Optimizing Vitamin E Purification from Unsaponifiable Matter of Palm Fatty Acids Distillate by Low Temperature Solvent Crystallization

Kgs Ahmadi¹, Sri Kumalaningsih², Susingsih Wijana² and Imam Santoso²

1. Agroindustry Technology, Agriculture Postgraduate Program, Brawijaya University, Malang, Indonesia

2. Departement of Agroindustry Technology, Brawijaya University, Malang, Indonesia

Received: July 14, 2012 / Published: October 20, 2012.

Abstract: Palm fatty acid distillate (PFAD), a by-product of deodorization in palm oil refining, contains about 0.7%-1% vitamin E. The advantage of PFAD over other vitamin E sources is higher amount of tocotrienols than that of tocopherols. Vitamin E purification of unsaponifiable matter of PFAD was aimed to remove other impurities to obtain high vitamin E concentration, mainly tocotrienols. This research used low temperature solvent crystallization to purify vitamin E. To optimize response of vitamin concentration, a response surface method was applied with three factors, i.e., the ratio between solvent and unsaponifiable matter (A), crystallization temperature (B), and crystallization time (C). The relation of three factors was quadratic with equation $Y = -128.54361 + 41.33904A - 0.87995B + 1.58941C + 0.00290AB - 0.044324AC + 0.00120BC - 3.33113A^2 - 0.039535B^2 - 0.02710C^2$. The optimum crystallization condition was obtained at ratio of solvent to unsaponifiable matter of 6.04:1, crystallization temperature of -10.54 °C, and crystallization time of 24.16 hours. Vitamin E enriched fraction from optimum crystallization conditions contained vitamin E of 20.13% (w/w).

Key words: Low temperature solvent crystallization, palm fatty acid distillate, vitamin E enriched fraction, unsaponifiable matter.

1. Introduction

Palm fatty acid distillate (PFAD) is produced about 3%-5% during palm oil processing [1] which has a valuable unsaponifiable named vitamin E. Vitamin E of PFAD consisted of about 70% tocotrienol and 30% tocopherol [2]. The high amount of tocotrienol compound in PFAD is thought very important due to its potential to be used as natural vitamin E source. This valuable PFAD presently is not used properly and sold as raw material at low prices.

Vitamin E concentrates were produced by molecular distillation in combination with fractionation ethanol fractionation [3]; methylation [4, 5], adsorption [6], and winterization-saponification [7]. Previously, Ahmadi [8] developed a process to separate vitamin E from PFAD by using saponification and adsorption by

zeolite. The unsaponifiable matter contained vitamin E and sterol [9]. According to Gapoor et al. [10], phytosterol could be separated from other fraction in PFAD by crystallization, and the remaining fractions in non crystallized fractions that contained tocopherols and tocotrienols. However, the unsaponifiable matter presence in the PFAD become the main hindrance in the development of this material as a potential source of natural vitamin E. Purification of PFAD unsaponifiable matter by low temperature solvent crystallization is thought beneficial not only for providing high yield of natural vitamin E but also protecting the functional properties of vitamin E as antioxidant.

Low temperature solvent crystallization is one of separation processes [11]. This method minimized thermal damage due to low temperature [12], and was applied to separate wax from rice bran oil [13].

Corresponding author: Kgs Ahmadi, MSc., research field: lipid technology. E-mail: kgs.ahmadi@yahoo.com.

Unsaponifiable matter from oils contained phytosterols [14], and unsaponifiable fraction of pea oils contained hydrocarbons C₃₀, C₃₂, squalene, tocopherol [15]. Its content was similar to unsaponifiable matter of olive oil [16]. The predominant unsaponifiable fraction was sitosterol. It is supposed that based on different melting points of such components of unsaponifiable matter, vitamin E could be separated by low temperature solvent crystallization. Previous study [17] indicated that the ratio of solvent to solid and time had a significant effect on the extent of low temperature solvent crystallization. This finding is consistent with Chen and Ju [12] who reported that low temperature solvent crystallization was also affected by crystallization temperature.

Vitamin E and other dietary antioxidants have been put to the forefront of the medical and nutrition sciences because of significant advances in understanding of the relationship of oxidative stress in its various forms to the onset and control of many chronic diseases. Of many such dietary components, vitamin E has commanded most interest because of its availability, strong marketing potential, overall health impact, and central role in preventing oxidation at cellular level [18]. Vitamin E has health beneficial effect because of its function as antioxidant, anticancer, and hypocholesterolemic, beside it prevents brain cell from neurodegradative process [11].

This research aimed to get optimum conditions among ratio of solvent to unsaponifiable fractions, crystallization time, and crystallization temperature by using Response Surface Methodology (RSM). Optimized response was vitamin E content of vitamin E enriched fraction.

2. Materials and Methods

2.1 Materials

Materials used in this research were PFAD (kindly given by Salim Ivomas Pratama Limited, Surabaya, East Java province, Indonesia), standard of vitamin E (α tocopherol, α tocotrienol, γ tocotrienol, and δ tocotrienol; Santa Cruz Biotechnology Inc.), KOH,

hexane (technical grade), DPPH, and other chemical reagents (pro analysis, Merck).

2.2 Saponification of PFAD

Saponification was performed by using modified method of John et al. [19]. About 20 g PFAD was mixed with 100 mL ethanol. The mixture was sprayed by nitrogen for 30 s to remove air and then 12 mL KOH 40% was added. This mixture was heated in waterbath at 69.1 °C for 38 min. After cooling to ambient temperature, 200 mL aquadest and 500 mL hexane were added. The mixture was shaking slowly. Saponifiable (water soluble) and unsaponifiable matters (hexane soluble) were separated by decantation until two phases appeared. Hexane was removed from unsaponifiable matter by vacuum evaporation.

2.3 Low Temperature Solvent Crystallization

Low temperature solvent crystallization was employed by using three factors (solvent ratio between unsaponifiable matter, crystallization temperature, and crystallization time). Five gram unsaponifiable fraction of PFAD was diluted with a solvent. The volume of solvent used was according to the treatment (Table 1). This mixture leads to crystallize at different temperature and time depended on the treatment (Table 1). Crystal from the mixture was separated by filtration. The filtrate was evaporated to remove solvent and the remaining was vitamin E enriched fraction.

2.4 Experimental Design

The optimizing low temperature solvent crystallization conditions were determined by RSM. Three independent variables were ratio between solvent and unsaponifiable matter (A), crystallization temperature (B), and crystallization time (C) (Table 1). Dependent variable (Y) or the optimized response was vitamin E concentration in vitamin E enriched fraction. Response surface model of the second order used the formula of $Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \epsilon$.

Table 1 Central composite design to optimize crystallization conditions.

Independent variable	Symbol		Coded factor				
	Actual	Code	- α	-1	0	1	α
Solvent: unsaponifiable matter	A	x_1	4.318:1	5:1	6:1	7:1	7.682
Crystallization temperature (°C)	B	x_2	-26.82	-20	-10	0	6.82
Crystallization time (hour)	C	x_3	3.816	12	24	36	44.184

The results were then analyzed by the vitamin E enriched fraction. Tocopherol and tocotrienol by HPLC [20], the antioxidant activity by DPPH [21], peroxide value and FFA content [22], and the yield was calculated based on total amount of unsaponifiable matter obtained divided with weight of PFAD.

2.5 Vitamin E Analysis by HPLC

Analysis of vitamin E was referred to method of Ball [19] by using HPLC (Shimadzu LC20AT), with column VP-ODS 4.6 mm $\times$ 25 mm, detector UV (SPD 20A), mobile phase of methanol:water 95:5, flowrate of 1 mL/min, and injection volume of 100 μ L.

3. Results and Discussions

3.1 Characteristics of PFAD and Unsaponifiable Matter

The major component of PFAD was free fatty acid and PFAD contained vitamin E 1313 ppm (Table 2). This result showed that PFAD had more vitamin E than of the other vitamin E sources such as rice bran 300 ppm [23], cereal and berry, respectively 84-318 and 56-140 ppm [24], and olive oil about 100-270 ppm [25]. The high content of vitamin E in PFAD was consistent with finding obtained by Goh [26] which reported about 150-8,500 ppm of vitamin E found in PFAD.

Composition of vitamin E from PFAD was α tocopherol (33.84%), α tocotrienol (21.26%), δ tocotrienol (6.43%), and γ tocotrienol (38.47%). Each vitamin E concentration was obtained by dividing each vitamin E concentration (in ppm) by total vitamin E concentration (in ppm) (Table 2). Tocotrienols were the major components of vitamin E in PFAD that reached 66.67% and the remaining was tocopherol. Musalmah et al. [2] stated that the excellency of PFAD was mainly vitamin E in the form of tocotrienol

Table 2 Characteristics of palm fatty acid distillate (PFAD) and unsaponifiable matters.

Characteristics	PFAD	Unsaponifiable matter
Free fatty acid (%)	95.75	7.99
Peroxide value (meq/kg)	0.27	0.22
Antioxidant activity (%)		81.51
Vitamin E (ppm)	1312	35,000
δ Tocotrienol (ppm)	84 (6.40%)	2,250.5
γ Tocotrienol (ppm)	505 (38.49%)	13,464.5
α Tocotrienol (ppm)	279 (21.27)	7,441
α Tocopherol (ppm)	444 (33.84%)	11,844
Total tocotrienols (ppm)	868 (66.16%)	23,156

(70%). According to Gapoor et al. [10], composition of vitamin E in palm oil during refining process were α tocopherol (14%-17%), α tocotrienol (22%-24%), γ tocotrienol (49%-53%), δ tocotrienol (6%-7%), and tocomonoenol (3%).

Compared to PFAD, unsaponifiable matter had lower free fatty acid content (Table 2) due to saponification could not remove free fatty acids (FFA) properly. Many factors influenced saponification such as alkali concentration, saponification temperature, time, and amount of alkali added [27]. In this study, such affecting factors were not optimized to obtain free fatty acids of unsaponifiable matter. FFA was undesirable component in unsaponifiable matter of PFAD and it was removed in the form of saponifiable fractions. Saponifiable fraction could be further processed into soap and other non food products. Saponification raised concentration of vitamin E in unsaponifiable matter about 26.67 times compared to PFAD. According to Mitei et al. [28], the major components of unsaponifiable matter were phytosterol and vitamin E. Hui [1] revealed that unsaponifiable matter of PFAD contained free fatty acid, aldehyde, ketone, degradable carotenoid, sterol, hydrocarbon, tocopherol, and tocotrienol. Other components of

unsaponifiable were vitamin E, wax, hydrocarbon, and sterol. The components of unsaponifiable matter had different melting points. Hence, we used low temperature solvent crystallization to separate vitamin E.

Unsaponifiable matter had 1,312 ppm of tocopherol that comprised of 33.84% tocopherol and 66.16% tocotrienol. This composition was comparable to that found in PFAD. Heating during saponification could contribute to degradation of bioactive compounds including vitamin E. According to Hidalgo et al. [29], tocopherol decreased as a function of time and temperature, following first order kinetics. Unsaponifiable matter had antioxidant activity of 81.51%, indicating PFAD contained a significant amount of antioxidant compounds that contributed mainly from vitamin E. The yield of unsaponifiable matter based on original PFAD was 3.75%. This was a high value because according to Hui [30], the concentration of unsaponifiable matter of PFAD was 1.6%-3.7%. This

indicated that the saponification process was optimum to produce high yield.

3.2 Response Surface and Optimum Condition

Low temperature solvent crystallization raised vitamin E concentration significantly, from 3.50% in unsaponifiable matter to about 7.27%-21.45% in vitamin E enriched fraction. This concentration depended on three variables combination (Table 3).

All three factors gave significant effect on vitamin E concentration of vitamin E enriched fraction in quadratic manner. The equation of quadratic model was $Y = -128.54361 + 41.33904A - 0.87995B + 1.58941C + 0.00290AB - 0.044324AC + 0.00120BC - 3.33113A^2 - 0.039535B^2 - 0.02710C^2$ with A = ratio of solvent: unsaponifiable fraction; B = crystallization temperature (°C); C = crystallization time (hour). The response surface of the relationship of the ratio between solvent and unsaponifiable matter, crystallization temperature, and crystallization time was

Table 3 Vitamin E concentration after low temperature solvent crystallization process.

No.	Actual variable			Coded variable			Vitamin E concentration (% w/w)
	A Solvent: unsaponifiable matter	B Crystallization temperature (°C)	C Crystallization time (hour)	x ₁	x ₂	x ₃	
1.	5:1	-20	12	-1	-1	-1	9.19
2.	5:1	-20	36	-1	-1	1	9.67
3.	5:1	0	12	-1	1	-1	7.27
4.	5:1	0	36	-1	1	1	9.17
5.	7:1	-20	12	1	-1	-1	10.48
6.	7:1	-20	36	1	-1	1	16.95
7.	7:1	0	12	1	1	-1	9.19
8.	7:1	0	36	1	1	1	8.45
9.	6:1	-10	24	0	0	0	21.45
10.	6:1	-10	24	0	0	0	19.63
11.	6:1	-10	24	0	0	0	19.76
12.	6:1	-10	24	0	0	0	19.72
13.	6:1	-10	24	0	0	0	20.45
14.	6:1	-10	24	0	0	0	19.76
15.	4.318:1	-10	24	-1.682	0	0	9.86
16.	7.682:1	-10	24	1.682	0	0	10.79
17.	6:1	-26.820	24	0	-1.682	0	8.75
18.	6:1	6.820	24	0	1.682	0	8.38
19.	6:1	-10	3.816	0	0	-1.682	8.53
20.	6:1	-10	44.184	0	0	1.682	9.99

maximum. This was indicated by minus sign (-) of coefficient of A^2 , B^2 , and C^2 . Optimum crystallization conditions were achieved at the ratio of solvent to unsaponifiable matter of 6.04:1, crystallization temperature of -10.54 °C, and crystallization time of 24.16 hours with predicted response of vitamin E concentration was 20.13%.

The ratio between solvent and unsaponifiable matter was a parameter that affecting viscosity of system. The viscosity of system affected mass and heat transfer. Dilute solution would easily transfer heat/energy and mass from the solute. The increase of vitamin E concentration occurred to the ratio of solvent: unsaponifiable matter of about 6:1 as ratio that is close to optimum response (Figs. 1 and 2). Increasing ratio of solvent to unsaponifiable led to decrease in vitamin E concentration.

Krishnamurthy and Kellens [11] stated that the ability of the system to initiate the crystal nucleus formation was inversely correlated with the viscosity of the system. At the low ratio (below 5:1), it was supposed that the solution system was too viscous that limit the motion of the molecule. It led to inhibition of mass and heat transfer that implicated to the disturbance in crystallization process. The low viscosity of the system at the ratio of solvent to unsaponifiable matter more than 7:1 led to high rate of mass and heat transfer to the crystal nucleus that made the formation of more crystal nucleus. This phenomenon was supposed to induce vitamin E crystallization. According to Myerson and Ginde [31] crystallization from solution can be thought as a two-step process. The first step is the phase separation, or "birth" of new crystals. The second step is the growth of these crystals to larger sizes. These two processes are known as nucleation and crystal growth, respectively. Crystal nucleation was strongly affected by viscosity of the system, in this study, it was represented by ratio of solvent to unsaponifiable matter. Furthermore, Myerson and Ginde [31] explained that there was a relation of the degree of nucleation to

crystal growth that controls the product crystal size and size distribution. Therefore, at low viscosity (high ratio of solvent to unsaponifiable matter), high rate of nucleation produced high crystal nuclei that could lead to vitamin E crystallization.

Crystallization temperature would determine the crystallization process. Temperature of 0 °C was too high to transfer heat from mixture (unsaponifiable matter and solvent) to environment. Therefore, crystal nucleus formation was limited. Also, mass transfer to nucleus limited due to heat transfer was not a maximum. The crystal formation was not optimum that made other component rather than vitamin E still in solution. Therefore, at high crystallization temperature the vitamin E concentration was low (Figs. 1 and 3) due to other component did not crystallize well and still in solution. Meanwhile, too low temperature -20 °C made the formation of many crystal nucleus. The growth of crystal nucleus needed mass from the solution system. During crystal growth, the compounds with low solubility would tend to migrate into crystal nucleus and crystallized that make some vitamin E crystallized. According to Myerson and Ginde [31], for several systems, the nucleation rate declined with increasing temperature for a given supersaturation. Thus crystal formation was limited and caused low vitamin E concentration of vitamin E enriched fraction.

Crystallization time was a crucial factor in crystal formation. The formation of crystal would continue until the balance of the system reached. The crystal would continue to grow as the system was not supersaturation or supercooling, and the molecules in the system still had high mobility to migrate into crystal matrix [32]. The overall crystallization was divided into two steps, nucleus formation and crystal growth. Once the crystal nucleus formed, crystal would continue to grow Gosh [13]. Some factors affecting crystallization were cooling rate, cooling temperature, and super saturation of the system [33, 34]. Time was needed for crystal growth. In this study, the optimum crystallization process was achieved at 24.16 hours.

Optimizing Vitamin E Purification from unsaponifiable Matter of Palm Fatty Acids Distillate by Low Temperature Solvent Crystallization

Fig. 1 The relation of ratio between solvent and unsaponifiable matter and temperature at 24 hours of crystallization time.

Fig. 2 The relation of ratio between solvent and unsaponifiable matter and crystallization time at crystallization temperature of -10°C .

Fig. 3 The relation of crystallization temperature and crystallization time at ratio between solvent and unsaponifiable matter 6:1.

Before optimum crystallization time was achieved, other undesirable components from unsaponifiable matter still in solution that caused low vitamin E concentration (Figs. 2 and 3). Meanwhile, increasing crystallization time after optimum was reached, also lead to decrease in vitamin E concentration. This was due to some vitamin E tended to crystallize.

4. Conclusions

Saponification in combination with low temperature solvent crystallization could be used to purify vitamin E from PFAD. The optimum conditions of low temperature solvent crystallization to obtain high vitamin E content in vitamin E enriched fraction was ratio between solvent and unsaponifiable matter of 6.04:1, crystallization temperature of -10.54°C , and crystallization time of 24.16 hours. At this optimum condition, vitamin E enriched fraction contained vitamin E of 20.13% (w/w).

References

- [1] Y.H. Hui, Encyclopedia of Food Science and Technology, John Wiley&Sons Inc., New York, 1992, pp. 1993-2145.
- [2] M. Musalmah, M.Y. Nizam, A.H. Fairuz, N. Aini, A.I. Azian, M.T. Gapor, et al., Comparative effects of palm vitamin E and α tocopherol on healing and wound tissue antioxidant enzyme levels in diabetic rats, *Lipids* 40 (2005) 575-580.
- [3] T. Nagao, Y. Hirota, Y. Watanabe, T. Kobayashi, N. Kishimoto, T. Fujita, et al., Recovery of sterol as fatty acid steryl esters from waste material after purification of tocopherols, *Lipids* 39 (2004) 784-794.
- [4] D.G. Hayes, Enzyme-catalyzed modification of oilseeds materials to produce eco-friendly products, *J. Am. Oil Chem. Soc.* 81 (2004) 1077-1103.
- [5] M.H. Ng, Y.M. Chao, A.H. Ma, C.H. Choah, M.A. Hashim, Separation of vitamin E (tocopherol, tocotrienol, and tocomonoenal) in palm oil, *Lipids* 39 (2004) 1031-1035.
- [6] B.S. Chu, B.S. Baharin, Y.B. Man, S.Y. Quek, Separation of vitamin E from palm fatty acid distillate using silica: I Equilibrium of batch adsorption, *J. Food Eng.* 62 (2004) 97-103.
- [7] J. Lewis, Process for the production of tocotrienol, US Patent, 2001.
- [8] K. Ahmadi, Natural zeolite activation and its use for tocopherol purification from palm fatty acid distillate,

- Berkala Penelitian Pasca Sarjana UGM 10 (1997) 247-258.
- [9] Y. Watanabe, T. Nagao, Y. Hirota, M. Kitano, Y. Shimada, Purification of tocopherols and phytosterols by two-step in situ enzymatic reaction, *J. Am. Oil Chem. Soc.* 81 (2004) 339-345.
- [10] A. Gapoor, W.H.W. Hassan, M. Sulong, Phytochemical for nutraceutical from the by product of palm oil refining, *Palm Oil Development* 36 (2002) 13-19.
- [11] R. Krishnamurthy, M. Kellens, Fractionation and winterization, in: Y.H. Hui (Ed.), *Bailey's Industrial Oil and Fat Products: Edible Oil and Fat Products: Processing Technology*, 5th ed. Vol. 4, A John Wiley & Sons, Inc., New York, 1995, pp. 301-337.
- [12] T.C. Chen, Y.H. Ju, Polyunsaturated fatty acids concentrates from borage and linseed oil fatty acids, *J. Am. Oil Chem. Soc.* 78 (2001) 485-488.
- [13] M. Gosh, S. Bandyopadhyay, Studies on crystal growth of rice bran wax in a solvent medium, *J. Am. Oil Chem. Soc.* 82 (2005) 229-231.
- [14] M. Schröder, M.W. Vetter, Investigation of unsaponifiable matter of plant oils and isolation of eight phytosterols by means of high-speed counter-current chromatography, *J. Chromatogr.* 1237 (2002) 96-105.
- [15] H.M. El-Saied, M.M.A. Amer, A. Gabran, Unsaponifiable matter and fatty acid composition of pea oil, *Ernährungswiss* 20 (1981) 132-138.
- [16] H.T. Khor, R. Rajendran, M. Gopalakrishnan, The role of unsaponifiable components in the lipidemic property of olive oil, *Mal. J. Nutr.* 4 (1998) 73-80.
- [17] K. Ahmadi, Low Temperature Solvent Crystallization for Omega-3 Fatty Acid Enriched Oil Preparation from by-product of Sardinia (*Sardinella longiceps*) Canning Processing, Indonesia Young Scientist Research Report, Tribhuwana Tungadewi University, Malang, Indonesia, 2006, p. 65.
- [18] R. Eitenmiller, J. Lee, *Vitamin E: Food Chemistry, Composition, and Analysis*, Marcel Dekker Inc., New York, 2004, pp. 1-13.
- [19] K.G.K. John, B. Lisa, C. Robert, A. Roman, M.R.K. Krishna, A rapid method for determination of vitamin E forms in tissues and diet by high performance liquid chromatography using a normal phase diol column, *Lipids* 32 (1997) 323-330.
- [20] G.F.M. Ball, *Fat-Soluble Vitamin Assays in Food Analysis*, Elsevier Applied Science, London, 1988, pp. 234-257.
- [21] O.S. Kim, Radical scavenging capacity and antioxidant activity of the E vitamin fraction in rice bran, *J. Food Sci.* 70 (2005) 208-213.
- [22] AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th ed., Broadmaker Drive, Champaign, Illinois, 1989.
- [23] Z. Xu, N. Hua, J.S. Godber, Antioxidant activity of tocopherols, tocotrienols, and γ -oryzanol components from rice bran against cholesterol oxidation accelerated by 2, 2-azobis(2-methylpropionamidine) dihydrochloride, *J. Agric. Food Chem.* 49 (2001) 2077-2081.
- [24] H. Kallio, B. Yang, P. Peippo, R. Tahnoven, R. Pan, Triacylglycerols, glycerophospholipids, tocopherol, and tocotrienols in berries and seeds of two subspecies (ssp. *sinensis* and *mongolica*) of sea buckhorn (*Hippophae rhamnoides*), *J. Agric. Food Chem.* 50 (2002) 3004-3009.
- [25] S.C. Cunha, J.S. Amaral, J.O. Fernandes, M.B.P.P. Oliveira, Quantification of tocopherols and tocotrienols in Portuguese olive oils using HPLC with three different detection systems, *J. Agric. Food Chem.* 54 (2006) 3351-3356.
- [26] S.H. Goh, Y.M. Choo, S.H. Ong, Minor constituent of palm oil, *J. Am. Oil Chem. Soc.* 62 (1985) 237-240.
- [27] S.M. Oluwatoyin, Quality of soaps using different oil blends, *J. Microbiol. Biotech. Res.* 1 (2011) 29-34.
- [28] J.C. Mitei, J.C. Ngila, S.O. Yeboah, L. Wessjohann, J. Schmidt, Profiling of phytosterol, tocopherol, and tocotrienol in selected seed oils from Botswana by GC-MS and HPLC, *J. Am. Oil Chem. Soc.* 86 (2009) 617-625.
- [29] A. Hidalgo, A. Brandolini, C. Pompei, Kinetics of tocopherol degradation during the storage of einkorn (*Triticum monococcum* L. ssp. *monococcum*) and breadwheat (*Triticum aestivum* L. ssp. *aestivum*) flours, *Food Chemistry* 116 (2009) 821-827.
- [30] Y.H. Hui, *Bailey's Industrial Oil and Fat Products*, 5th ed. Vol. 3, John Wiley & Son Inc., New York, 1996, p. 549.
- [31] A.S. Myerson, R. Ginde, Crystals: Crystal Growth, and Nucleation, in: S.A. Myerson (Ed.), *Handbook of Industrial Crystallization*, 2nd ed., Elsevier Science & Technology Books, London, 2001, pp. 33-64.
- [32] R.W. Hartel, *Crystallization in Foods*, A Wolters Kluwer Co., USA, 2001, p. 325.
- [33] S. Martini, M.C. Anon, Determination of wax concentration in sunflower seed oil, *J. Am. Oil Chem. Soc.* 77 (2000) 1087-1092.
- [34] S. Martini, M.C. Anon, Crystallization of sunflower oil waxes, *J. Am. Oil Chem. Soc.* 80 (2003) 525-532.