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The Effect of Minor Components of Wheat Germ Oil on Thermal Behavior, Crystallization Characteristics and Oxidative Stability of Butter Oil

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Abstract

Minor lipid components (MLCs) can be added to fats to improve some of their chemical and physical properties and reduce the health risks associated with them. The effect of adding MLCs extracted from wheat germ oil (WGO) on oxidative stability (OS), thermal behavior, slip melting point (SMP), solid fat content, and crystallization properties of butter oil (BO) was studied. The MLCs were added to butter oil (BO) at a rate of 0.25, 0.5, 0.75, 1.0, and 1.5%. The main phytosterols in the MLCs of WGO were β-sitosterol and campesterol, with concentrations of 806.25±24.76 and 374.6±117.61 mg/100 g, respectively, whereas the main tocopherols were α- and γ-tocopherols. The addition of MLCs could significantly affect the melting and crystallization properties of BO. The SMP of BO increased as the concentration of MLCs increased; it increased from 33.3°C in pure BO to 35.4 °C in BO containing 1.5% MLCs. A pure BO had smaller and more numerous spherical crystals than any other BO sample containing MLCs; however, BO containing 1.5% MLCs showed a notably larger crystal aggregation. The addition of MLCs to BO could significantly affect its thermal behavior and OS. The ΔH decreased from 46.19±1.11 J/g in pure BO to 20.15±1.23 and 16.21±1.02 J/g in BO containing 0.25 and 0.50% MLCs, respectively. The OS increased in proportion to the MLC content; this increase was more noticeable in BO containing 0.25 and 0.5% MLCs, which were respectively two and three times greater than pure BO.

Keywords: Wheat germ oil, butter oil, minor lipid components, sterol fractions, thermal behavior, oxidative stability

1. Introduction

Fat is an important ingredient in milk. It is known that milk fat consists of 97–98% tri-acylglycerols (TAG) and 2–3% minor components [1]. Nutritionally, milk fat has a high content of cholesterol and hypercholesterolemic fatty acids (FAs), namely C12:0, C14:0, and C16:0, which make up 27.6% of the total [2]. Technologically, butter has low structural stability at room temperature and an unspreadable consistency compared to margarine after being removed from the refrigerator. It also shows oiling-off and moisture migration [3]. The principal minor components that are indigenous or added to oils and fats include free fatty acids (FFAs), sterols, tocopherols, phenolic compounds, mono-acylglycerols (MAG), di-acylglycerols (DAG), and phospholipids [4, 5]. Concentrations of FFA, DAG, MAG, and sterols can vary largely between fats and even between different batches of the same fat. The interest in these components was high due to their ability to both contribute to the positive influence on human health and their technological properties in the food industry as emulsifiers and emulsion stabilizers [6]. According to epidemiologic and experimental research, dietary sterols may protect against the most common cancers, such as colon, breast, and prostate cancer, but their main effect is to lower circulating cholesterol levels. Tocopherol has also powerful antioxidant and anti-inflammatory effects, reduces skin damage, promotes healthy ageing, and boosts

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immunity [2]. Contarini & Povolo [1] reported that phospholipids have a positive effect on the health, nutritional, major constituents of the brain, and technological properties of milk.

The physical properties of fats, such as crystallization, microstructure, and solid fat content (SFC), rely greatly on minor lipid components (MLCs). MLCs can affect induction times and crystallization rates by being removed from or added to milk fat. Lopez [7] reported that many factors, including cooling speeds, thermal history, shear, MLCs, and changes in the composition of FAs and TAG, can affect the crystallization properties of milk fat. When crystallizing a 10% milk fat and 90% cocoa butter mixture, longer induction times and slower crystallization rates were observed [8]. Mazzanti et al. [9] showed that the transformations of β-crystals as well as the crystallization process are both affected by the addition of minor lipid components. Phospholipids act as nucleation sites and may help improve the spherulite size of the crystals or maintain clarity at various concentrations lower than 2%. However, the microstructure at concentrations above 2% was formed of small and uniformly distributed fat crystals, as well as an increase in the SFC [10]. Chen et al. [11] discovered that the addition of saturated phosphatidylcholine and phosphatidylethanolamine to neutralized and bleached cocoa butter or molten and recrystallized commercial chocolate at 0.1%, followed by rapid cooling to 20 °C in the absence of shear, accelerates crystallization, stabilizes the desirable form V polymorph, and induces the formation of chocolate with an optimal microstructure, surface gloss, and mechanical strength. In addition, butter manufacturers have long considered the possibility of using additives, including MAG, to improve spreadability and decrease hardness. Abd El-Aziz et al. [12] found that blends of butter oil (BO) with refined palm oil (RPO) exhibited a lower solid fat content than BO and RPO at 0 and 10 °C, while at 25 °C, the SFC of oil blends was close to that of pure. Palm oils high in free fatty acids tend to have lower melting points because of the formation of eutectics between the TAG and the DAG that are present [3].

On the other hand, oxidation of lipids is one of the major reactions resulting in a decrease in food quality and acceptability because it reduces the nutritional value and generates rancidity, causing undesirable flavors [13]. Spěváčková et al. [14] found that MG retarded lipid oxidation in margarine emulsion, a system of three phases (two limitations: miscibility liquids and solid phase). In addition, the phospholipids are able to affect lipolysis; little experimental evidence is available in this respect. When lecithin and cephalins are added to milk, lipase activity is found to reduce as a result of an interaction between the phospholipid and lipase [15]. They have hydrophilic and hydrophobic groups in the same molecule and prefer to be concentrated on the surface of edible oils. Therefore, this study aimed to investigate the effect of the addition of minor lipid components (MLCs) extracted from wheat germ oil (WGO) on the crystallization properties, crystal morphology, thermal behavior, and oxidative stability of butter oil.

2. Materials and Methods

2.1 Materials

Wheat germ oil (WGO) was purchased from a local market in Moscow, Russia. Fresh butter was obtained from the Animal Production Institute, Ministry of Agriculture, Cairo, Egypt. The butter was melted at 60 °C and centrifuged at 2500 xg for 5 min to separate the protein and other materials. The top butter oil (BO) layer was decanted and stored at -20 °C until use. Both hexane and ethanol (HPLC grade) were purchased from Fisher Scientific UK, Bishop Medow Road, Loughborough, UK. All chemicals and reagents were analytical grade and came from various sources. As standards, tocopherol fractions (β- and γ-tocopherol) and sterol fractions (β-sitosterol, campesterol, stigmasterol, and 1,25-dihydroxyvitamin D3) were obtained from Merck, Darmstadt, Germany.

2.2 Methods

3.2.1 Preparation of minor lipid components

Minor lipid components (MLCs) were extracted from WGO as described by Miraliakbari & Shahidi [16], with minor modifications. Fifty grams of WGO were combined with 400 mL of hexane in a 1000 mL separator funnel. About 200 mL of methanol was added to the mixture, and the separator funnel was sealed and agitated for 5 min with periodic venting. The separator funnel was then sealed with nitrogen and stored at 4 °C for 1 h. The methanol fraction was

decanted into a 1 liter round-bottom flask. The methanol extraction process was repeated four times, and the pooled extracts were evaporated in a rotary vacuum evaporator (Rota-vapor R110, Buchi, Switzerland) at 50 °C. The concentrated extracts (MLCs) were stored at -80 °C for up to one week before use.

3.2.1.1 Sterol and tocopherol fractions

The sterol fractions (β-sitosterol, campesterol, stigmasterol, and 1,25-dihydroxyvitamin D3) and tocopherol fractions (α-tocopherol and γ-tocopherol) in the MLCs extract were prepared according to the method described by Abd El-Aziz et al. [12] and measured using HPLC (Knauer, Germany) equipped with a UV detector at 250 nm. A 250 4.6 mm Gemini-Nx-Nx, C18, column was used. Isocratic elution with a mobile phase of methanol and water (95:5) at a flow rate of 0.7 ml/min was used to separate the sterol fractions. The injection volume was 20 μL, and the column temperature was set at 35 °C. While the mobile phase for the tocopherol fractions was a 99:1 v/L mixture of hexane and isopropanol flow at a rate of 1.5 mL/min. By comparing the peak areas of the sterols and tocopherols in the MLCs extract with the peak area of the standard about concentration, the concentration of each was determined.

3.2.2 Experiment design

The experiment was conducted in December 2022 at the National Research Centre and Regional Centre for Food and Feed Labs (Egypt), as well as the Moscow State University of Food Production (Russia). MLCs extract of WGO was added to BO at rates of 0.25, 0.50, 0.75, 1.0 and 1.5% (w/w). At the same time, a control sample (pure BO) was conducted with no additions. The samples were stored at a refrigerator temperature (4±0.1 ºC) for 21 days. Various analyses of the samples were performed.

3.2.2.1 Chemical analysis

Free fatty acid content (FFA as % oleic acid), peroxide value (PV, meq O2/kg oil), refractive index (RI) and iodine value (IV, g I_2/100 g oil) were determined according to the analytical methods described by AOCS [17].

3.2.2.2 Thermal analysis

The thermal analyses of BO samples were studied by differential scanning calorimeter (DSC, Model 7, Perkin Elmer, Norwalk, CT). The DSC was calibrated with Indium (m.p.156.60 °C, ΔHf 28.45 J/g) and Gallium (m.p.29.78 °C, ΔHf 80.09 J/g). The system was purged with nitrogen gas at 20 mL/min during the analysis, and liquid nitrogen was used as a refrigerant to cool the system. AOCS method Cj-94 [17] was followed with a modification. A sample of 9–10 mg was hermetically sealed in a 30 mL capacity aluminium pan (Perkin Elmer, Norwalk, CT), with an empty sealed pan used as a reference. Samples were rapidly heated (100 °C/min) from room temperature to 80 °C and held at this temperature for 5 min to destroy any previous crystalline structure, before being cooled to -50 °C at a rate of 10 °C/min to obtain the crystallization curves. After 15 min holding at -50 °C, melting curves were generated by heating the samples to 70 °C at a rate of 10 °C/min. Thermograms were analyzed for peak maxima temperature (°C) and enthalpy (J/g). Data analysis was performed by the software provided with the DSC (Pyris software, Perkin Elmer, Shelton, CT).

2.2.2.3 Crystal morphology

An Olympus BH polarized light microscope (Olympus, Tokyo, Japan) was used to observe the fat microstructure of BO samples. Samples were crystallized from the melt on glass slides under glass coverslips at 25 °C. A CCD digital video camera (Efston Science Inc., Toronto, Canada) was used to record images on videotape, which were then digitized using Rainbow Runner software (Matrox Graphics Inc., Dorval, Québec, Canada).

2.2.2.4 Slip melting point

The slip melting point (SMP) of the BO samples was determined by the capillary tube [17]. Ten mm of melted fat was drawled into a thin-walled capillary tube (1 mm), and the fat-filled end of the tube was sealed in a small flam. Tubes containing fat were held overnight (~16 h) in a refrigerator at 4–10 °C. The tube was attached to a 0.2 °C accurate thermometer, which was immersed (30 mm) in a 600 mL beaker half-filled with water. The bath temperature was started at 8–10 °C below the SMP of the sample and then increased slowly (0.5 °C/min). The SMP temperature at which the substance becomes

transparent was measured.

2.2.2.5 Solid fat content

The solid fat content (SFC) of the BO samples was determined by Nuclear Magnetic Resonance (NMR, Model: MARAN–SFC, Company: Resonance Instruments Ltd) according to the method described in IUPAC [18]. The temperatures of the tested samples were 10, 20, 25, 30, 35, and 40 °C. The sample in the NMR tube was melted at 70 °C for 30 min and then chilled at 0 °C for 90 min and held at the measuring temperature for 60 min before measurement.

2.2.2.6 Oxidative stability

The rancimat Metrohm instrument (Ud. CH-9100 Herisau, Switzerland, Model 679) was used to estimate the oxidative stability (OS) of the BO samples as the induction period (h) under accelerated conditions (110°C, airflow at 20 L/h) according to the method described by El-Hadad et al. [19]. The maximum change in oxidation rate was defined as the point of oxidative stability.

2.2.3 Statistical analysis

The GLM procedure was used to statistically analyze all of the obtained data using SAS software, version 9.1 [20]. Analysis of variance (ANOVA) and Duncan's multiple comparison procedure were used to compare the means. A probability of $P \leq 0.05$ was used to establish statistical significance. presentation achieved in this Word® document.

3. Results and Discussion

3.1 Sterol and tocopherol fractions

Phytosterols have been used as a food additive because of their capacity to lower plasma cholesterol levels and their potential to offer protection against the most common cancers. Along with other compounds that are present in relatively large amounts, such as tocopherols, squalene, and other hydrocarbons, they make up the unsaponifiable lipid fraction of oils. On a daily basis, adults consume 150–400 mg of plant sterols, with most of this quantity coming from β-sitosterol, campesterol, and stigmasterol [21, 22]. The sterol and tocopherol fractions in the MLC extract of the WGO are presented in Table 1. β-sitosterol, campesterol, and stigmasterol were the three main sterols contained in MLCs extract, with concentrations of 806.25±24.76, 374.61±17.61, and 185.67±11.48 mg/100 g, respectively. These findings were consistent with those of Rodrigues et al. [2], who found that the predominant sterols found in commercial phytosterol esters were β-sitosterol, campesterol, and stigmasterol. Barnes [23] also mentioned that WGO exhibits a range of sterols. Campesterol, β-sitosterol, triterpenoid alcohols, and 4-methyl sterols are the main components. Vitamin E (β-tocopherol and γ-tocopherol) and vitamin D (1,25-dihydroxyvitamin D3), which represent 49.23±11.01, 46.83±8.59, and 1.52±0.67, respectively, are also present in substantial amounts in MLCs extract. Vitamin E affects gene expression, regulates the activity of enzymes like protein kinase C (PKC), which is involved in the growth of smooth muscle, and acts as a radical scavenger by delivering a hydrogen (H) atom to free radicals [24, 25]

Table 1. Some sterol and tocopherol fractions in the wheat germ oil extract.

Items	Formula	Concentration (mg/100 g extract)
β-Sitosterol	$C_{32}H_{58}O$	806.25 ±24.76
Campesterol	$C_{31}H_{56}O$	374.61 ±17.61
Stigmasterol	$C_{32}H_{56}O$	185.67 ±11.48
1,25-Dihydroxyvitamin D3	$C_{30}H_{52}O_3$	1.52 ± 0.67
α-Tocopherol	$C_{32}H_{58}O_2$	49.23 ±11.01
γ-Tocopherol	$C_{31}H_{56}O_2$	46.83 ±8.59

Table 2 shows some of the chemical properties of butter oil (BO) as affected by different concentrations of MLCs extracted from WGO. The refractive indices (RI) of BO samples containing various MLC concentrations were not significantly different ($P > 0.05$). The values of RI recorded in BO samples are

3.2 Chemical properties of BO

similar to those of a wide range of lipids (butter fats and oils), whereas the values ranged from 1.4539 to 1.4532 for samples treated with 0.5 to 1.5%, respectively. The obtained data agrees with the data shown by Nikolova et al. [26]. The RI of fats and oils was correlated with saturated fatty acids, free fatty

acid (FFA) content, and oxidation state [27]. The peroxide values (PVs) and FFA (%) of BO samples were also not significantly affected ($P > 0.05$) by the addition of MLCs. Because PV is an indicator of peroxidation, a high PV of the oil indicates a low resistance to oxidation during storage and a hint of deterioration [28]. PVs were ranged between 0.62 and 0.69 mEq O_2/kg, in the acceptable range set by the Codex Alimentarius Commission [29]; the maximum acceptable value is 10 mEq O_2/kg. FFAs can catalyze the oxidative decay of oils by enzymatic and or chemical hydrolysis to form off-volatile components. Lipase efficacy is indicated by the FFA value [30]. The FFA (%) of all samples was at acceptable levels and ranged from 0.16 to 0.17%. The iodine value (IV) is a measure of the relative degree of unsaturation in oil components as determined by the uptake of halogen. Because the melting point and oxidative stability are related to the degree of unsaturation, IV provides an estimation of these quality factors. The greater the iodine value, the more unsaturation and the higher the susceptibility to oxidation [31]. The data obtained showed that IV, which ranged from 36.01±1.0 to 37.11±0.34 g/100 g of BO, was not significantly affected ($P > 0.05$) by the addition of MLCs.

Table 2. Some chemical properties of butter oil as affected by the addition of minor lipid components extracted from wheat germ oil.

Parameters	The concentration of MLCs added to the BO (%)					
	Control	0.25	0.50	0.75	1.00	1.50
RI (at 25±0.5 °C)	1.4537^a±0.001	1.4537^a ±0.001	1.4539^a ±0.001	1.4536^a ±0.001	1.4535^a ±0.001	1.4532^a ±0.001
I (g I_2/100 g BO)	37.11^a ±0.89	36.01^a ±1.00	36.90^a ±0.67	36.47^a ±0.31	36.47^a ±0.21	36.80^a ±0.34
FFA (% as oleic acid)	0.17^a ±0.01	0.16^a ±0.01	0.16^a ±0.01	0.17^a ±0.01	0.16^a ±0.01	0.17^a ±0.01
PV (mEq. O_2/kg)	0.64^a ±0.10	0.62^a ±0.11	0.66^a ±0.11	0.69^a ±0.10	0.65^a ±0.10	0.66^a ±0.11

Means (±SE) with the same letters in the same row are not significantly different at $P \leq 0.05$; BO, butter oil; MLCs; Minor lipid components; RI, Refractive index; IV, Iodine value; FFA, Free fatty acid; PV, Peroxide value

3.3 Slip melting point and solid fat content

The slip melting point (SMP) of BO samples treated with MLCs changed significantly ($P < 0.05$), as shown in Fig 1. The SMP of the BO samples containing 0.25% MLCs was 33.50 ºC and this value was increased to 35.40 ºC significantly ($P < 0.05$) by increasing the concentration of MLCs to 1.5%. A similar observation was made by Chai et al. [32], who concluded that the removal of MLCs did not affect triglyceride composition, but the existence of the MLCs did increase the SMP, which enhanced the start of crystallization. Solid fat content (SFC) is an important characteristic for predicting fat functionality at different stages of manufacturing [33]. It also provides a reference for the hardness and spreadability (i.e., cold spreadable butter) [34]. The SFC significantly decreased as temperature increased ($P < 0.05$); the rate of decrease was comparable among all BO samples (Table 3). However, the SFC of BO slightly increased as the addition of MLCs increased; the increase was more pronounced ($P < 0.05$) at an addition level of 1.5% compared to pure BO. These findings confirm those in Fig 1 of the SMP, which indicates that the MLCs may affect the equilibrium SFC of BO at high concentrations [32].

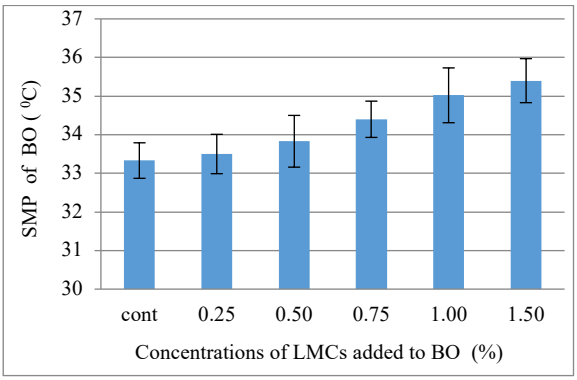

Fig. 1. Slip melting point (SMP) of butter oil as affected by the addition of minor lipid components extracted from wheat germ oil.

Similarly, adding soy lecithin as an emulsifier led to an increase in SFC and crystal formation in palm oil. Emulsifiers may therefore be useful tools for managing crystallization in compositions produced with fluidized palm oil [35]. Inversely, Rodrigues et al. [2] reported that at low temperatures, milk fat containing phytosterol ester displayed a softer consistency and a lower SFC than pure milk fat. In another study, the SFC of BO and palm oil (PO) blends was lower at 0.0 and 10 °C than it was for either pure PO or BO separately, while at 25 °C, the SFC of oil blends was comparable to that of pure BO [12]. Shazly et al. [36] suggest that the concentration of USFA as well as other lipid-minor components like sterols and vitamin E affect the physical characteristics of AMF, such as SFC.

Table 3. The solid fat content (SFC) of butter oil is affected by the addition of minor lipid components extracted from wheat germ oil.

Temperature (°C)	The concentration of MLCs added to the BO (%)					
	Control	0.25	0.50	0.75	1.00	1.50
	---------- (%) ----------					
10	44.1^{b}±0.12	44.1^{ab}±0.25	44.2^{ab}±0.30	44.3^{ab}±0.25	44.5^{ab}±0.20	45.0^{a}±0.30
20	20.9^{b}±0.20	20.9^{b}±0.15	21.2^{ab}±0.20	21.1^{ab}±0.15	21.3^{ab}±0.25	21.6^{a}±0.20
25	13.2^{a}±0.00	13.3^{a}±0.20	13.4^{a}±0.20	13.2^{a}±0.05	13.5^{a}±0.20	13.8^{a}±0.25
30	6.3^{b} ±0.00	6.3^{b} ±0.05	6.5^{b} ±0.20	6.7^{ab} ±0.10	6.9^{ab} ±0.10	7.3^{a} ±0.15
35	1.7^{c} ±0.10	1.8^{bc} ±0.10	1.8^{bc} ±0.05	2.0^{b} ±0.00	2.0^{b} ±0.00	2.3^{a} ±0.10
40	0.0^{b} ±0.00	0.0^{b} ±0.00	0.0^{b} ±0.00	0.0^{b} ±0.00	0.0^{b} ±0.00	0.2^{a} ±0.00

Means (±SE) with the same letters in the same row are not significant different at P ≤ 0.05; BO, butter oil; MLCs; Minor lipid components

3.4 Thermal behavior

Thermal profiles explain the transition of temperatures and heat in terms of the melting and crystallization behavior of fats and oils and provide integral results for lipid profiles. The melting and crystallization curves of BO with different concentrations of MLCs are shown in Fig 2. In general, the exothermic thermogram is only affected by the lipid profile of oils and fats, instead of the elementary crystallization case [32]. The DSC cooling thermogram of pure BO with a starting crystallization temperature of 54.85°C showed two peaks at 35.88 °C (peak 1) and 49.49 °C (peak 2). Table 3, represents high melting and high melting fractions, with the released enthalpy variation (ΔH, J/g) of 41.54 and 4.65 J/g, respectively. After the MLCs were added at various concentrations (0.25, 0.5, 0.75, 1.0 and 1.5% W/W), the starting crystallization of BO decreased to 52.51, 41.55, 50.01, 45.02, and 47.51°C ($P < 0.05$). On the other hand, the peak shifting was noticed for different concentrations of MLCs addition to 32.36; 46.13 (two peaks), 33.34 (one peak), 32.29; 44.60 (two peaks), 29.99; 39.11 (two peaks), and 29.76; 39.95 °C (two peaks), respectively. As a result, the addition of MLCs resulted in a slight shift of endothermic peaks towards lower temperatures. Moreover, the ΔH of the first peak decreased to 15.00, 16.21, 27.27, 17.34, and 22.50 J/g, respectively, and the other decreased to 5.15, 4.46, 2.97, and 3.33 J/g for ratios 0.25, 0.50, 0.75, 1.0 and 1.5% %, respectively, the exception 1.0% ratio was one peak (Table 4).

Table 4. Differential scanning calorimetric (DSC) melting peak enthalpy of butter oil as affected by the addition of minor lipid components extracted from wheat germ oil.

Treatments	ΔH_f (J/g)		$\Delta H_{f,\,total}$ (J/g)
	First melting peak	Second melting peak	
Control	41.54^{a} ±0.91	4.65^{ab}±0.25	46.19^{a} ±1.11
0.25	15.00^{d} ±1.00	5.15^{a} ±0.34	20.15^{d} ±1.23
0.50	16.21^{d} ±1.10	--	16.21^{e} ±1.02
0.75	27.27^{b} ±0.90	4.46^{ab}±0.15	31.73^{b} ±0.98
1.00	17.34^{d} ±0.86	2.97^{c} ±0.15	20.31^{d} ±1.06
1.50	22.50^{c} ±1.05	3.33^{b} ±0.16	25.83^{c} ±1.21

Means (±SE) with the same letters in the same column are not significantly different at P ≤ 0.05; BO, butter oil; MLCs; Minor lipid components

Chai et al. [32] suggested that the existence of minor polar components contributed to the crystallization of fully hydrogenated palm kernel oil. Smith et al. [4] added minor components in milk fat

and found that the drop melting point and equilibrium SFC do not change but that the onset of crystallization is delayed at low undercooling. The changes in the form of the melting behavior could be indicative of changes in polymorphism. This suggested that the addition of MLCs led to changes in polymorphic shape. Lipid crystallization is affected by a wide range of external as well as internal factors. Pressure, sonication, shear, additives, and heat treatment are important external variables [37].

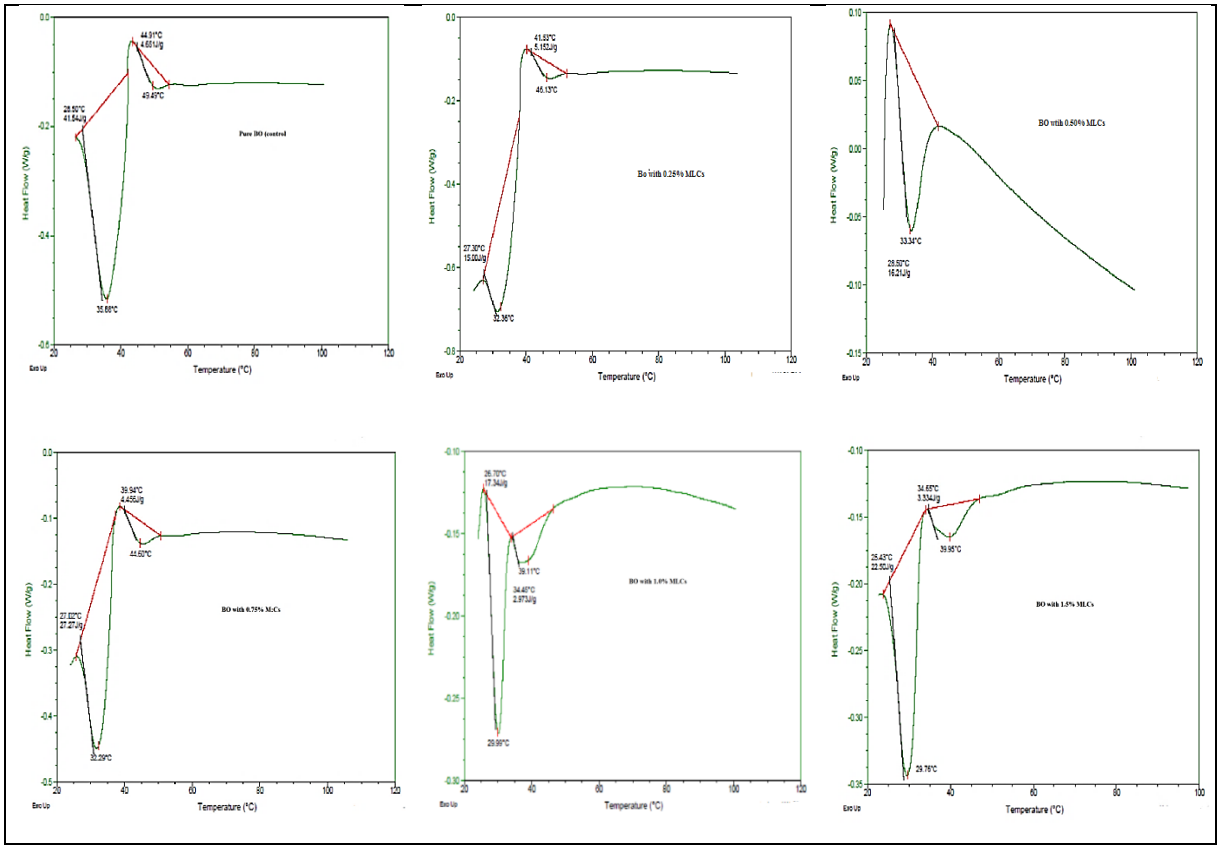

Fig. 2. Differential scanning calorimetry (DSC) melting peak maximal temperatures and enthalpies of butter oil as affected by the addition of minor lipid components extracted from wheat germ oil.

3.5 Oxidative stability

Oxidative stability (OS) is generally used as a quick and easy method to predict the storage stability of fats and oils [38]. Table 5 displays the induction periods (IP) at 110 °C for pure BO and the other BO samples that contained MLCs. In general, as the level of MLCs increased, the IP of BO significantly increased ($P < 0.05$). The IP increased from 7.67±0.54 h in pure BO to 15.3±1.09, 22.43±1.97, 24.5±0.99, 26.87±1.62, and 28.13±1.78 h in BO containing 0.25, 0.50, 0.75, and 1.0%, respectively. However, the rate of increase was more pronounced in BO containing 0.25 and 0.5% MLCs ($P < 0.05$), which were respectively 2 and 3-fold higher than pure BO. Hence, from the previous results, it could be concluded that the addition of MLCs extracted from WGO to BO increased the oxidative stability of BO. Similarly to this, the ethanol extract of the Arjuna plant (*T. Arjuna*) was found to have a substantial ability to increase the antioxidant potential of both cow and buffalo ghee [39]. Merai et al. [40] found that ghee made from butter and 0.6% Tulsi (Ocimum sanctum) leaf extract had equal stability to ghee containing 0.02% BHA after 8 days of high-temperature storage (80±2 °C). El-Hadad et al. [19] reported also that the addition of DHQ reduced the oxidation process in BO, which caused prolongation of the shelf life (1.9 to 3.53 times) in a concentration-dependent manner (50 – 200 ppm). On the other hand, as the induction time increased, all butter samples showed increasing trends in the SFC [41].

3.6 Crystal morphology

The microstructures of fat samples were determined by polarized light microscopy to evaluate the effects of the addition of MLCs on the BO crystal network structure. Micrographs obtained after crystallization at 25°C are shown in Fig 3. Some significant differences in the crystal number and crystallite diameter were observed among the different BO samples. A pure BO sample showed smaller and more numerous spherical crystals compared to other BO samples with MLCs. For example, compared to BO containing 1.0 and 1.5% MLCs, the crystals of BO (1.5% MLCs) had great differences in morphology. Because of the existence of MLCs, the crystalline particles of BO tended to be more arranged, aggregate, and grow into more ordered and larger microstructures. However, the aggregation of BO (1.5% MLCs) crystals was comparatively larger after the addition of the MLCs, resulting in a variety of fat crystal shapes. These results indicated that MLCs could play a role in the seeding of the fat crystals, lead the development of the fat crystal network, and contribute to the formation of various crystal microstructures [10, 32]. These results suggest that MLCs could participate in crystallization and boost the nucleation and growth processes to be more ordered.

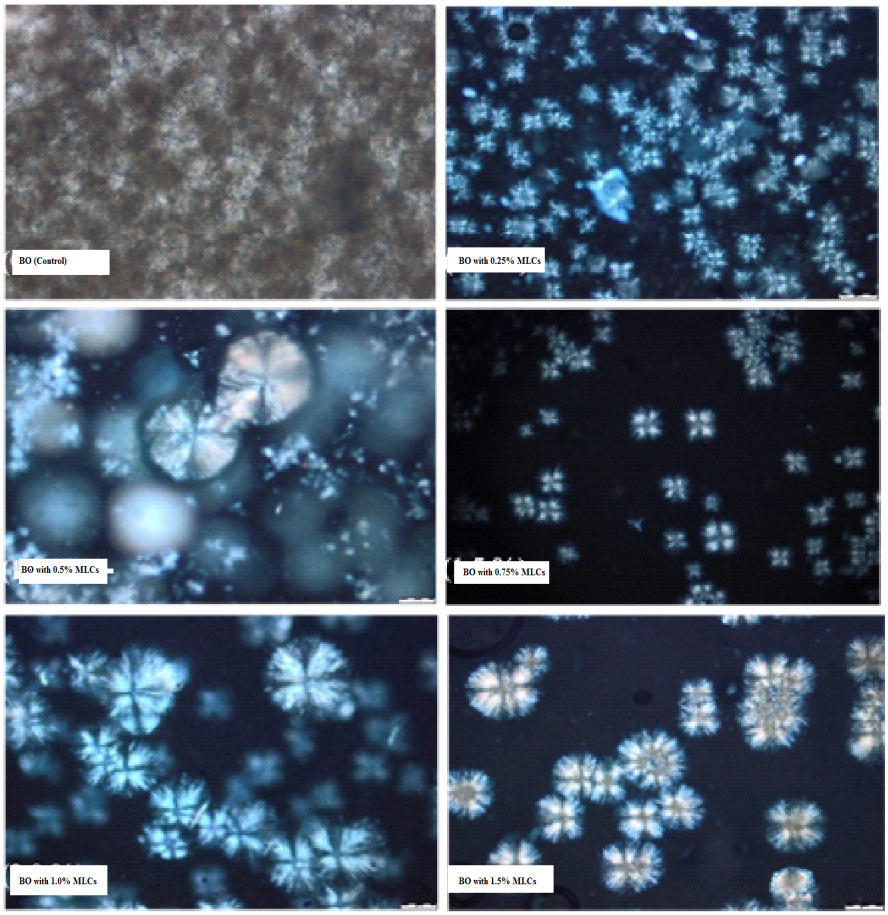

Fig. 3. Microscope images of BO crystals by polarized light microscopy as affected by the addition of minor lipid components extracted from wheat germ oil

Table 5. Oxidative stability (induction period) of butter oil as affected by the addition of minor lipid components extracted from wheat germ oil.

BO treatments	Induction periods at 110 °C	
	Time (h)	Increasing rate (fold)
Control	7.67^{e} ±0.54	1.00^{e} ±0.00
0.25	15.30^{d} ±1.09	1.99^{d} ±0.14
0.50	22.43^{c} ±1.97	2.92^{c} ±0.25
0.75	24.50^{cb}±0.99	3.19^{cb} ±0.13
1.00	26.87^{ba}±1.62	3.50^{ab} ±0.21
1.50	28.13^{a} ±1.78	3.67^{a} ±0.24

Means (±SE) with the same letters in the same column are not significantly different at $P \leq 0.05$); BO, butter oil; MLCs; Minor lipid components.

4. Conclusion

It was found that the existence of MLCs could significantly affect the melting and crystallization properties of BO. The presence of MLCs increases the SMP and enhances the start of crystallization. Changes in microstructure and polymorphic diversion took place after the addition of MLCs, resulting in changes in the fat crystal network. The oxidative stability of BO was significantly impacted by the addition of MLCs; it was three times higher than the control sample. The SFC of BO is not affected significantly by the addition of MLCs. However, the existence of MLCs caused the transformation from the rough crystal form to the unified crystal form in the microstructure network. Finally, the MLCs could engage in and influence the physiochemical properties as well as the crystallization behavior of BO, leading to the formation of different structural properties of fat crystals and physical properties of fat, which may be helpful for different applications in the food industry. Nutritionally, consuming foods high in plant sterols and tocopherols can also help reduce blood cholesterol, reduce obesity, and boost immunity.

Conflicts of interest

The authors have declared no conflicts of interest for this article.

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