ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Optimized Ultrasound Assisted Tempering Method For Dark Chocolate Preparation.

Florentina Priyangini, Aishwarya Ravichandran, Niveda Kumar, Richa Cyriac, and Chidambaram Ramalingam*

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

ABSTRACT

Tempering chocolate is an essential step for making glossy, smooth chocolates. An alternative to the thermal chocolate tempering is the use of non-thermal processing (ultrasonicator) that negates the effect of excessive time consumption by the thermal process and enhances the quality of chocolate. Optimized condition was set for 2 secs and 3 secs based on scanning electron microscopy (SEM) results. X-ray diffraction (XRD) confirmed that the fifth form of chocolate is the most stable and desired form of cocoa butter polymorphism. Atomic force microscopy (AFM) denoted that the tempered form revealed relative absence of bloom with a smooth surface. Further, the 2 secs and 3 secs tempered chocolate contained 61.93±0.3% and 48. 98±0.2% free radical scavenging activity and 2.66±0.6 mg GAE $g^{-1}$and 2.57±0.1 mg GAE g^{-1} phenolic content respectively. The results suggest that the optimized tempering process enhanced the shelf life and acceptability of the tempered chocolate.

Keywords: Tempering; Ultrasonicator; Surface topology; Polymorphism; Sensory analysis

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Chocolate is made of sugar and cocoa solid in a hydrophilic phase. The chocolate matrix is rich in cocoa-butter and the crystal structure of the fat decides the sensory perception and macroscopic properties. For the cocoa butter to have good solidifying characteristics, molding properties, foam stability, gloss and shelf life characteristics, the process of shearing the chocolate mass at controlled temperature to promote the crystallization of triacylglycerol is a very crucial step. About 5-6 different polymorphs of cocoa butter are reported [1].These polymorphs are generally identified using scanning electron microscopy (SEM) and x-ray diffraction (XRD). The most stable form of chocolate is the fifth form of chocolate [2].The currently available industrial scale tempering machines comprise of multi stage heat exchangers in which there are controls for temperature and other adjustments to enhance the formation of appropriate crystals which are stable. These tempering techniques prolong equipment standardization with significant effects on the processing time and the quality of the product. This study focuses on non-thermal technique *i.e*. ultrasonication [3]. Sonication is a preferred technique because it reduces the processing time, conserves energy and is eco-friendly[4].Our main purpose was to assess the effects of sonication process and at the same time maintain the quality of chocolate.

Thermal techniques are the traditional way to manufacture chocolates as they raise the shelf life but the physiochemical and nutritional properties are compromised in it. By using non-thermal technique, a trend has been started by which the physiochemical and nutritional properties are kept intact along with its shelf life characteristics [5]. In this experiment we have dealt on reducing the particle size of chocolate using an ultrasonicator and the results were assessed using SEM analysis. This study hence focuses on knowing more about the effects of tempering and the fat crystallization behaviors.

MATERIALS AND METHODS

Materials

Good quality of cocoa beans were procured from Campco chocolate factory, Mangalore, India. Soy lecithin (E322) was used as an emulsifier.

Equipment

Mechanical grinding process of the cocoa beans was done in a 3-stone mixer. Tempering was carried out in an ultrasonicator (Sonics, Vibra Cell). Scanning electron microscope (ZEISS EVO18) was used for studying structural. X-ray diffraction (Bruker-D8 Advance, Germany) was used for polymorphism analysis. High resolution images of the surface characteristics of chocolate were obtained using atomic force microscopy (Nanosurf Easy scan 2, Switzerland). The chocolate samples were incubated in an environment test chamber (Remi programmable chamber-396LAG, India) for shelf life analysis.

Preparation of chocolate

The chocolate sample was prepared with slight modification in the procedure previously described by Are *et al*. [6]. The beans were refined to reduce the particle size and were grounded aseptically in a mixer at a low speed for a period of 6 hours. The chocolate was subjected to conching by adding the emulsifier-soy lecithin (E322) to evenly distribute the cocoa butter within the chocolate. The conched chocolate was further tempered and the samples were stored in air tight plastic containers.

Tempering of chocolate

Conched samples were tempered using ultrasonicator. The chocolate sample was subjected to tempering at different time intervals of 0-30 mins at a standard temperature of 32 °C and pulse rate at 2, 3, 5, 10, 25, 50 secs on-off. The optimized condition for tempered and untempered chocolate was set to 5 mins[7]. This optimized sample was stored and further subjected to different analysis and tests [8].

Characterization of tempered chocolate

The structure and morphology of the tempered and untempered chocolate was analyzed using SEM [9]. Total phenolic content of dark chocolate was determined using the method described by Singleton and Rossi [10]. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the antioxidant properties of the chocolate [11].The various crystal polymorphs of cocoa butter were investigated using XRD [12]. The tempered chocolate sample was incubated in an environment test chamber for a period of 10 days at 50 °C and at 65-70% relative humidity for the shelf life analysis. The aim was to keep a check on the microbial contamination during intervals of 5 days by determining the total plate count. The taste, appearance, aroma and other organoleptic properties of the chocolate were also analyzed. High resolution images of the surface characteristics of chocolate were obtained using AFM [13].The sensory characteristics of the products were deduced using quality descriptive testing (QDT) employing the free choice profile (FCP) which tested 7 different characteristics, *viz*., appearance, odor, texture, color, blooming, masticating properties and sensation post mastication. 7 point hedonic testing pertaining to three parameters mainly preference, texture and appearance of chocolate was assessed on a number scale of 1-7 [14] [15].

RESULTS AND DISCUSSION

Chocolate sample

The cocoa beans were grinded in the mixer and stored in air tight containers after lecithin was added. The chocolate samples after being tempered [16] for 0-30 mins at pulse rate 2, 3, 5, 10, 25, 50 secs on-off were observed and it was confirmed that the chocolate sample tempered for 5 mins – 2 secs on-off, 3 secs on-off exhibited a glossy appearance. The fine quality chocolate was prepared after it was optimized using ultrasonicator and was further molded as shown in Fig. 1.

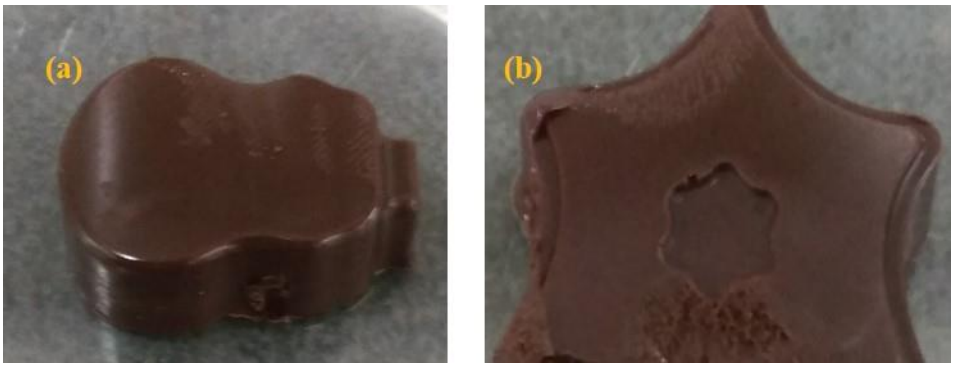

Fig 1: Molded chocolate: (a) 2 secs (b) 3 secs.

Optimization using SEM

The SEM analysis confirmed that 2 and 3 secs tempered chocolate had the optimum texture and required particle size and these samples were chosen for further studies (Fig. 2). Fig. 2(a), 2(b) & 2(c) were the samples that underwent higher pulse rates and for a longer period of time. We noticed a smoky odor for these samples and also the SEM analysis for these samples did not show very positive results as their surface was not smooth and glossy. Whereas, Fig. 2(i) & 2(j) showed the best results as we had reduced the pulse rate as well as the time and we were able to confirm with the SEM analysis that these particles were much more uniform when compared to the other images obtained.

Bioactivity of antioxidants

DPPH assay was conducted on the chocolate samples *i.e.* 2 secs and 3 secs tempered chocolate and the following results were obtained. 2 secs tempered chocolate showed 61.93$\pm$0.3% DPPH free radical scavenging activity whereas 3 secs tempered chocolate showed 48.98$\pm$0.2% DPPH free radical scavenging activity. This clearly showed that the chocolate that was tempered at 2 secs had better antioxidants property than compared to the chocolate that was tempered for 3 secs. The total phenolic content obtained revealed

ISSN: 0975-8585

that 2 secs tempered chocolate had 2.66±0.6mg GAE g^{-1} and 3 secs tempered chocolate had 2.57±0.1mg GAE g^{-1} phenolic content. In this case too, we have deduced that the 2 secs tempered chocolate had higher phenolic content than the chocolate tempered for 3 secs.

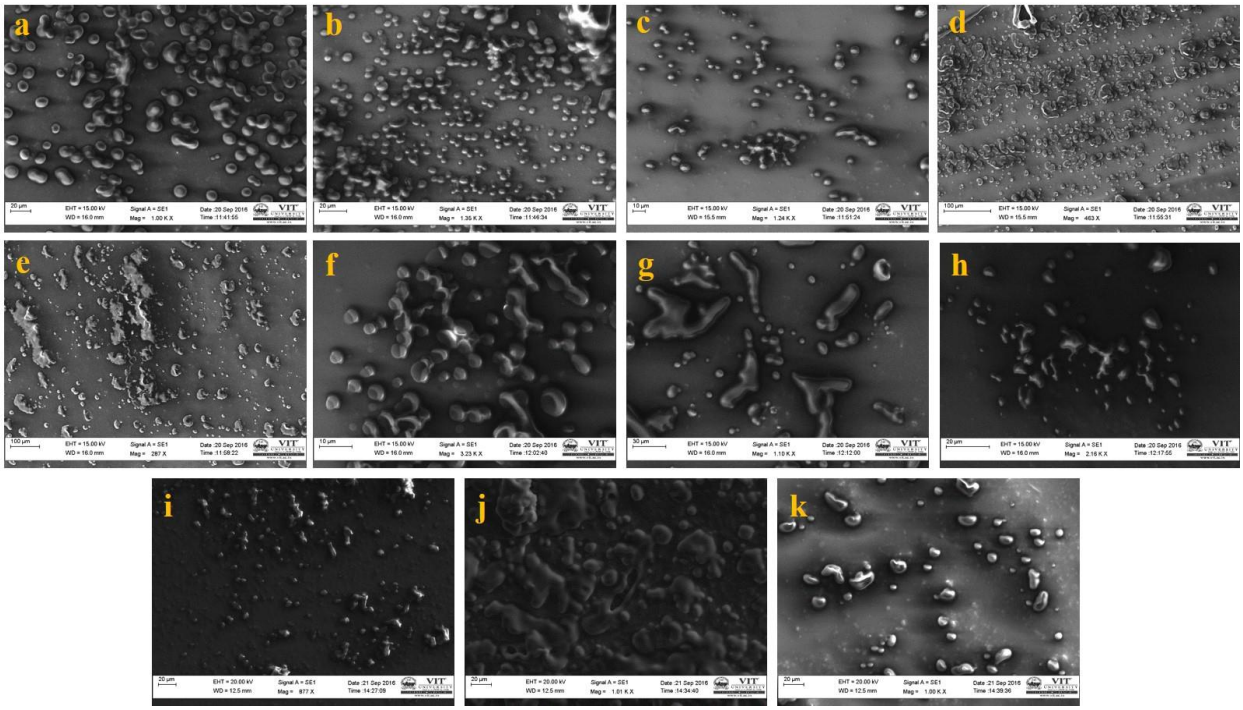

Fig 2:SEM images (a) control (b)25/3min (c) 25/6min (d) 25/9min (e) 50/3min(f) 10/3min (g) 10/6min (h) 10/9min (i) 10/2sec (j) 10/3sec (k) 10/6sec.

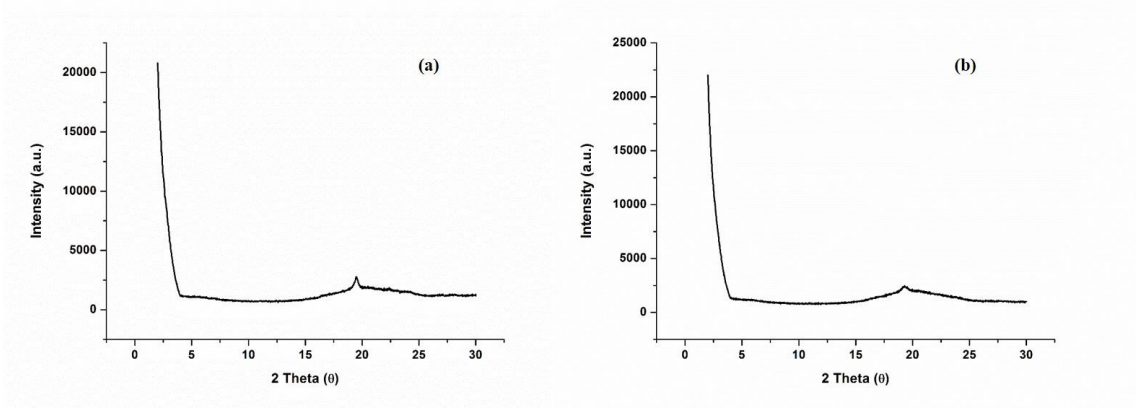

Fig 3: X-ray diffractogram of (a) 2 secs tempered chocolate (b) 3 secs tempered chocolate.

XRD analysis

XRD results showed that the peaks were obtained between 19 and 20 for both the tempered chocolate samples and hence substantiating the tempering procedure (Fig. 3(a) & 3(b)). In both the cases, the peaks were obtained between 19 and 20 which confirmed that both the forms of chocolate were in the fifth form of crystallization which means tempering has been efficient. According to Schenk and Peschar[17], the d-spacing of the chocolate tempered for 2 secs is 4.56 and the d- spacing for the chocolate tempered for 3 secs is 4.59 and these values nearly match the theoretical value of 4.58 which showcases the peak for the strongest form of the fifth form of chocolate. This showed that the chocolate prepared matched the standards of the theoretical and practical aspects[18].

ISSN: 0975-8585

AFM analysis

High resolution images of the surface characteristic of chocolate by AFM were carried out which showed smooth and glossy surfaces of the chocolates[19] (Fig. 4(a) & 4(b)). This proved that the tempering process had been done right.

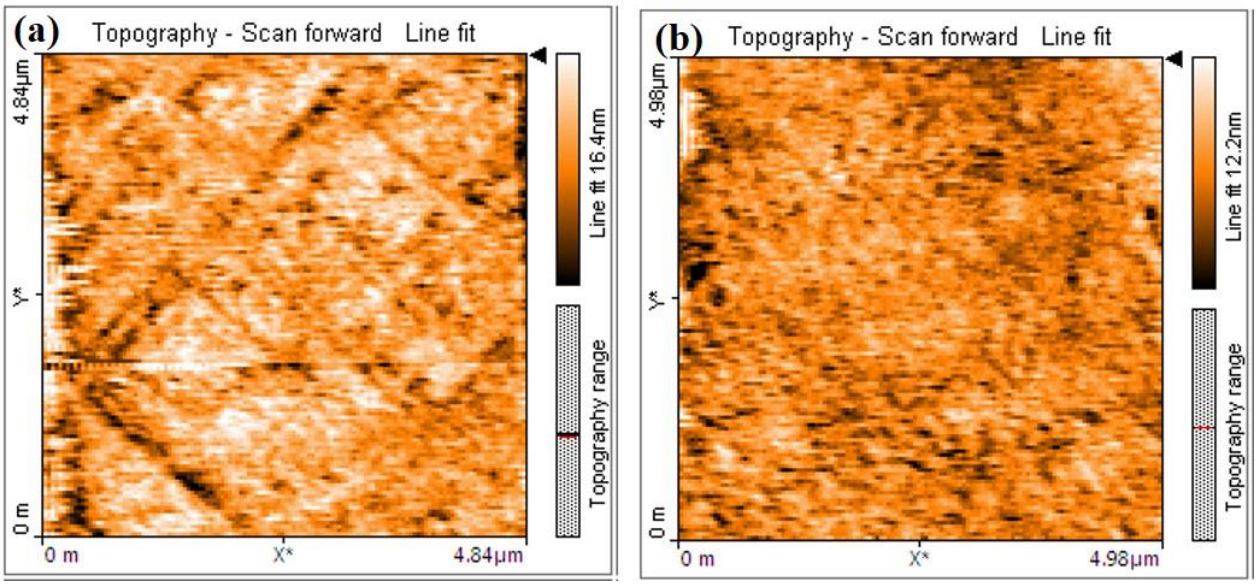

Fig 4: AFM images a) 2secs(5x5 µm) b) 3secs(5x5 µm).

Shelf life studies

Fig. 5(a1), 5(a2) &5(a3) are the plate studies for the chocolate tempered for 2 secs and Fig. 5(b1), 5(b2) & 5(b3) are the plate studies for the chocolate tempered for 3 secs. The plate studies were carried out for a span of 10 days[20]. On the 0^{th} and 5^{th} day the plates were free of any contamination but on the 10^{th} day a slight mat was formed on both the plates which indicated that the shelf life of these chocolates was found to be 15 days based on 1:3 time ratio.

Sensory analysis

This analysis was carried out by selecting 20 people to taste and rate the prepared chocolate. The two tests include quantitative descriptive testing (Fig. 6(a) & 6(b)) and seven point hedonic scale testing (Fig.7(a)& 7(b)). In both the quantitative descriptive testing and seven point hedonic scale testing, majority of the people were in favor of the tempered chocolate based on the various parameters. The conclusion based on this analysis is that we were successful in contenting the public demand by choosing a non-traditional method of manufacture that is the non-thermal technique.

ISSN: 0975-8585

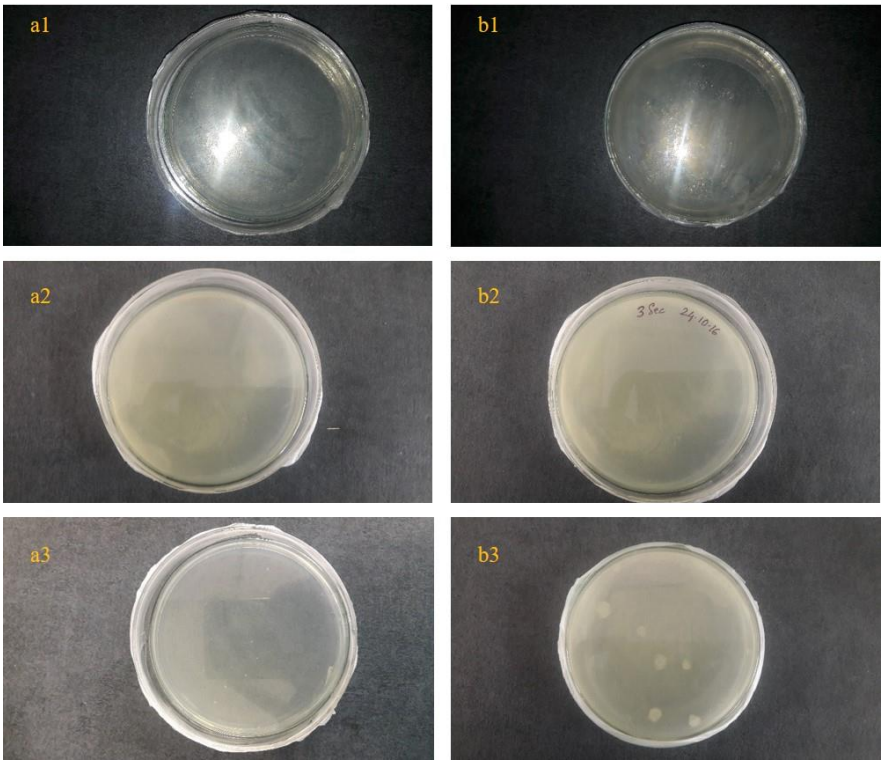

Fig 5: 2 secs tempered chocolate on (a1) 0th day (a2) 5th day (a3) 10th day. 3 secs tempered chocolate on (b1) 0th day (b2) 5th day (b3) 10th day.

Fig 6: Quantitative description testing (QDT) for (a) 2 secs tempered chocolate. (b) 3 secs tempered chocolate.

ISSN: 0975-8585

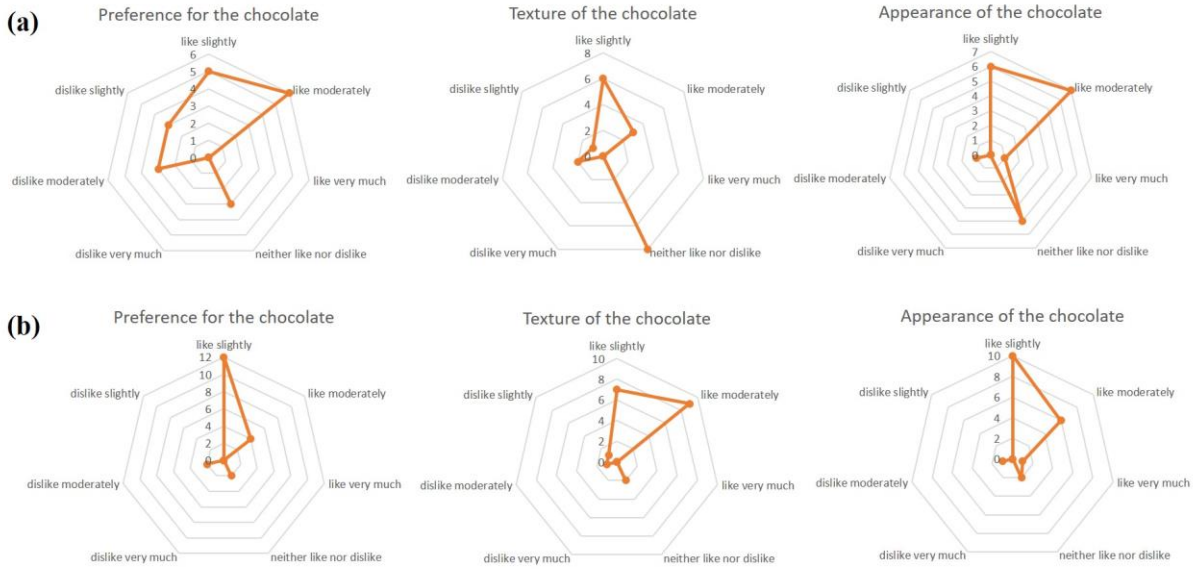

Fig 7: Seven point hedonic scale(a) 2 secs tempered chocolate. (b) 3 secs tempered chocolate.

CONCLUSION

Our main aim through this project was to bring to light the non-thermal technique *i.e.* ultrasonicator and how it could be used in the manufacture of good quality chocolate. Our future studies would include understanding about the rheological factors of the chocolates prepared. We would also like to dwell upon the manufacture process of different types of chocolate and how we can substitute cocoa butter for a healthier variant.

ACKNOWLEDGEMENT

This work was entirely carried out in VIT University, Vellore at the Instrumental and Food Analysis Laboratory. We thank the University for granting us approval to use some of the sophisticated equipment required for the experimental procedures.

REFERENCES

[1] Sato K, Koyano T, Garti N, DekkerM.Crystallization processes in fats & lipid systems. CRC Press, USA, 2001, pp. 429-456.
[2] Benjamin JD, Reverend L, Fryer PJ, Coles S, BakalisS. J. Am. Oil Chem. Soc2010; 87: 239-246.
[3] Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S, Zhang X, ZengX. Ultrason Sonochem2013; 20: 1182-1187.
[4] Chemat F, Khan MK. Ultrason Sonochem2011; 18: 813-835.
[5] Basri NF, Aziz FA. AJOFAI2012; 5(01): 71-78.
[6] Are LA, Gwynne-Jones DRG.Cacao in West Africa. Oxford, England, 1974.
[7] Higaki K, Uenom S, Koyano T, SatoK.J. Am. Oil Chem. Soc 2001; 78: 513-518.
[8] Afoakwa EO, Paterson A, Fowler M. Trends Food Sci Technol2007; 18: 290-298.
[9] Glicerina V, Balestra F, Rosa MD, RomaniS. J. Food Eng2016; 169: 165-171.
[10] Singleton V, Rossi J. Am. J. Enol. Vitic1965; 16: 144-158.
[11] Yu L, Perret J, Harris M, Wilson J, Haley S. J Agric Food Chem2003; 51: 1566-1570.
[12] Afoakwa EO, Paterson A, Fowler M, Vieira J. J. Food Eng 2008; 89: 128-136.
[13] Hodge SM, Rousseau D. J. Am. Oil Chem. Soc2002; 79: 1115-1121.
[14] Waghray K, Gulla S, Santhosh C, Kumar M, Kumar A. Stud Home Community Sci 2012; 6: 179-181.
[15] Beinner MA, Soares ADN, Barros ALA, Monteiro MAM. JFST 2010; 30(2): 516-519.
[16] Bund RK, Pandit AB. Chem Eng Process2007; 46(9): 846–850.
[17] Schenk H, Peschar R. Radiat Phys Chem2004; 71: 829–835.

[18] Klug HP, Alexander LE. X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials. John Wiley & Sons, Inc, USA, 1974.
[19] Rousseau D, Sonwai S. Food Biophys2008; 3: 273-278.
[20] Nattress LA, Ziegler GR, Hollender R, Peterson DG. J. Sens. Stud2004; 19: 133-138.