Interesterified blend-based and physical blend-based special fats: storage stability under fluctuating temperatures

Ting-Wei Zhu, a Xia Zhang, a,b Min-Hua Zong, a Robert J Linhardt, c Bing Li a,b,* and Hong Wu a,b,*

Abstract

BACKGROUND: Temperatures that special fat faces in a real environment fluctuate, thus, understanding the property changes of special fats under fluctuating temperatures will be helpful in guiding how to keep its high quality in the production and application process. Therefore, a comparative study was carried out on the storage stability of physical blend-based and interesterified blend-based special fats (PBSFs and IBSFs) and their oxidative stability, crystallization and physical properties were studied under fluctuating temperatures.

RESULTS: The peroxide values of IBSFs and PBSFs were less than 10.0 mmol kg$^{-1}$ after 4 weeks of storage, and IBSFs had better oxidative stability. There was little change in the solid fat content, and the hardness decreased when IBSFs and PBSFs were stored for 4 weeks. X-ray diffraction results indicated that PBSFs had only $\beta$-crystal, but IBSFs had $\beta$- and $\beta'$-crystal after storage. Moreover, in IBSFs, the transformation from $\beta'$- to $\beta$-form in PS:RO-IBSF was more obvious than that in PS:SO-IBSF (PS, palm stearin; SO, soybean oil; RO, rapeseed oil) after 4 weeks of storage, and the good integrity of crystalline network in fast-frozen special fats during fluctuating temperature storage followed the order: IBSF $>$ PBSF, PS:RO-PBSF $>$ PS:SO-PBSF.

CONCLUSION: The results suggest IBSF can better maintain its quality during fluctuating temperature storage than PBSF. © 2019 Society of Chemical Industry

Keywords: interesterified blend; oxidative stability; crystallization characteristics; physical property; fluctuant temperature storage

INTRODUCTION

Fast-frozen special fat is an essential plastic fat widely applied in the preparation of traditional Chinese fast-frozen foods such as those including sweet dumpling balls (rice balls) and dumplings. 1,2 At present, most of the fast-frozen special fats used are all-purpose plastic fats, like margarines and shortenings, which are mostly prepared by using refined animal and/or vegetable fats and oils, hydrogenated vegetable oil, or the physical blends of these as base oil. 3,4 However, these base oils with high melting points, are rich in abundant saturated fatty acids, and the granular crystal which related to the $\beta$-polymorph transformation impairs the consistency, plasticity and mouthfeel of fat products in the application. 5 Trans-fatty acids are inevitably generated in the partial hydrogenation of vegetable oils and, thus, the intake of such fat products would increase the risk of cardiovascular diseases. 6

Enzymatic interesterification, a promising oil and fat modification technology, has received increasing attention in production of trans-free plastic fats, and the characteristics of the interesterified blends such as slip melting point, plastic range and its crystals can provide desirable properties and smoother mouthfeel for food products. 1,7 It was found that tightly packed crystals of small size in the interesterified-blend, which are related to the $\beta'$-crystal, provide the fat with plasticity, resulting in better performance than the corresponding physical blend-based product when it was applied in fast-frozen food production. 1,8 In fact, except for the fat itself, the storage process also has an impact on the quality of plastic fats. 9

Compared to the corresponding physical blend-based fast-frozen special fat (PBSF), the interesterified blend-based fast-frozen special fat (IBSF) stored at constant temperature (4 $^\circ$C) for 8 weeks still maintained good quality when applied in the preparation of fast frozen sweet dumplings. 4 Indeed, the constant temperature storage mentioned earlier was the ideal situation during storage, and the temperature that the special fat faced in a real environment will fluctuate due to the external environment, such as the temperature difference between day and night and the processing environment of products during the application of the special fat. Hence, it is necessary to understand the property changes of IBSF under fluctuating temperature conditions, and this should be helpful in guiding how to keep high quality of fast-frozen special fats.

* Correspondence to: BLi@scut.edu.cn (Li); bbhwu@scut.edu.cn (Wu)

a School of Food Science and Engineering, South China University of Technology, Guangzhou, China

b Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou, China

c Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York, USA
fat in the production and application process. However, to date, there are few reports on the property changes of fast-frozen special fats under fluctuating storage conditions.

Therefore, to obtain a deep insight into the property changes of fast-frozen special fat stored under fluctuating temperature conditions, in this study, two types of IBSFs (PS:SO-IBSF, PS:RO-IBSF, where PS, palm stearin; SO, soybean oil; RO, rapeseed oil) and PBSFs (PS:SO-PBSF, PS:RO-PBSF) were chosen for comparison, and the storage stability of IBSFs and PBSFs, including their oxidative stability, crystallization characteristics and physical properties, were investigated. Since fast-frozen special foods are generally stored at \(-20^\circ C\) and thawed at 4 \(^\circ C\) or at room temperature (25 \(^\circ C\)) before application, two fluctuating temperatures (\(-20^\circ C\) to 25 \(^\circ C\) for 12h) were selected based on the application environment of fast-frozen foods. Moreover, the oxidative stability about peroxide values (POVs) and induction period (IP) index for IBSFs and PBSFs were measured by iodometric procedure method and Rancimat method, respectively. Subsequently, relevant physical properties, including solid fat content (SFC) and hardness, were monitored through a low-resolution pulse nuclear magnetic resonance (pNMR) spectrometer and a texture analyzer, respectively. Crystal polymorphism and crystal microstructure were studied by X-ray diffraction (XRD) and polarized light microscopy (PLM), respectively.

MATERIALS AND METHODS

Materials
Palm stearin (PS, slip melting point 52 \(^\circ C\), iodine value: 406 g I kg\(^{-1}\)) was supplied by Shenzhen Jingyi Co. (Shenzhen, China), and soybean oil (SO) and rapeseed oil (RO) were purchased from a local grocery store. Lipzyme TL IM (1,3-specific immobilized lipase) was purchased from Novozymes (China) Biotechnology Co., Ltd (Guangzhou, China). All other reagents and solvents were analytical or chromatographic grade and were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Preparation of fast frozen special fats
The specific steps of making IBSF are shown in Fig. 1. First, the interesterified blend was obtained through lipzyme TL IM-catalyzed interesterification. The enzymatic interesterification was carried out in a fluidized-bed reactor (column length 35 cm, internal diameter 1.2 cm) and 10 g of immobilized lipzyme TL IM was added into the internal column. A peristaltic pump was used to feed the substrate mixture of 210 g PS and 90 g SO or 90 g RO (PS:SO, PS:RO 7:3, wt%) into the reactor and the reaction temperature was 60 \(^\circ C\). The blend drained out of the fluidized-bed reactor was collected. Then, 84 g of the earlier interesterified blend, 15 g water and 1 g emulsifier (Span-60/trimethylene glycol ester/soybean lecithin 5:3:2, wt%) was fully mixed at 60 \(^\circ C\) and 2000 \(\times\) for 20 min. After that, the resulting mixture was maintained at 40 \(^\circ C\) for 10 min, and then kept at \(-20^\circ C\) for 120 min followed by maintaining at 25 \(^\circ C\) for 48 h. The corresponding physical blend (210 g PS and 90 g SO, 210 g PS and 90 g RO) were used as the base oil to prepare PBSF following the steps mentioned earlier.

Storage protocol
The IBSFs and PBSFs were stored at fluctuating temperatures (\(-20^\circ C\) to 25 \(^\circ C\) for 12h and 4 \(^\circ C\) to 25 \(^\circ C\) for 12h) for 4 weeks, respectively. Three parallel experiments were performed.

Oxidative stability
POV was collected according to the method of iodometric procedure.\(^{10}\) IP index was measured by using Rancimat.\(^{11}\) Briefly, the sample, weighing 3.0 g, was added into the reactor tube of the instrument and the reaction temperature was 120 \(^\circ C\) and the airflow rate was 20 L h\(^{-1}\).

SFC determination
A low-resolution pNMR spectrometer (Bruker, Karlsruhe, Germany) was used to determine the SFC according to the American Oil Chemist’s Society (AOCS) Official method Cd 16b-93.\(^{12}\)

Hardness analysis
The hardness of the sample was determined by using a TA.XT-Plus texture analyzer (TA Instrument Inc., New Castle, UK). A 45° conical probe was inserted into the sample to a penetration depth of 10 mm at 2 mm s\(^{-1}\). The maximum recorded force (g) was defined...
as the hardness of the sample. The sample was prepared in triplicate for each analysis.

XRD spectroscopy
The polymorphic form of fat crystal in the sample was determined by XRD using Cu KR radiation with a Ni filter (voltage 40 kV; current 40 mA). The sample was scanned from 10° to 30° (2θ scale) at a rate of 2.0° min⁻¹.

PLM
The morphology of the sample was imaged using PLM (Axioskop40pol, Leica, Wetzlar, Germany) with an attached Canon A640 digital camera (Canon, Tokyo, Japan). The desired amount of the sample (approximately 50 mg) was placed on a carrier glass slip pre-cooled to the desired temperature. A cover glass slip was then placed parallel to the plane of the carrier slide and centered on the drop of sample to ensure uniformity. The photomicrograph of the crystal was captured at a 500x magnification.
Statistical analysis
The experiments were performed in triplicate (n = 3), and the data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Oxidative stability during fluctuating temperature storage

Lipid oxidation affects the food quality through changing in color, flavor, texture and nutritional value. Two approaches including measuring the POV and IP were utilized to investigate the oxidative stability of IBSFs and PBSFs during fluctuating temperature storage and the results are shown in Fig. 2. As shown in Fig. 2A–D), the fluctuating temperature had no significant effect on POV of IBSFs and PBSFs ($P > 0.05$), while increasing the storage time (from 1 week to 4 weeks), the POV increased and the POV was less than 10.0 mmol kg$^{-1}$ after 4 weeks storage. The results indicated that all these fast-frozen special fat samples still maintained good quality as the POVs was in accord with the safety limit (10.0 mmol kg$^{-1}$) enacted by Codex Alimentarius Commission (CAC). In addition to the POV, the IP was obtained by the Rancimat method for a better estimation of lipid oxidation. The results were presented in Fig. 2(E).

The storage time and storage temperature had no significant effect on the IP of IBSFs and PBSFs, which indicates these storage conditions had little effect on the oxidation stability of fat ($P > 0.05$) (Fig. 2(E)). The IP of fat can be used as a parameter to judge the antioxidant capacity. The longer the IP value, the greater the stability observed for a fat. For IBSFs, the IP values of PS:SO-IBSF and PS:RO-IBSF were 2.0 h to 2.5 h and 4.0 h to 5.0 h, respectively, indicating PS:RO-IBSF has better oxidation stability than PS:SO-IBSF. One possible explanation was the different base oils, in which rapeseed oil (RO) showed better oxidation stability because of its indigenous antioxidants. These results were also consistent with the observed POV.

Physical properties of IBSFs and PBSFs during fluctuating temperature storage

SFC

The SFC is responsible for plastic fat product properties, including spreadability and oil exudation. Therefore, the SFC changes in IBSFs and PBSFs during storage under fluctuating temperatures as shown in Table 1. Apparently, the SFC of IBSFs was low in comparison with that of PBSFs under the same conditions due to the SSS-type triacylglycerols (TAGs) (S: saturated fatty acid) such as PPP (palmitic acid) decreased after interesterification, which was indicated in our previous studies. Moreover, it was found that there were no significant changes in the SFC of samples with increased storage time at a given temperature ($P > 0.05$). Fluctuating temperature during 4 weeks of storage also had little effect on the SFC and the SFC of PS:SO-IBSF and PS:RO-IBSF were about 20% and 14%, respectively. Different TAGs composition and crystallization behavior could affect the SFC and the storage conditions provide a recrystallization environment due to the insufficient super-cooling temperature. In addition, the value of SFC, more than 10% at room temperature (between 20 °C and 25 °C), is good for preventing oiling off. The SFC of both IBSFs and PBSFs were over 10% when stored for 4 weeks, which indicated that the IBSFs and PBSFs still maintained a good consistency after storage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(−20 (12 h)–25 (12 h))</td>
</tr>
<tr>
<td>PS:SO-PBSF</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>28.02 ± 1.13</td>
</tr>
<tr>
<td>1</td>
<td>28.29 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>30.59 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>30.65 ± 0.86</td>
</tr>
<tr>
<td>4</td>
<td>30.26 ± 0.92</td>
</tr>
<tr>
<td>PS:SO-IBSF</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20.93 ± 0.44</td>
</tr>
<tr>
<td>1</td>
<td>21.33 ± 0.34</td>
</tr>
<tr>
<td>2</td>
<td>20.66 ± 0.20</td>
</tr>
<tr>
<td>3</td>
<td>20.19 ± 0.30</td>
</tr>
<tr>
<td>4</td>
<td>20.38 ± 0.21</td>
</tr>
<tr>
<td>PS:RO-PBSF</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>18.46 ± 0.19</td>
</tr>
<tr>
<td>1</td>
<td>19.29 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>20.02 ± 0.29</td>
</tr>
<tr>
<td>3</td>
<td>19.71 ± 0.25</td>
</tr>
<tr>
<td>4</td>
<td>20.56 ± 1.05</td>
</tr>
<tr>
<td>PS:RO-IBSF</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16.37 ± 0.17</td>
</tr>
<tr>
<td>1</td>
<td>15.35 ± 0.47</td>
</tr>
<tr>
<td>2</td>
<td>15.53 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>13.19 ± 0.30</td>
</tr>
<tr>
<td>4</td>
<td>13.29 ± 0.23</td>
</tr>
</tbody>
</table>

Note: The same letter in the individual column indicates no significant difference ($P > 0.05$) between each parameter tested.

**Hardness**

The hardness of plastic fat is responsible for its appearance, mouth feel and workability. The hardness of IBSFs and PBSFs during storage under fluctuating temperatures is presented in Fig. 3. Under storage conditions of fluctuating temperatures, the hardness of both IBSFs and PBSFs decreased with prolonged storage time. For PBSFs, the hardness was greater and after 4 weeks of storage the hardness of PS:SO-PBSF and PS:RO-PBSF decreased from 90 g to 74 g, and from 65 g to 49 g, respectively. Changes in IBSFs hardness was less and the hardness decreased from 45 g to 35 g for PS:SO-IBSF and from 22 g to 18 g for PS:RO-IBSF. These results were attributed to the occurrence of an aggregated crystall network in fat during storage. The crystals involved in the crystalline network become aggregated into larger crystal aggregates, which in turn affect the hardness of the fat product. The varying degree of crystalline network structure destruction might result in a decrease in hardness. Previous studies found that plastic fat with a completed and compacted crystalline network structure exhibited high hardness values under the same SFC value conditions. Based on these results, we presume that these hardness changes are caused by different crystalline networks as well as SFC. Therefore, the microscopic crystallization behavioral changes of IBSFs and PBSFs during storage were further investigated.

Crystallographic characteristics of IBSFs and PBSFs during fluctuating temperature storage

Crystal polymorphism

Crystal polymorphism ($α$-, $β$-, and $β$-forms) can be characterized according to the short spacing using XRD. The short spacing of...
α-form appears near 4.15 Å, and the β-form and β′-form have a strong short spacing at 4.6 Å and two strong short spacings near 3.8 Å and 4.2 Å, respectively. The sub-β-form had three short spacings near 3.6 Å, 3.9 Å and 4.5 Å. The crystal polymorphism of IBSFs and PBSFs, stored at different fluctuating temperatures over different times, were investigated by XRD and the results are presented in Fig. 4.

The peaks at 4.59–4.60 Å, 4.44–4.45 Å, 4.33 Å, 4.19–4.20 Å, 3.89 Å and 3.81 Å appeared in IBSFs and PBSFs before storage, which are attributed to the β′- and β-form (Fig. 4). The intensity of the characteristic peaks of β′-form was stronger in IBSFs than that in PBSFs, indicating the coexistence of β′- and β-form in both PBSFs and IBSFs, but primarily the β′-form in IBSFs. Moreover, there was an obvious change in the short spacing peak of IBSFs and PBSFs during fluctuating temperature storage patterns (−20 °C 12 h to 25 °C 12 h and 4 °C 12 h to 25 °C 12 h). Specifically, for PBSFs, the peak at 4.2 Å disappeared with increasing storage time. This was due to the gradual transformation from metastable β′-form to β-form or the disappearance of β′-form during a period of storage. After 4 weeks of storage, only β-crystal form was present in the PBSFs. However, in IBSFs, during storage at fluctuating temperatures, the intensity of peaks at 4.3 Å and 4.2 Å gradually became weaker and the intensity of peak at 4.6 Å gradually became stronger with increased storage time, indicating that the β′-form was partly transformed into the β-form. The changes were even more obvious after 2 weeks of storage. In our previous studies, it was found that the TAGs composition such as the ECN (equivalent carbon number) 42-type, ECN 48-type and ECN 50-type TAGs had notable influence on the crystallization profile of blends, especially the increase of ECN 50-type TAGs would significantly enhance the formation of β′-crystal (P < 0.05). Other research also reported that the PLO (P, palmitic; L, linoleic; O, oleic) and PPS (P, palmitic; S, stearic) which belonged to the ECN 50-type TAGs were tended to form β′ crystal. The IBSFs had higher content of ECN 50-type TAGs and more β′-form crystal existed in the IBSFs than the PBSFs, suggesting that the more content of ECN 50-type TAGs in IBSFs is helpful to reduce its crystal transformation from β′- to β-form. Furthermore, the fatty acids on the ECN 50-type TAGs skeleton tend to form relatively diversified arrangements, which are beneficial for forming the β′-form crystal.

In addition, by comparing with the data of our previous study, the transformation rate of crystal from β′- to β-form was higher when IBSFs was stored at fluctuating temperatures than at low temperature (4 °C). The β′-form with small crystals is desirable for providing special fat with good texture and properties, as the plastic fat with β′-form can incorporate relatively large amounts of liquid oil in the crystal network. The fluctuating temperatures could destroy the desirable crystal form in special fat, while this phenomenon can be enhanced to some extent in IBSFs.

Microstructure
The crystal size and density of crystals in the crystalline network could affect the hardness and viscoelasticity of plastic fats. Thus, the crystalline network microstructures of IBSFs and PBSFs under the fluctuating temperature storage conditions were analyzed by PLM and the results were provided in Fig. 5.

Some significant differences were observed in the IBSFs and PBSFs under the two fluctuating temperature storage conditions.
including the changes of crystal size, density and crystal morphology (Fig. 5). As for PBSFs, relatively large and rod-like crystals were distributed in the crystalline network before storage. Bigger size crystals appeared through aggregation and large flake-like crystals began to appear during the storage. This phenomenon was even more obvious when PBSFs was stored for 4 weeks, and large-block fluffy crystals were unevenly distributed within the crystalline network. Changes in the fluctuating temperature storage conditions, −20 °C 12 h to 25 °C 12 h, were even more severe than the fluctuating temperature storage conditions, 4 °C 12 h to 25 °C 12 h.

In IBSFs, the crystals of small size were uniformly distributed in the crystalline network and a large amount of small spherical crystals was densely present in a network before storage. For PS:SO-IBSF, small areas of flocculent crystals appeared after 2 weeks of storage and the crystals were unevenly distributed in a loose crystalline network after 4 weeks of storage. In PS:RO-IBSF, the crystalline network including the crystal size, density and crystal morphology dramatically improved during storage. When PS:RO-IBSF was stored for 3 weeks, the crystal size became larger and the crystals were uniformly dispersed. In addition, small amounts of flocculent

Figure 4. X-ray diffraction (XRD) spectra of fast-frozen special fats during fluctuating temperature storage.
crystals appeared in the crystalline network. Previous research showed that the crystal size and the morphology were essential for the consistency and acceptability of plastic fat products, and crystals with small size and needle-like structure was $\beta'$-form and the crystal aggregated to form spherulite of larger sizes corresponded to $\beta$-form.\textsuperscript{15,27} These results were attributed to the presence of more $\beta'$-form in the IBSFs, especially in PS:RO-IBSF, which was consistent with the XRD results. On the whole, the good uniformity and small crystal size of crystals in the crystalline network during the fluctuating temperature storage showed the order: IBSFs > PBSFs, PS:RO-PBSF > PS:SO-PBSF.

CONCLUSIONS
Fluctuating temperatures caused different effects in the stability of the fast-frozen special fats, IBSFs and PBSFs. IBSFs had better storage stability during fluctuating temperature storage than the corresponding PBSFs. Both kinds of IBSFs showed good oxidative stability (PS:RO-IBSF > PS:SO-IBSF) and the POV for both types...
of IBSFs were less than 10.0 mmol kg\(^{-1}\). The physical properties measured for both fast-frozen special fats during the fluctuating temperature storage showed that the SFC exhibited unobvious changes, while hardness decreased with increased storage times, due to the different changes in the crystallization characteristics. During the storage process, only \(\beta\)-crystal was present in PBSFs after 4 weeks of storage. However, in IBSFs, the \(\beta\)-form was partly transformed into the \(\beta\prime\)-form and the transformation from \(\beta\)- to \(\beta\prime\)-form in PS:SO-IBSF was more obvious than that in PS:RO-IBSF. Moreover, large flake-like crystals appeared through crystal agglomeration in PBSFs during the storage process, but small amounts of flocculent crystals appeared in the crystalline network when IBSFs was stored for 4 weeks. The good uniformity and small crystal size of crystals in the crystalline network during the fluctuating temperature storage showed the order: IBSFs > PBSFs, PS:RO-PBSF > PS:SO-PBSF.

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