Probiotic Level

Vitality level of probiotics were determined after 1 day of production according to the method reported by Konar et al. [23]. For this aim melted chocolate samples were plated on convenient media (De

Man, Rogosa and Sharpe Agar (Oxoid CM 361)) and after incubation at 37.5 ºC for 48 h, LAB were counted.

2.4. Texture Analyse

Hardness parameter of chocolates was determined by texture analyser equipment (Stable Micro Systems, TA-XT Plus, UK) depending on the method described by Toker et al. [25]. The force required to break chocolate sample was determined using time versus distance graph. Hardness values of the chocolate samples was measured 7 times for each sample.

2.5. Rheological Analyses

Rheological properties of the chocolate samples were determined using stress/strain controlled rheometer Çikolata (Antonpaar MCR 302, Australia). Rheological analyses were conducted at 40°C according to the method described by ICA (International Confectionery Association). The method is composed of 4 steps as the following:

1. Step: Shear rate at 5 s^{-1} was applied for 500 s to homogenise the samples and to equilibrate the temperature
2. Step: Shear rate increased from 2 s^{-1} to 50 s^{-1} within 180 min.
3. Step: The chocolate samples were sheared at for 60 s.
4. Aşama: Sheared rate decreased from 50 s^{-1} to 2 s^{-1} within 180 s.

Casson model parameters (yield stress and plastic viscosity), seen in Eq. 1, were calculated using the data obtained from analyses.

$$\tau^{0.5} = \tau_0^{\,0.5} + \eta_{pl}\,\dot{\gamma}^{n} \qquad (1)$$

where τ is shear stress; $\dot{\gamma}$ is shear rate, τ_0 is yield stress and η_{pl} is plastic viscosity

2.6. Moisture Content

Moisture content of the produced chocolate samples was determined by the method described by Lonchampt and Hartel [26].

2.7. Water Activity

Water activity values of the samples were determined using a_w-meter (Novasina, Switzerland) depending on the method reported by Konar [4].

2.8. Color Measurement

Color parameters (L: brightness, a: ±red-green and b: ±yellow-blue) of the chocolates were measured by colorimeter (Chroma Meter CR-400, Konica Minolta, Japonya). Chroma (C*), hue angle ($h°$) and whiteness index (WI) were calculated using the following equations (Eq. 2-4) [27]:

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

$$h° = \arctan\ (b*/a*) \qquad (3)$$

$$WI = \sqrt{(100-L)^2 + a*^2 + b*^2} \qquad (4)$$

2.9. Sensory Analyses

Sensory properties of the chocolate samples were investigated by multiple-comparison technique [28]. For this aim, 8 trained panelists were evaluated the effect of β_v seed concentration on the sensory parameters of synbiotic chocolates. The evaluated parameters are sweetness, bitterness, fattiness, cacao taste, particle size, lightness, end taste present in mouth, first bite, color, melting in mouth, smootness and apperance which were scored to between 1 (very bad) and 10 (very good).

2.10. Statistical Analyses

All of the results were expressed as average ± standard deviation. The results were analysed with ANOVA and the significance level between samples were determined by Tukey's Test.

Statistical analyses were performed by MINITAB-Express (Minitab Inc., State College, PA, ABD) and MSTAT (Michigan State University, East Lansing, MI, ABD) software programs (p<0.05).

3. RESULTS AND DISCUSSION

Spesific surface area and D_{90} values are critical parameters of particle size analyses which are related with the quality characteristics of chocolates. Beckett [29] reported that these two parameters are also important in production process. Particle size affects the sensory, textural and rheological properties of chocolates. In addition, it also plays an important role in pumping, mixing, transportation and stability of solid-liquid mixtures [30].

In the present study, in order to clearly observe the effect of β_V seed concentration on the quality properties of the samples, the other factors such as particle size was standardized for abolishing its effect. D_{90} parameter of chocolate samples was found to be 20.33 and addition level of β_V did not significantly influence this parameter (P>0.05) since very low amount of seed was added to the formulation, which did not result in remarkable differences in the D_{90} value. Similar particle size of the chocolates prepared with conventional and seeding tempering methods is important for the desired quality of the samples. In addition, similar production process can be used for the chocolates be manufactured by seeding technique.

3.1. Probiotic Vitality Level

As mentioned above, *L. acidophilus* was inoculated at concentration of 9.0 log cfu/25 g to dark chocolate samples including maltitol and inulin. There are different views for definition of probiotic foods. For example in Canada and Italy, for probiotic statement in label, the probiotic level should be 9.0 log cfu/serving size or day and general acceptance for serving size of the chocolate is 25 g [16]. However, Maragkoudakis et al. [31] reported that 5 – 8 log cfu/g probiotic load can be acceptable for dairy products. In the present case, stability of probiotics during process and shelf life as well as inoculation level is of capital importance.

In this study, regarding for all chocolate groups, the loss of probiotic vitality due to production process was found to be approximately 0.50 – 1.50 log cfu/25 g (Table 1). This loss level is consistent with the previous studies where different probiotics were used in chocolate or chocolate-based products [21, 23, 32]. According to the results, pre-crystallization technique also influenced the probiotic vitality level (P<0.05). Highest vitality level was observed in dark chocolate sample prepared with conventional tempering process and application of seeding technique resulted in decrease in vitality level of probiotics.

In the production process of the chocolate tempered with β_V seed crystals, mixing process required after seed addition should be optimized depending on formulation of the products or ingredinets present in the media [33]. Mechanical force applied during mixing process can adversely affect the vitality level of probiotics. Change percentage of vitality level due to applied process is shown in Table 1. Probiotic dark chocolate production can be achieved by adjusting inoculation level considering the losses observed during manufacturing. In conventional tempering, flow is conducted at different temperature zones without mechanical forces; therefore there is no mechanic stress for probiotics. However, production of synbiotic or probiotic chocolate with β_V seeding technique, probiotic loss can be decreased by optimizing mixing process employing for achievement of desired TI values (4.0-6.0 TI).

Table 1. Vitality level of *Lactobacillus acidophilus* in dark chocolate samples and loss of them during processes.

β_V Crystal Amount	*L. acidophilus* enumaration (log cfu/25 g)	Loss in Probiotic Level (%)
0.00 g/100g (Control)	8.556 ± 0.126 [a]	4.93
0.50 g/100g	7.543 ± 0.034[c]	16.18
0.75 g/100g	7.992 ± 0.131[b]	11.20
1.00 g/100g	7.964 ± 0.099[b]	11.51
1.50 g/100g	8.042 ± 0.187[b]	10.64

Different letters show significant differences between corresponding quality parameters of the sample (p<0.05).

3.2. Moisture Content and Water Activity

Water activity can provide information about microbiological, physical and chamical stability of the food products. This crucial parameter is also taken into consideration for controlling of raw materials used in the product and for determining production process effects. Water activity and content of the chocolates are influenced by raw material, specific surface are of the products and temperature levels which applied during refining and conching processes [34, 35]. In addition water activity affects the storage stability of the products especially the fat bloom observed.

As water activity of the dark chocolates including maltitol veried between 0.182-0.212, moisture content was between 0.79 g/100 g – 0.86 g/100 g (Table 2). Generally, chocolate products have water activity lower than 0.40. Also, it is aimed for all chocolate samples with water content lower than 1.5 g/100 g. Chocolates produced with polyols instead of sucrose had lower water activity values [36], which can associated with hydrophilic characteristics of ingredients. In addition, using inulin in chocolate also resulted in decrease in water activity [4].

Regarding β_v seeding technique, remarkable reduction was observed in the chocolates especially for the ones prepared by 1.00 and 1.50 % seeds, no significant differences was found between water content of the samples (P<0.05). After proper tempering, fat crystal network is fimly packed in chocolate, which reduces water and fat transportation [37]. The results showed that seeding technique is a good alternative to conventional method for synbiotic dark chocolate production in terms of water activity and moisture content parameters.

Table 2. Rheological parameters, water activity, water content and textural properties of dark chocolate samples.

β_V Crystal Amount	Yield stress (σ_0, Pa)	Plastic viscosity (Pa.s)	a_w	Water content (%)	Hardness (g)
0.00 g/100g	31.20 ± 2.95[a]	3.050 ± 0.341[a]	0.212 ± 0.005[a]	0.81 ± 0.05[a]	8526 ± 796[a]
0.50 g/100g	27.87 ± 2.98[a]	2.724 ± 0.217[ab]	0.212 ± 0.009[a]	0.86 ± 0.02[a]	8665 ± 381[a]
0.75 g/100g	26.49 ± 3.17[a]	2.590 ± 0.198[ab]	0.206 ± 0.004[a]	0.80 ± 0.03[a]	8804 ± 361[a]
1.00 g/100g	26.09 ± 4.21[a]	2.551 ± 0.201[ab]	0.195 ± 0.010[ab]	0.79 ± 0.03[a]	8826 ± 414[a]
1.50 g/100g	24.05 ± 3.76[a]	2.351 ± 0.119[b]	0.182 ± 0.006[b]	0.81 ± 0.05[a]	7594 ± 640[a]

Different letters show significant differences between corresponding quality parameters of the sample (p<0.05).

3.3. Textural Properties

Texture is another quality parameter for chocolate and its derivatives, the snap sound formed during breaking of chocolate is related with textural characteristics. Hardness is important textural parameter for chocolate which is equal to the required force necessary for penetrating of probe into chocolate at desired level or for breaking of the sample. Product formulation, production process and parameters affect hardness of the end product [14, 23, 29, 33]. Generally, sugar-free chocolates had lower hardness values when compared with the ones including sucrose [38].

Hardness values of the synbiotic chocolate produced in the study were found to between 7595 g and 8826 g (Table 2). Applied pre-crystalization technique and seeding level did not significantly affect hardness value (P>0.05). Fatty acids crystallise as binary or tertnary chain depending on the triglyceride composition and position. β_V crystal form enables ternary closed packaging system; therefore, thermodynamic stability increases [8]. Therefore, there can be relation between hardness and fatty acid composition [39]. In addition, hardness is an important indicator of tempering efficiency and formation of fat crystal network. Hardness value of chocolate depends on crystallization of cacao butter, crystal size and morphology and polymorphic behavior [38]. According to the results, seeding technique can be satisfactorily used in production of synbiotic bitter chocolates including maltitol.

3.4. Rheological Properties

Flow behavior of melted chocolates takes part among main quality parameters. Rheological parameters of chocolate influence designing of production process (temperature level of processes, mixing, transporation, pumping), and removal of water and air during production. Therefore, these quality characteristics direclty affects the quality parameters. Applied shear rate versus shear stress data for chocolate were modelled with Casson (especially), Herschel-Bulkley and Bingham models. Yield stress and plastic viscosity parameters were evaluated for rheological characterization. Importance of rheological parameters in sensory charactersistics and designing of production process is forced to be considered them during the manufacturing of the products with desired quality [4].

Slight but not significantly important decreased was observed in yield stress values of the synbiotic chocolate including maltitol as a result of β_V seed used for pre-crystallization purpose (P<0.05). This result can be seed as an advantage for seeding technique since no significant production process change is required. Plastic viscosity values also decreased with increasing β_V seed concentration (Table 2) (P<0.05). Texture analysis results are in accordance with rheologica parameters [4]. Deviations of these parameters from suitable ones can be eliminated by increasing PGPR level to decrease yield stress and change in lecithin level for modifying viscosity of samples [23].

3.5. Color Properties

Visual characteristics such as brightness, roughness, turbidity, transparency and color properties can be used for defining of chocolate appearance [39]. The preliminary parameter being associated with the food quality is visual characteristics, mainly color, affect positively or negatively consumer acceptability of the products. Regarding chocolate, color and lightness are the most important quality parameters. For this aim, in the study, brightness (L*), chroma (C*), hue angle ($h°$) and whiteness index (WI) values were investigated (Table 3).

Table 3. Color properties of synbiotic dark chocolates.

β_V Crystal Amount	$L*$	$C*$	$h°$	WI
0.00 g/100g	26.9 ± 0.21^{b}	7.31 ± 0.25^{a}	0.72 ± 0.03^{a}	73.4 ± 0.19^{a}
0.50 g/100g	27.2 ± 0.13^{ab}	7.61 ± 0.10^{a}	0.72 ± 0.02^{a}	73.2 ± 0.12^{ab}
0.75 g/100g	27.2 ± 0.25^{ab}	7.28 ± 0.45^{a}	0.73 ± 0.05^{a}	73.2 ± 0.23^{ab}
1.00 g/100g	27.8 ± 0.20^{a}	7.33 ± 0.19^{a}	0.69 ± 0.01^{a}	72.6 ± 0.21^{b}
1.50 g/100g	27.2 ± 0.48^{ab}	7.65 ± 0.13^{a}	0.70 ± 0.01^{a}	73.2 ± 0.47^{ab}

Different letters show significant differences between corresponding quality parameters of the sample (p<0.05).

For all chocolate types, surface brightness positively affected consumer perception [4]. Chocolate brightness increased with seed usage (P<0.05); however, concentration is not significantly affect this parameter. Pre-crystlization technique and seed concentration did not significantly influence C^* and h° parameters (P>0.05). WI is included in the scope of this study since it is correlated with fat and sugar bloom, main quality defects of chocolate. Conducting pre-crystallization with seeding technique resulted in reduction of WI values (P<0.05). No regular trend was observed between *WI* values and seed concentration; however, using seed at 1.00 g/100 g level is the most suitable level regarding all the color parameters analysed.

Moisture content, pH, ingredients of chocolate as well as tempering process also affect the color parameters [40]. Color of foods gives clues about various factors such as poperties of raw materials, applied production technology, storage conditions and period. Regarding chocolate, color change is generally resulted from variation in formulation and process conditions [38]. Proper tempering of chocolate improves brightness, color and moulding properties of chocolates [40]. The results of the present study implied that employind seeding technique can provide advantage in production of synbiotic dark chocolate including maltitol in terms of color parameters.

3.6. Sensory Properties

Chocolate quality is also determined by subjective evaluaitons such as sensory characteristics in addition to objective analyses such as texture, rheology and color measurements. Chocolate quality can be defined by appearance, taste, texture, last taste perception in mouth and satisfaction level of consumers. Origin, variety, harvesting and processing conditions of cacao as well as chocolate production steps can generate variation in sensory properties of chocolates such as taste, texture and appearance. Preference of conscious/unconscious consumers is based on the sensory characteristics of the products [28, 32]; therefore, it is inevitable part of product development process.

In the present study, sensory evaluation was performed by research and development experts using multiple comparision technique. Sweetness, bitterness, cacao taste, fattiness, particle size, lightness, last taste in mouth, first bite, color, melting in mouth, roughness, texture and appearance properties were scored with high points (Figure). Generally, no significant difference was observed between sensory results for all parameters evaluated (P>0.05). Sensory analysis results also indicated that seeding technique can be used as an alternative method in synbiotic dark chocolate production as well as in other quality parameters considered.

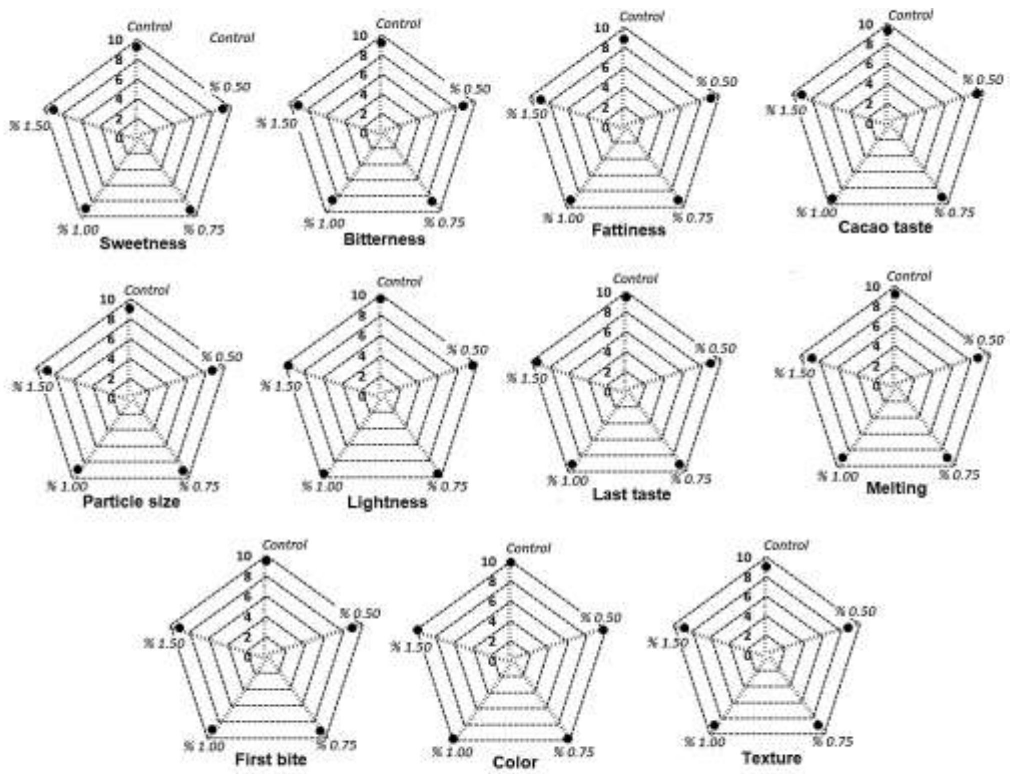

Figure. Sensory properties of synbiotic dark chocolate including βV seeds at different concentrations.

4. CONCLUSIONS

In functional food development studies, the improvement of products having similar quality characteristics with conventional ones is of great importance for consumer acceptance. At the same time, the consumers' expectation is that the bioactive components of these foodstuffs have stability over their shelf life and have the potential to show potential effects, especially those declared by the label. As a result of this study, probiotic and synbiotic chocolate development studies, the effects of the changes tending to process optimization or using alternative technologies on the viability of probiotics have been demonstrated. Although there are no negative changes in quality characteristics, the determination of higher probiotic viability in the products manufactured with conventional tempering may be indicated as a disadvantage for the use of seeding techniques in functional chocolate development studies. However, this effect needs to be examined in different chocolate matrices. In addition, researches aimed at eliminating this disadvantage are particularly important with the optimization studies carried out in the application of seeding for pre-crystallization process. This is because the use of alternative pre-crystallization techniques has the potential to significantly increase the availability of chocolate production with improved storage stability as well as high efficiency production at low energy costs. Depending on the seeding level, production process (mixing time and rate) should be optimized. The results of the present study also indicated that faster production is possible by using seeding technique, which can provide advantage to the manufacturers.

Acknowledgment

The present study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No: TEYDEB-3140541).

REFERENCES

[1]. Belscak-Cvitanovic A., Komes Dujmovic M., Karlovic S., Biskic M., Brncic M. and Jezek D., Physical, bioactive and sensory quality parameters of reduced sugar chocolates formulated with natural sweeteners as sucrose

alternatives, Food Chemistry, 167 (2015) 61-70.

[2]. Olinger P.M., New options for sucrose-free chocolate. The Manufacturing Confectioner, 74(5) (1994) 77–84.

[3]. Sokmen A. and Gunes G., Influence of some bulk sweeteners on rheological properties of chocolate, LWT-Food Science and Technology, 39 (2006) 1053–1058.

[4]. Konar N., Influence of conching temperature and some bulk sweeteners on physical and rheological properties of prebiotic milk chocolate including containing inulin, European Food Research and Technology, 23 (2013) 135-143.

[5]. Konar N., Ozhan B., Artik N. and Poyrazoglu E.S., Using polydextrose as a prebiotic substance in milk chocolate-Effects of process parameters on physical and rheological properties, CyTA Journal of Food, 12-2 (2014) 150-159.

[6]. Konar N., Ozhan B., Artik N., Dalabasmaz S. and Poyrazoglu E.S., Rheological and physical properties of ınulin-containing milk chocolate prepared at different process conditions. CyTA Journal of Food, 12-1 (2014) 55-64.

[7]. Melo L.L.M.M., Bolini H.M.A. and Efraim P., Storage time study of sugar-free and reduced calorie milk chocolates. Department of Food and Nutrition FEA, UNICAM Campinas, Brazil, CEP (2008) 13083-130862.

[8]. Afoakwa E.O., Paterson A. and M. Fowler M., Factors influcing rhelogical and textural qulities in chocolate- a review, Trend in Food Science and Technology, 18 (2007) 290-298.

[9]. Dhonsi, D. and Stapley A.G.F., The effect of shear rate, temperature, sugar and emulsifier on the tempering of cocoa butter, Journal of Food Engineering, 77 (2006) 936-942.

[10].Glicerina V., Balestra F., Rosa M.D. and Romani S., Rheological, textural and calorimetric modifications of dark chocolate during process, Journal of Food Engineering, 119-1 (2013) 173-179.

[11].Afoakwa E.O., Paterson A., Fowler, M. And Vieira J., Particle size distribution and compositional effects on textural properties and appearance of dark chocolates, Journal of Food Engineering, 87 (2008) 181-190.

[12].Dahlenborg H., Millqvist-Fureby A. and Bergenstahl B. Effect of shell microstructure on oil migration and fat bloom development in model pralines, Food Structure, 5 (2015) 51-65.

[13].Debaste F., Kegelaers Y., Liegeois S., Ben Amor H. and Halloin V., Contribution to the modeling of chocolate tempering process, Journal of Food Engineering, 88 (2008) 568-575.

[14].Afoakwa E.O., Paterson A., Fowler M. and Vieira J., Effects of tempering and fat crystallization behaviour on microstructure, mechanical properties and appearance in dark chocolate systems, Journal of Food Engineering, 89 (2008) 128-136.

[15].Bolliger S., Breitschun B., Stranzinger M., Wagner T. and Windhab E.J., Comprasion of precrystallization of chocolate, Journal of Food Engineering, 35 (1998) 281-297.

[16].Konar N., Toker O.S., Oba S. and Sagdic O., Improving functionality of chocolate: A review on probiotic, prebiotic, and/or synbiotic characteristics, Trends in Food Science & Technology, 49 (2016) 35-44.

[17].Maillard M. and Landuyt A., Chocolate: an ideal carrier for probiotics. Agro Food Industry Hi-Tec, 19-3 Suppl. (2008) 13–15.

[18].Coman M.M., Cecchini C., Verdenelli M.C., Silvi S., Orpianesi C. and Cresci A., Functional foods as carriers for SYNBIO, a probiotic bacteria combination, International Journal of Food Microbiology, 157 (2012) 346-352.

[19].Erdem O., Gultekin-Ozguven M., Berktas I., Ersan S., Tuna H.E., Karadag A., Ozcelik B., Gunes G. and Cutting S.M., Development of a novel synbiotic dark chocolate enriched with Bacillus indicus HU36, maltodextrin and lemon fiber: Optimization by response surface methodology, LWT – Food Science and Technology, 56 (2014) 187-193.

[20].Champagne C.P., Raymond Y., Guertin N. and Belanger G., Effects of storage conditions, microencapsulation and inclusion in chocolate particles on the stability of

probiotic bacteria in ice cream, International Dairy Journal, 47 (2015) 109-117.

[21].Lalicic-Petronijevic J., Popov-Raljic J., Obradovic D., Radulovic Z., Paunovic D., Petrusic M. and Pezo L., 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, Journal of Functional Foods, 15 (2015) 541-550.

[22].Raymond Y. and Champagne C.P., The use of flow cytometry to accurately ascertain total and viable counts of *Lactobacillus rhamnosus* in chocolate, Food Microbiology, 46 (2015) 176-183.

[23].Konar N., Palabiyik I., Toker O.S., Genc Polat D., Sener S., Akcicek A. and Sagdic O., Effect of Inulin DP on various properties of sugar-free dark chocolates containing *Lactobacillus paracasei* and *Lactobacillus acidophilus*. International Journal of Food Engineering, doi: 10.1515/ijfe-2017-0045 (2017).

[24].Beckett S.T. The science of chocolate (2nd Ed.) London, Cambridge, UK: Royal Society of Chemistry (2008).

[25].Toker O.S., Sagdic O., Konar N., Zorlucan T. and Dağlıoğlu O., Some physicochemical, rheological and melting properties and volatile compound profile of compound chocolate and cocolin samples: Effect of particle size, European Food Research and Technogy, 242-8 (2016) 1253-1266.

[26].Lonchampt P. and Hartel R.W., Surface bloom on improperly tempered chocolate, European Journal of Lipid Science and Technology, 108-2 (2006) 159-168.

[27].Periche A., Heredia A., Escriche I., Andrés A. and Castelló M.L., Potential use of isomaltulose to produce healthier marshmallows, LWT - Food Science and Technology, 62 (2015) 605-612.

[28].Shah A.S., Jones G.P. and Vasiljevic T., Sucrose-free chocolate sweetened with Stevia rebaudiana extract and containing different bulking agents effects on physicochemical and sensory properties, International Journal of Food Science and Technology, 45 (2010) 1426-1435.

[29].Beckett S.T. Industrial chocolate manufacture and use, (3rd Ed.). Blackwell, Oxford, 1999.

[30].Ziegler G. and Hogg R., Particle size reduction, In: Industrial chocolate manufacture and use, Beckett ST. (Ed.), Chapman § Hall, New York, USA, (1999) 182-199.

[31].Maragkoudakis P.A., Miaris C., Rojez P., Manalis N., Magkanari F. and Kalantzopoulos G., Production of traditional Greek yoghurt using *Lactobacillus* strains with probiotic potential as starter adjuncts, International Dairy Journal, 16 (2006) 52-60.

[32].Zaric D.B., Bulatovic M.L.J., Rakin M.B., Krunic T.Z., Loncarevic I.S. and Pajin B.S., Functional, rheological and sensory properties of probiotic milk chocolate produced in a ball mill, RSC Advances, 6 (2016) 13934-13941.

[33].Konar N., Oba S., Toker O.S., Palabiyik I., Goktas H., Artik N. and Sagdic O., Rapid tempering of sucrose-free milk chocolates by β_V seeding: textural, rheological and melting properties, European Food Research and Technology, 243 (2017) 1849-1860.

[34].Vercet A. 2003. Browning of white chocolate during storage, Food Chemistry, 81 (2003) 371-377.

[35]. Rossini, K., Norena C.P.Z. and Brandelli A., Changes in the colour of white chocolate during storage: potential roles of lipid oxidation and non-enzymatic browning reactions, Journal of Food Science and Technology, 48-3 (2011) 305–311.

[36].Shadwell, N., Villalobos F., Kern M. and Hong M.Y., Blooming reduces the antioxidant capacity of dark chocolate in rats without lowering its capacity to improve lipid profiles, Nutrition Research, 33 (2013) 414-421.

[37].Afoakwa, E.O., Paterson A., Fowler M. and Vieira J., Microstructure and mechanical properties related to particle size distribution and composition in dark chocolate, International Journal of Food Science and Technology, 44-1 (2009) 111–119.

[38].Aidoo R.P., Afoakwa E.O. and Dewettinck K., Rheological properties, melting

behaviours and physical quality characteristics of sugar-free chocolates processed using inulin/polydextrose bulking mixtures sweetened with stevia and thaumatin extracts, LWT - Food Science and Technology, 62 (2015) 592-597.

[39]. Aidoo R.P., De Clerq N., Afoakwa E.O. and Dewettinck K., Industrial manufacture of sugar-free chocolates- applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development, Trends in Food Science and Technology, 32 (2013) 84-96.

[40]. Lindecrantz A. 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, Chalmers University of Technology, Gothenburg, Sweden, 2014.