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Effect Of Non-Thermal Processing On The Tempering Behavior Of Dark Chocolate.

Florentina Priyangini, Vuchooru Gayathri, Tesnia Grace George, Sruthi Prathapan, and Chidambaram Ramalingam*.

Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Tamil Nadu, India

ABSTRACT

Tempering chocolate fosters the development of the required form of cocoa butter crystals. In this study, the effect of ultrasonicator bath was used in the chocolate tempering process. Optimization of the chocolate sample was studied using scanning electron microscopy (SEM). X-ray diffraction (XRD) analysis confirmed the β2 form - the Vth cocoa butter polymorph conferring a d-spacing value of 4.59 Å which is noteworthy for its dense and stable crystal structure. Atomic force microscopy (AFM) signifies that the tempered form exhibited the relative absence of bloom with a smoothened surface. Further, the tempered chocolate was found to contain a percentage scavenging 48.08±0.2% in terms of free radical scavenging activity and 2.83±0.2 mg GAE/g phenolic content. The study suggests that the optimized tempering process enhanced the shelf life of chocolate on a 1:3 ratio timeline and also added to its sensorial attributes. In summary, a new perspective on the use of ultrasonicator bath as a non-thermal tempering technique that is a boon to the dark chocolate manufacturing process is studied.

Keywords: Tempering; Non-thermal; Surface topology; Free choice profile; Polymorphism

Corresponding author

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INTRODUCTION

Chocolate is a heterogeneous concoction of fine solid particles, viz., cocoa solids, sugar and milk proteins, dispersed in a continuous phase constituting fats, customarily cocoa butter and milk fat, based on the type of chocolate[1]. The production of chocolate involves the use of cocoa bean (Theobroma cacao) as the crude source for its fabrication, following fermentation to shed the pulp adjoining the beans and aids in unfolding characteristic tastes and flavors of chocolate[2]. Chocolates are solid at ambient (20–25 °C) and melt at oral temperature (37 °C) during consumption giving a smooth suspension of particulate solids in cocoa butter and milk fat[3] [4].Chocolate, intended to give a sensorial experience to its consumers in view of its rich, smooth, creamy and sweet taste, includes mainly three types: white, milk and dark chocolate. Dark chocolate contains at least 35% chocolate liquor, cocoa butter and sugar (Code of federal regulations Title 21). The cocoa butter is accountable for the crunch, crispiness, lustrous appearance and melting properties of chocolate[5]. Cocoa butter, largely composed of triglycerides, can crystallize in 6 different polymorphic forms, denoted by roman numerals I-VI, which is governed by its triacylglycerol composition and conditions specific to its crystallization prerequisites, viz., temperature, rate of cooling, etc. Of these polymorphs, the beta crystal (Form V) is reckoned as the most stable confirmation creating a chocolate possessing the coveted finish and consistency that is not subject to degradation over time[6]. This β form is acquired through a tempering process that is subject to a very fixed temperature profile[7]. The more compact the organization of the crystals, the more prominent will be the stability, quality and refinement of the chocolate, inversely affecting the sensory characteristics, viz., taste, color, odor, texture and in turn, a highly sought-after and superior grade chocolate [3].

The routine process of tempering incorporates melting of the chocolate at elevated temperatures followed by cooling of the melted chocolate, leading to the production of crystal nuclei around which the remaining fatty acids continue to crystallize[8]. One of the major obstacles overlaying the tempering process is the emergence of fat bloom, which is signalized by an off-white or whitish haze on the surface giving rise to a disfigured and non-glossy chocolate that also modifies the internal attributes of the chocolate. Presence of ruptured triacylglycerol's and storage of tempered chocolate at intense temperature leads to the form VI polymorph further aggravating bloom formation[9].

According to previous literature[10], the traditional thermal tempering of chocolate is accompanied by excessive time lag, depletion of vital nutrients and vitamins, color, texture and flavors. The intent of this research was to assess an alternative to the conventional approach employing non-thermal tempering of chocolate which would yield chocolate having improved consumer appeal, shelf life, attractiveness, increased palatability, taste and health perks without compromising on the textural and chemical attributes[10].We report here the use of ultrasonicator bath as a means for the non-thermal tempering of dark chocolate representing a novel and innocuous technique, which is susceptible to changes in crystal size and polymorphism[11]. Extended studies were performed for the effective understanding of the tempered chocolate characteristics.

MATERIALS AND METHODS

Preparation of chocolate

Commercially available good quality fermented dry cocoa beans were procured from Campco chocolate factory (Mangalore, India) as cocoa beans are the basic raw material for the production of chocolate which has a notable role in ascertaining the composition and flavor of the chocolate. The chocolate was prepared according to the method stated by Are et al.[12] by imparting few modifications. The beans were refined to reduce the particle size and were ground aseptically in a grinder at low speed for a period of 6 hours. The chocolate was subjected to conching by addition of emulsifier- soy lecithin (E322) for the even distribution of cocoa butter within the chocolate. The conched chocolate was further tempered and samples were sealed in air tight plastic containers until further use.

Tempering of chocolate

The tempering of the conched chocolate samples was carried out by an ultrasonicator bath (BVN Instruments, Madras Private Ltd, India) which contained an agitation unit consisting of a reaction tank

operating at a temperature of 32°C and possessing a uniform frequency throughout the process. Conched chocolate samples containing sealed tubes were placed in the ultrasonic reaction tank. The samples were subjected to tempering for a time period of 30 minutes under the above mentioned operating conditions. Subsequently, the samples were collected at an interval of 5 minutes and stored.

Characteristics of tempered chocolate

The optimized condition for tempering chocolate employing ultrasonicator bath was determined by further scrutiny using SEM analysis and was assessed using extended characterization techniques.

SEM analysis

The prepared chocolate smear samples were sputter coated before they were analyzed by scanning electron microscopy (ZEISS-EV018, USA). The sample was then visualized at different magnifications for determining its microstructure[13]. The structural behavior of the tempered chocolate was unveiled at various time intervals so as to attain a better understanding of the optimized conditions of non-thermal tempering process by means of ultrasonicator bath.

Chocolate polymorphism

The various crystal polymorphs of cocoa butter in the molded and freezed chocolate, after it was non-thermally tempered were investigated using X-ray diffraction (XRD) analysis by means of X-ray powder diffractometer (Bruker D8 Advance, Germany) which commonly inculcates the use of Cukα radiation of wavelength 1.54Å[14].

Surface topology

Another fresh set of sample smears were prepared and taken from the environmental chamber and were subjected to surface topology analysis using atomic force microscopy (AFM). High resolution images of 5× 5µm and 10 × 10 µm areas of the chocolate were obtained using an Atomic Force Microscope (Nanosurf, Switzerland) for evaluating the surface topographical characteristics of chocolate[15]. AFM was operated at 0.8 seconds under static force operating mode. The tip voltage employed was of 0 nV which comprised of a cantilever type ContAl-G. Chocolate roughness was determined using the software (Software ver. 3.1.0.22) provided by the AFM manufacturer.

Shelf life analysis

The tempered chocolate sample was incubated in an environmental test chamber (Remi programmable chamber-396LAG, India) for a period of 10 days at 50°C and at a relative humidity of 65-70% for the shelf life analysis. Fresh chocolate extract was prepared and cultured in nutrient agar by means of spread plate method. This was done to check for microbial contamination after a 24 hour duration for 10 day period at an interval of 5 days for determining the shelf life of the chocolate prepared[16].

Sensory analysis

The quality and consumer acceptance of the prepared chocolate was assessed using the sensory evaluation test[17]. The sensory characteristics of the product were deduced using quantitative descriptive testing (QDT) employing the free choice profile (FCP) testing 6 different characteristics of chocolate, viz., appearance, odor, texture, color, bloom formation, and masticating properties, possessing two descriptors each namely, glossy or dull finish, chocolaty or roasted, smooth or soft uneven, even brown or uneven with white patch, no bloom or fat bloom and melty or chewy respectively. In this test, a group of 20 untrained panelists of different age groups, were provided with both molded untempered and tempered chocolate along with an evaluation form to record their freely perceived responses in terms of 12 descriptors. 7 point hedonic testing pertaining to three parameters namely preference, texture and appearance of chocolate was assessed on a number scale of 1-7 inferring ‘disliked very much’ (1) to ‘liked very much’ (7)[18].

Estimation of antioxidant activity and total phenolic content

Total phenolic content of prepared tempered dark chocolate was determined using the method described by Singleton & Rossi[19]. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed as per the method stated by Yu et al.[20]to determine the anti-oxidant properties of the prepared chocolate.

RESULTS AND DISCUSSION

Tempering of prepared chocolate employing ultrasonicator bath

The chocolate samples after being tempered for 5, 10, 15, 20, 25 and 30 minutes were observed and it was deduced that the chocolate sample tempered for 5 minutes exhibited a glossy appearance in contrast to the remaining samples which gave off a rough appearance and smoky odor. The glossy and polished fine quality chocolate bar was prepared after it was optimized at 5 minutes using ultrasonicator bath and was further molded as shown in Fig.1.

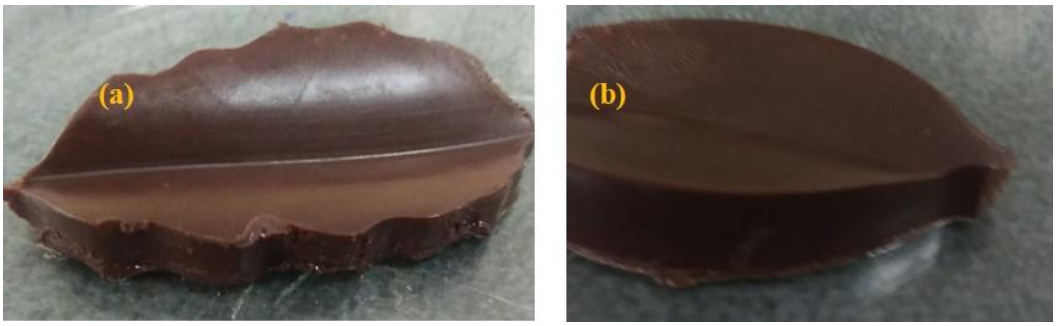

Fig 1: Molded chocolate:(a) untempered (b) tempered.

SEM analysis

Representations of the microstructures of tempered and untempered chocolate, optimized at varying time intervals are shown in Fig. 2. Images reveal marginal distinctions between the chocolate samples. The control sample was that of untempered chocolate having a rough rugged surface. In contrast, the tempered sample at 5 minutes rendered a well-defined and velvety surface. The samples optimized at the 10^{th}, 15^{th}, 20^{th}, 25^{th} and 30^{th} minute were displayed in the SEM images as disintegrated and corrugated surfaces. The visible spaces portray areas where the fat has been deposited. From Fig. 2.it is observed that there is a pronounced difference in the viscosity of the non-thermally tempered sample at the 5^{th} minute as compared to the untempered samples and samples tempered from the 10^{th} to the 30^{th} minute. The perceivable differences denoted that the sample optimized at 5 minutes had a preferable and superior higher-grade texture without any distortions in its structure and also exhibited a glossy appearance. Hence it was considered as a sample of choice, optimized to be utilized for further analysis and the results revealed that the mechanism underlying the non-thermal tempering of dark chocolate produced a chocolate sample where the solid particles were securely and compactly arranged giving rise to a uniform structure and network that combats deformation.

Chocolate polymorphism

Typical XRD diffractograms of both tempered and untempered chocolate samples were shown in Fig. 3a & 3b respectively. By employing Bragg's Equation, the corresponding d-spacing values were calculated for experimentally obtained 2θ values;

$$d = n\lambda/2\sin(\theta)$$

Where, λ is the wavelength of the source, d is the d-spacing and θ is the angle between the incident and the diffracted wave. The d-spacing value for the prominently visible highest peak was figured out as 4.59 Å which is in compliance with the expected standard value for β_2 V form peak[14]. Hence, the chocolate

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processed by means of non-thermal tempering is in the desirable β_2 V polymorph form, which reciprocates on its overall quality by virtue of reduced fat blooming problems and better melting points.

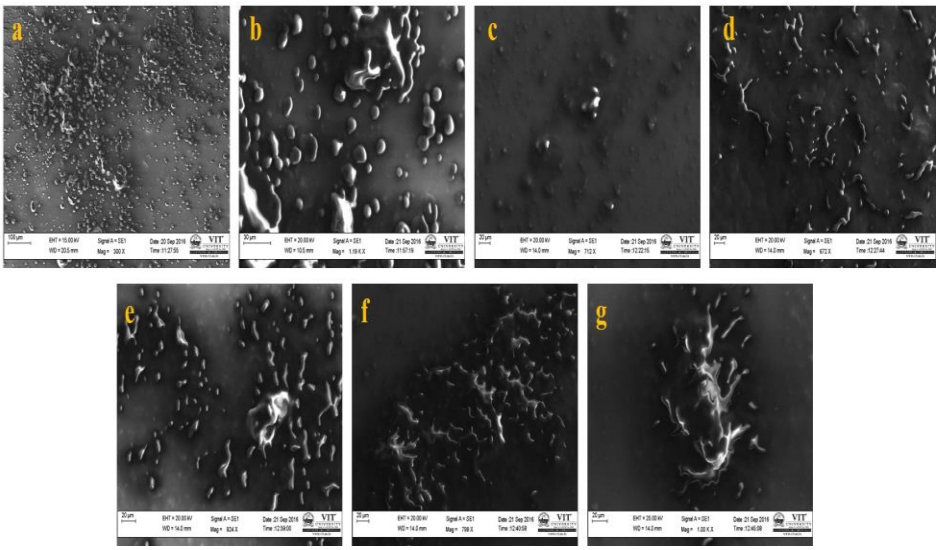

Fig 2: SEM images at various magnifications: (a) untempered chocolate. Tempered chocolate at (b) 5 mins. (c) 10 mins.(d) 15 mins.(e) 20 mins.(f) 25 mins. (g) 30 mins.

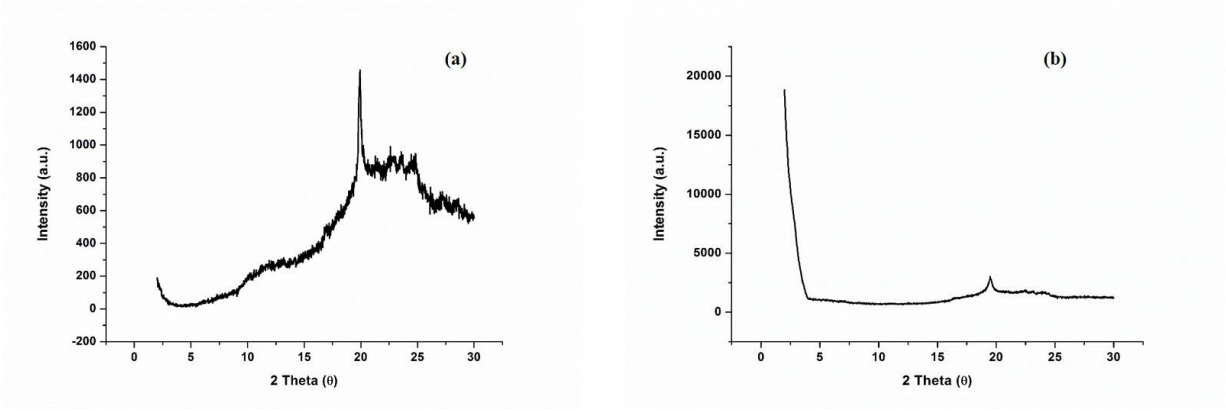

Fig 3: X-ray diffractogram of (a) untempered chocolate.(b) tempered chocolate.

Surface topology

The surface topography and structural elements of the dark chocolate were examined by an atomic force microscope (AFM) used to image 5 x 5 µm and 10 x 10 µm planes of the chocolate smear. The plane surface of the chocolate smear exhibiting negligent bloom displayed a smooth and sleek surface with uniformly dispersed crystals (Fig. 4b & 4d). The samples run at 32°C in the ultrasonicator bath revealed no observable presence of bloom The AFM images of the untempered chocolate smear sample revealed a slight manifestation of surface bloom, exhibiting a corrugated and rough surface texture, thus implying the presence of uneven and unstable crystal polymorphs when compared to that of tempered chocolate (Fig. 4a & 4c). The increase in roughness of the untempered chocolate can well be thought of as an indicator of fat bloom formation wherein bloomed chocolate consists chiefly of the form I-IV crystal polymorphs. These are considered fairly less stable in form and diminish quickly and act as templates for their own transformation into more stable polymorphs V and VI causing the occurrence of bloom. Yet another possible reason for the formation of bloom is the residence of triacylglycerides in the cocoa butter matrix, each possessing a distinct melting point. When the chocolate is subject to high inflated temperatures, the TAG's possessing low melting points melt to form a liquid phase suspension, which migrates through the pores and crevices of the chocolate internal structure, upto the surface enfolding as structures featuring bloom formation[15].

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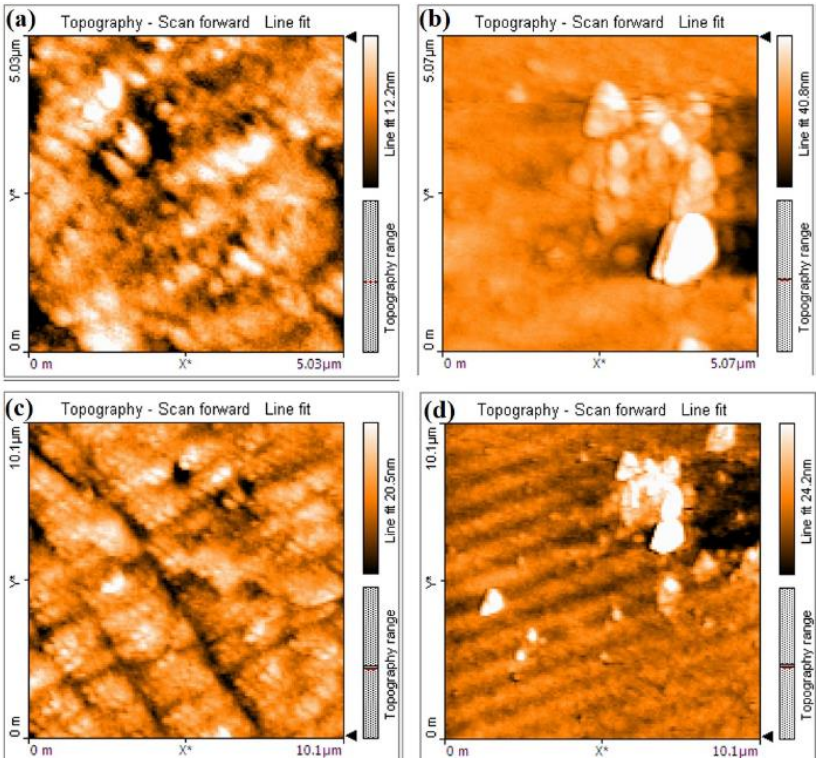

Fig 4: AFM images depicting surface roughness in(a) untempered chocolate (5x5 µm) (b) tempered chocolate (5x5 µm) (c) untempered chocolate (10x10 µm)(d) tempered chocolate (10x10 µm).

Shelf life analysis

Microbial analysis was performed for both the tempered and untempered samples to ascertain the shelf life of the chocolate to scrutinize the ability of chocolate to conserve its freshness, physical, chemical and microbiological qualities before the setting in of bloom or rancidity which would compromise on its sensorial quality and visual appeal. The nutrient agar plates were visually assessed on the 0^{th}, 5^{th} and 10^{th} day for both samples as shown in Fig. 5.

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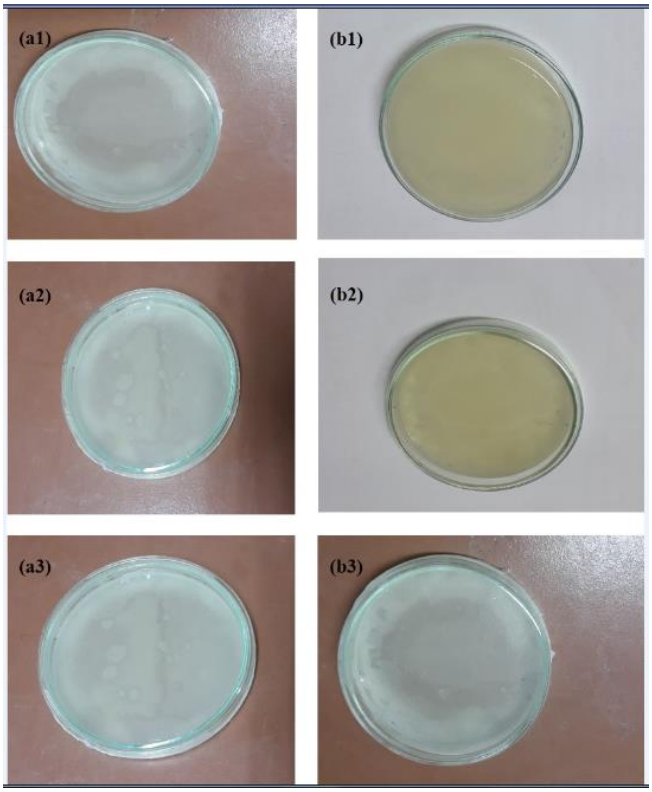

Fig 5: Untempered chocolate on (a1) 0th day (a2) 5th day (a3) 10th day. Tempered chocolate on (b1) 0th day(b2) 5th day (b3) 10th day.

As observed from Fig. 5b1, 5b2 & 5b3, the tempered chocolate samples showed a reduction in the microbial content. The tempered chocolate samples visualized on day 0 and day 5 exhibited negligible contamination while the sample visualized on day 10 revealed the onset of microbial inhabitation. In contrast, the untempered chocolate samples showed an increase in microbial contamination visible in Fig. 5a1, 5a2 & 5a3. The shelf life of chocolate is dependent on extrinsic factors such as the time-temperature outline of the tempering process, temperature conditions provided for storage and environmental microbial colonization. The key players mediating microbial colonization are improper tempering, moisture migration, pH storage temperatures and oxidative rancidity which cause deterioration in sensory attributes of chocolate as is visible to the human eye by an inferior taste, odor and texture contributed by the action enzymes produced by the spoilage microbes. Increased incidence of exposure of the chocolate to high temperatures, cause fat crystallization unveiling the presence of fat surface bloom[21]. Chocolate stored at extremely low temperatures exhibit sugar bloom characterized by moisture condensation to the chocolate surface causing its absorption by the sugar, leading to the formation of sugar crystal formation on moisture evaporation. Therefore, an appropriate temper and proper storage conditions are needed for an extended shelf life. The novel method approached in this study, provided optimized time-temperature profiles for the non-thermal tempering process and enhanced the shelf life of the chocolate in a 1:3 time ratio. The tempered chocolate was found to have a shelf life of 30 days during which it superlatively retained its desired characteristics.

Sensory analysis

The illustrative elucidation and sensory evaluation of chocolates were examined by quantitative description testing (QDT) and 7-point hedonic testing employing free choice profile (FCP) to analyze the degree to which the chocolate would be acceptable to the consumer. The sensorial analysis of the chocolate samples conducted, brought forth results portraying the perceiver's sensorial insight and contentment to the chocolate. Fig. 6 outlines the results of the sensory analysis denoted in percentages, given to a team of 20 untrained panelists for their evaluation, pertaining to the variations of chocolate possessing a glossy or a dull finished appearance, chocolaty or roasted odor, smooth or soft uneven texture, evenly brown colored or uneven with white patch, no bloom or fat bloom and melty or chewy mouth-feel.

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From Fig. 6a, for untempered chocolate, the appearance descriptors showed 40% variability whereas odor and texture descriptors exhibited 60% variability. Predominantly, color descriptors presented 80% variability. To a great extent, variability of 100% was prevailed in the case of bloom and masticating property descriptors. In the case of tempered chocolate from Fig. 6b, the appearance, odor, texture, color, bloom, masticating property descriptors displayed 50%, 60%, 60%, 30%, 100%, 100% variabilities respectively.

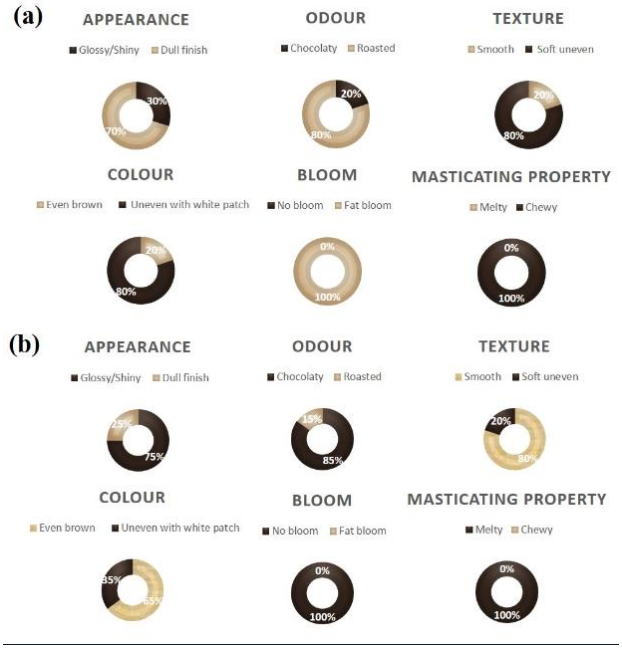

Fig 6: Quantitative description testing (QDT) for (a) Untempered chocolate.(b) Tempered chocolate.

Hence, the results depict that tempered chocolate was distinguished from the untempered chocolate as it was marked by a preferable appearance, color, odor, texture and masticating property, whereas the formation of bloom was escalated in untempered chocolate but negligible in tempered chocolate.

Corresponding to the QDT, there were notable disparities existent in the sensory quality of the tempered and untempered chocolate samples. On the whole, the panelists showed higher affinity towards the tempered than to the untempered chocolate.

Hedonic rating using 7-point scale was performed for both tempered and untempered chocolate to assess the degree to which the chocolate samples possessed consumer acceptance and satisfaction (Fig. 7). The results helped to interpret the value of responses in terms of degree of liking/disliking[22]. By means of the 7-point hedonic testing, the scale reciprocating the consumer preference was 2-point hedonic scale in the case of untempered chocolate whereas for tempered chocolate, it was 6-point scale which was appreciable. Similarly, the texture and appearance of the untempered chocolate were 'disliked very much' which bears 1-point scale but the same parameters were 'liked very much' for tempered chocolate which bears an extreme and notable 7-point scale. Hence, hedonic testing exhibits that the tempered chocolate has a remarkable insight in the fields of production and marketing.

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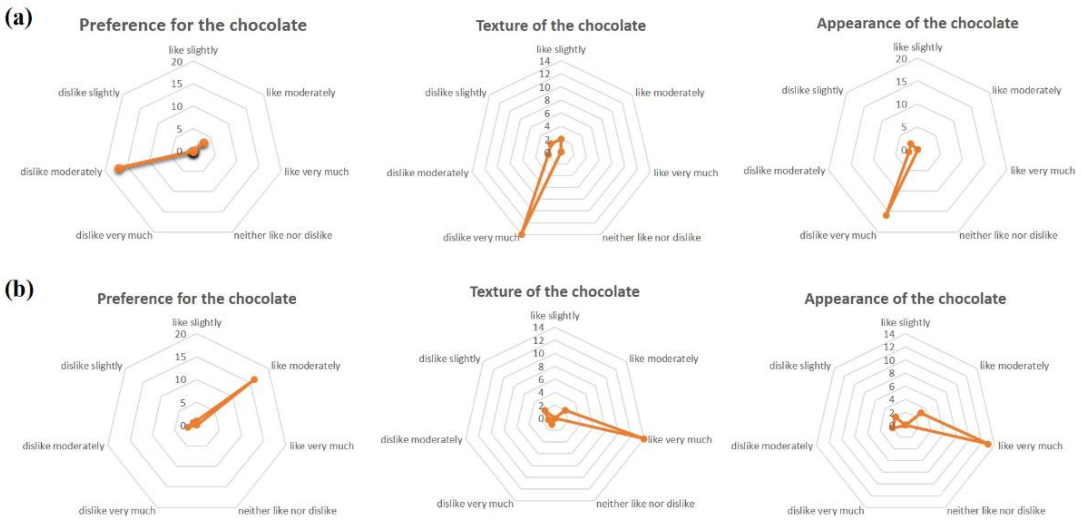

Fig 7: Sensory descriptors characterizing the chocolate from (a) untempered. (b) tempered samples in percentages by means of 7-point hedonic testing.

Estimation of antioxidant activity and total phenolic content

The present study was aimed to determine the total phenolic content and DPPH scavenging effect in tempered and untempered chocolate. The presence of polyphenols in chocolate is dependent upon the techniques relied upon for the chocolate processing, viz., fermentation, tempering etc. Additionally, the occurrence of NFCS (non-fat cocoa solids) in dark chocolate accounts for a higher proportion of phenols in dark chocolate when compared to other forms[22]. Both the tempered and untempered chocolate exhibited antioxidant activity. It was deduced that the phenolic content of tempered chocolate was 2.83±0.2 mg GAE/g and that for untempered chocolate was 3.33±0.2 mg GAE/g. The results obtained from the DPPH assay showed a percentage scavenging of 48.08±0.2% in tempered chocolate differing from a 53.82±0.5% in the untempered chocolate. The obtained results reveal that the process of tempering yielded negligible changes to the ability of the phenolic compounds present in chocolate to scavenge the free radicals and thus does not alter the nutritional composition of the chocolates.

CONCLUSION

Our study has unveiled a novel strategy to retain the nutritive value of chocolate. Based on the study, the non-thermal tempering of dark chocolate using ultrasonicator bath was found to be successful. Multiple analysis of the chocolate depicted better sensorial and shelf life characteristics in contrast to untempered chocolate. XRD analysis revealed the desired β_2 crystal polymorph in our chocolate sample further having a low surface roughness value as evaluated by AFM. Further studies are needed to assess the rheological characteristics of tempered chocolate using both milk and dark chocolate.

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