



## $\beta$ -Cyclodextrin ( $\beta$ -CD): A new approach in bread staling

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### ABSTRACT

In this study, the impact of the addition of  $\beta$ -cyclodextrin ( $\beta$ -CD) on bread staling was investigated by texture profile analysis (TPA), X-ray diffraction (XRD), and differential scanning calorimeter (DSC). Results showed that the retardation effect of  $\beta$ -CD on bread staling was found to be significant as less changes of hardness, cohesiveness and springiness were observed during the storage. The addition of  $\beta$ -CD also significantly decreased recrystallization rate  $k$ , and increased Avrami exponent  $n$ , indicating that the nucleation type was transformed. Investigated by X-ray diffraction, changes of crystalline patterns occurring in crust and crumb were retarded by the development of A + V and B + V intermediate patterns for stored crust and stored crumb, respectively. This retardation was attributed to the amylose–lipid– $\beta$ -CD complex formation observed and analyzed by DSC technique, resulting in nucleation type transformation and lowering the rate of bread staling.

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### 1. Introduction

The economic loss resulting from bread staling encourages research to control or retard staling process. Among the proposed techniques, the addition of glucose oxidase [1], xylanase [2], whey protein [3], soy protein [4], pentosans [5], arabinoxylans [6], hydroxypropylmethylcellulose [7], as well as the use of emulsifiers, have been well experimented on anti-aging of bread. Small molecule materials, such as ribose, xylose, maltose, and fructose, were also considered as anti-staling additives in the case of these sugars transforming the crystalline type, leading to a lower retrogradation rate of wheat starch [8]. Further Miyazaki et al. [9] found the addition of dextrin with low molecular weight produced less staling endothermic enthalpy compared to that of native bread; however, a large amount of addition would debase bread quality.

$\beta$ -Cyclodextrin ( $\beta$ -CD) is a cyclic oligosaccharide consisting of seven glucopyranose units, which are joined together by  $\alpha$  (1  $\rightarrow$  4) linkage forming a torus-shaped ring structure. Its hydrophobic core can complex with various organic molecules through host–guest

interaction: the interior cavity of the molecule provides a relatively hydrophobic environment, into which an apolar pollutant can be trapped. This interaction, in food industry, led to cholesterol removal from cream [10], undesired taste reduction, and food products shelf life extension as well [11]. It was also reported that the addition of  $\beta$ -CD would decrease the falling number of wheat starch and wheat flour suspensions in both the presence and absence of  $\alpha$ -amylase, and increase the loaf volume of bread [12]. Additionally,  $\beta$ -CD significantly increased swelling factor, solubility, amylose leaching and viscosity of wheat starch due to the disruption of existing amylose–lipid complex [13]; while this complex dissociation meant that  $\beta$ -CD might produce an amylose–lipid– $\beta$ -CD complex when interacting with starch lipid, resulting in the decrease in gelatinization enthalpy of wheat starch [14]. Therefore, it would be of importance to investigate the potential impact on the retrogradation of wheat starch or wheat flour. However, few studies were available in this field till far.

In this study, we aimed at investigating the influence of the addition of  $\beta$ -CD on bread staling by using texture profile analysis (TPA), X-ray diffraction (XRD) in combination with differential scanning calorimeter (DSC) method.

### 2. Experimental

#### 2.1. Materials

Wheat flour (13.6% moisture content, 12.5% protein) was obtained from Pengtai Flour Co., Ltd. (Hebei, China). Yanshan yeast, an industrial product in China (Hebei Mali Co., Ltd.), was used as a

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starter.  $\beta$ -CD was purchased from Seebio Biochemical, Inc. (Shanghai, China).

## 2.2. Breadmaking process

A basic bread formula consisted in wheat flour (2.0 kg), dry yeast (0.8%, flour basis), salt (1.0%, flour basis), and water (60%, flour basis, according to the amount needed for Brabender farinograph to achieve 500 Brabender Units) was used. Prior to mixing,  $\beta$ -CD was incorporated into the ingredients at a level of 1.5% (w/w) and the resultant blend was optimally mixed until dough development. Then the dough was divided into 200 g pieces, hand-rounded, mechanically molded, proofed at 30 °C and relative humidity (RH) of 85% for 90 min and baked into an electric oven at 195 °C for 16 min. Loaves were removed from the pans, cooled at room temperature for 1 h, packed in plastic bags and finally stored at 4 °C for aging studies.

## 2.3. Texture profile analysis (TPA)

A texture analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) was used to measure the force–time curve. In all experiments, fresh or stored samples were cut into cuboids form with the lengths of 3 cm  $\times$  3 cm  $\times$  2 cm (length  $\times$  width  $\times$  height) and compressed by a cylindrical probe (P25) at a test speed of 1 mm/s and a control force of 10 g. The deformation level was 85% of original sample height and the loaf was compressed twice. Samples in triplicates were tested for hardness, cohesiveness and springiness for the evaluation of bread staling. Finally, the data on hardness was collected and applied to perform Avrami theory analysis according to the method of Armero and Collar [15].

## 2.4. X-ray diffraction patterns

Prior to XRD test, crusts and crumbs were completely desiccated, finely triturated to powers (100 meshes) and hydrated at 75% relative humidity (RH) in a sealed vessel using saturated sodium chloride. Basically, the powders (0.8 g) were pressed into a pellet (10 mm  $\times$  25 mm) with hydraulic press and analyzed using a Bruker D8-Advance XRD instrument (Bruker AXS Inc., Germany). The diffractograms were collected under the conditions of 40 kV, 30 mA, with the scanning angle  $2\theta$  set from 3° to 35° at a scanning rate of 0.6°/min. Relative crystallinity (RC) of the staling bread and  $d$ -spacing of X-ray patterns were analyzed by Jade 5.0 software (Materials Data Inc., California).

## 2.5. Differential scanning calorimeter (DSC) measurement

Thermal analysis of crusts and crumbs were performed by using a Pyris 1 DSC (Perkin-Elmer Inc., USA) under ultrahigh-purity nitrogen atmosphere. The equipment was calibrated with indium and tin standards. The fresh samples, and aging samples stored at 4 °C for

three weeks, were gradually scanned from 25 °C to 120 °C at a constant rate of 10 °C/min to get the message of crystalline dissociation. An empty pan was used as a reference for all measurements.

## 2.6. Statistical analysis

The data were expressed as means of triplicate determinations. Statistical significance was assessed with one-way analysis of variance (ANOVA) using ORIGIN 7.5 (OriginLab Inc., USA) for Windows program. Treatment means were considered significantly different at  $P \leq 0.05$ .

## 3. Results and discussion

### 3.1. Textural properties

Table 1 shows the hardness changes of bread mixed with or without  $\beta$ -CD. Results showed the hardness of crumb gradually increased with increasing of storage time; meanwhile the addition of  $\beta$ -CD tended to significantly prevent the hardness increase during the whole storage ( $P \leq 0.05$ ), suggesting that the addition of  $\beta$ -CD would retard the bread staling process. This retardation effect of  $\beta$ -CD was partially attributed to an improvement of network formation in bread, probably resulting from the interaction of  $\beta$ -CD and starch. It also could be explained by the addition of  $\beta$ -CD might transform a nucleus of starch recrystallization from amylose to amylose–lipid– $\beta$ -CD complex since this complex might be formed in the case of incorporating  $\beta$ -CD into wheat starch [12], resulting in the decline of bread staling. Cohesiveness was more of an internal characteristic and depended on a combined effect of adhesive and cohesive forces, and others include viscosity and elasticity as well [16]. Generally, the cohesiveness markedly decreased with the increasing of storage time, and  $\beta$ -CD retarded this decrease probably resulting from prevention the loss of free water, which leaked out slowly during the bread aging, thus displaying the native stability of bread network. Springiness is a measure of how much the gel structure is broken down by the initial compression and it is also an important parameter to determine bread staling. Similar to the pattern of cohesiveness, the springiness of bread decreased during the storage, but the sample with  $\beta$ -CD obviously retarded this decline. It is generally considered that high springiness appears when the gel structure is broken into few large pieces during the first TPA compression, whereas low springiness results from the gel breaking into many small pieces [17]. Therefore, compared to the control, the presence of  $\beta$ -CD probably transformed the dissociation fraction of bread crumb from small pieces close to large one, as a result of offering the crumb relatively higher elasticity.

Avrami's theory has often been employed for the isothermal crystallization kinetic study of bread staling [15,18]. The Avrami equation of this theory can be written as follow:

$$1 - X(t) = \frac{F_{\infty} - F_t}{F_{\infty} - F_0} = \exp(-kt^n)$$

**Table 1**

Changes of hardness, cohesiveness and springiness of bread with storage at 4 °C in the presence or absence of  $\beta$ -CD.

Stored time (d)	Hardness (g)		Cohesiveness		Springiness	
	Control	$\beta$ -CD	Control	$\beta$ -CD	Control	$\beta$ -CD
0(fresh)	664 $\pm$ 8 <sup>a</sup>	600 $\pm$ 5	0.72 $\pm$ 0.03	0.79 $\pm$ 0.02	0.75 $\pm$ 0.04	0.82 $\pm$ 0.03
1	702 $\pm$ 6	625 $\pm$ 4	0.62 $\pm$ 0.02	0.74 $\pm$ 0.04	0.66 $\pm$ 0.03	0.78 $\pm$ 0.04
3	767 $\pm$ 3	699 $\pm$ 5	0.54 $\pm$ 0.01	0.68 $\pm$ 0.01	0.60 $\pm$ 0.01	0.74 $\pm$ 0.03
5	856 $\pm$ 5	775 $\pm$ 7	0.47 $\pm$ 0.02	0.63 $\pm$ 0.02	0.55 $\pm$ 0.02	0.71 $\pm$ 0.02
7	974 $\pm$ 7	857 $\pm$ 4	0.42 $\pm$ 0.02	0.58 $\pm$ 0.03	0.51 $\pm$ 0.03	0.68 $\pm$ 0.01
14	1079 $\pm$ 6	974 $\pm$ 6	0.37 $\pm$ 0.02	0.55 $\pm$ 0.01	0.47 $\pm$ 0.02	0.64 $\pm$ 0.03
35	1126 $\pm$ 8	1001 $\pm$ 8	0.31 $\pm$ 0.01	0.51 $\pm$ 0.02	0.44 $\pm$ 0.03	0.59 $\pm$ 0.02

<sup>a</sup> Values are means  $\pm$  standard deviations of at least triplicate determination.

**Table 2**  
Isothermal crystallization kinetic parameters of bread stored at 4 °C in the presence or absence of  $\beta$ -CD.

Samples treatment	Avrami parameters		
	$n$	$k$ ( $d^{-n}$ )	$R^2$
Control	$1.2812 \pm 0.0063a^*$	$0.0762 \pm 0.0015a$	0.9920
1.5% $\beta$ -CD	$1.4231 \pm 0.0072b$	$0.0604 \pm 0.0012b$	0.9952

\* Samples means with different lower case letters in the same column are significantly different at  $P \leq 0.05$ .

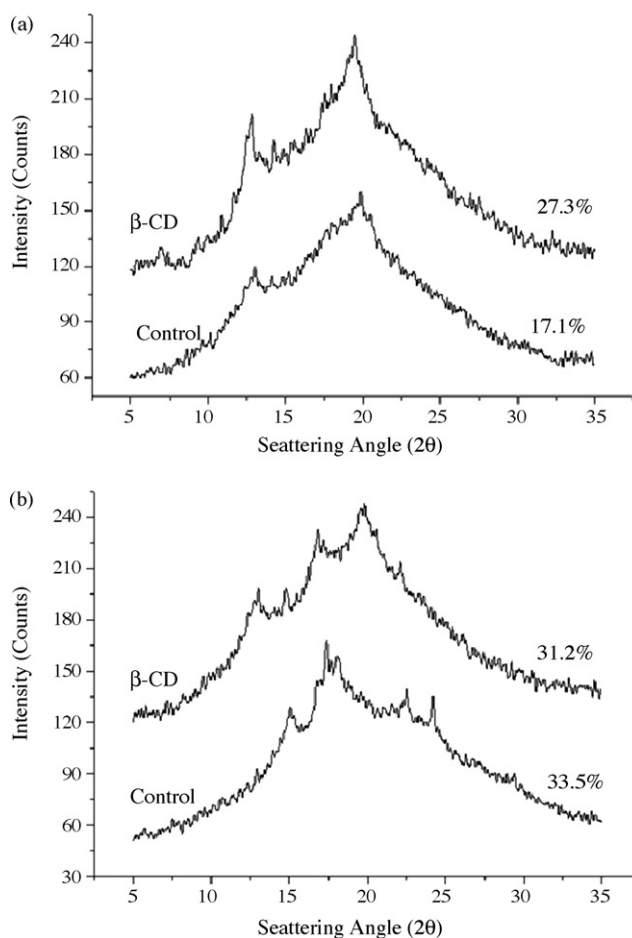
where  $X(t)$  is the fraction of the recrystallization still to occur,  $F_0$  is the crumb firmness of fresh bread,  $F_t$  is the crumb firmness at 't' time,  $F_\infty$  is the final crumb firmness (35 d for all samples),  $n$  is Avrami exponent in relation to the nucleation type of crystallization, and  $k$  is constant rate for the evaluation of bread firming rate.

Recrystallization kinetics data obtained at 4 °C were fit for the Avrami equation according to the correlative coefficient  $R^2$  values (Table 2). Compared to the control, the addition of  $\beta$ -CD significantly decreased ( $P \leq 0.05$ ) the value of the constant rate ( $k$ ) while significantly increased ( $P \leq 0.05$ ) the value of the Avrami exponent ( $n$ ). This lower  $k$  value associated with higher  $n$  for bread suggested that the addition of  $\beta$ -CD retarded crumb firming kinetics; this finding was confirmed by the previous study of Armero et al. [15]. Meanwhile, the increase of exponent indicated that the nucleation type of starch recrystallization was transformed from instantaneous nucleation to rod-like growth of crystals. This transformation could be induced by a change of electrostatic property in relation to the surface-activity of the surrounding  $\beta$ -CD molecules or  $\beta$ -CD could interfere with the re-association of starch, and their physical characteristics change might partly explain the nuclei type transformation.

### 3.2. X-ray diffraction (XRD) pattern analysis

The pattern of the fresh crust showed two weak peaks at 4.4, and 6.8 Å (Fig. 1a), indicating an unstable structure partly characterized by V-pattern crystallite. This crystallite type was generally granted by peaks around d-space of 4.4, 6.8, and 12 Å [19,20]. The addition of  $\beta$ -CD caused the increase of 4.4, and 6.8 Å peaks and a weak peak at around 5.1 Å, and increased the relative crystallinity (RC) value up by 10.2%, which developed a V-type crystalline. This pronounced V-pattern and the additional peak might be attributed to a novel amylose–lipid and  $\beta$ -CD complex formation because a V-type crystalline was believed to be mainly attributed to the formation of helical complex of amylose with lipid in gelatinized starch [21]. For the aging crust, the crystalline pattern was transformed from V- to A-pattern due to the formation of 3.9, 4.9, 5.2, and 5.9 Å peaks (Fig. 1b); meanwhile the addition of  $\beta$ -CD prevented this transformation according to decrease in RC by 2.3% and formation of a V + A pattern.

However, the pattern of the fresh crumb showed an A-type with the occurring 3.9, 4.9, and 5.2 Å peaks; meanwhile the addition of  $\beta$ -CD increased this pattern by the development of the relative peaks and the increase in RC value by 7.5% (Fig. 2a). The crucial cause of this A-pattern was corresponding to the pregelatinization fraction of crust still to occur, thus leading to A-pattern of native wheat starch. For the aging crumb, the XRD pattern changed to a more stable B-type via an amorphous state [21]. But this transformation was delayed by the addition of  $\beta$ -CD since the RC value was decreased and the V + B pattern was observed (Fig. 2b), indicating an additional crystalline was formed due to the outside hydroxyls of  $\beta$ -CD interacting with amylose and lipid. This interaction was different from that of amylose and lipid inserting into starch helix to complex with amylose [22], but occurred on both surface and inside



**Fig. 1.** Effect of 1.5%  $\beta$ -CD on XRD pattern of fresh crust (a) and stored crust (b) in the absence (control) or presence of  $\beta$ -CD.

of amylose molecules, as a result of influencing the properties of bread staling. Referring to the discrepancy in pattern between the stored crust and crumb, the development of A- or B-type diffraction patterns during aging was dependent on the amount of water presence. Water content lower than 29% led to an A-type pattern, while a B-type pattern development was observed with water content higher than 43% [23]. This finding confirmed the development of an A-type pattern was formed in the crust and a B-type pattern in the crumb during the storage. However, the addition of  $\beta$ -CD retarded this resultant pattern display in crust or in crumb due to an intermediate pattern of A + V or B + V presence, respectively. This result further demonstrated that the significant increase in exponent  $n$  value during bread staling was due to the nucleation core transformation in the presence of  $\beta$ -CD.

### 3.3. Thermal properties analysis

Typical DSC thermal properties for crust and crumb samples in the presence and absence of  $\beta$ -CD are shown in Table 3. Fresh crust showed a single peak phase transition at 100.8 °C, corresponding to dissociation of amylose–lipid complex. However, the addition of  $\beta$ -CD significantly increased this dissociation temperature to a high level (108.4 °C), as well as the enthalpy of  $\Delta H_2$  by 0.9 J/g. These indicated the formation of amylose–lipid– $\beta$ -CD complex. This explanation was in accordance with the previous findings that  $\beta$ -CD might disrupt amylose–lipid complex formation interacting with starch lipid [13]. However, two phase transitions were observed in the stored crust. The addition of  $\beta$ -CD showed an increase in  $T_0$ ,  $T_p$ , and  $T_c$ , but a significant decrease ( $P \leq 0.05$ ) in the

**Table 3**  
Thermal properties of fresh crust, stored crust, fresh crumb, and stored crumb in the presence or absence of  $\beta$ -CD.

Samples	The first phase transition				The second phase transition			
	$T_0$ ( $^{\circ}\text{C}$ )	$T_p$ ( $^{\circ}\text{C}$ )	$T_c$ ( $^{\circ}\text{C}$ )	$\Delta H_1$ (J/g)	$T_0$ ( $^{\circ}\text{C}$ )	$T_p$ ( $^{\circ}\text{C}$ )	$T_c$ ( $^{\circ}\text{C}$ )	$\Delta H_2$ (J/g)
Fresh crust								
Control	–	–	–	–	92.3 $\pm$ 0.3b	100.8 $\pm$ 0.4b	105.2 $\pm$ 0.2b	1.5 $\pm$ 0.2d
$\beta$ -CD	–	–	–	–	102.3 $\pm$ 0.2a	108.4 $\pm$ 0.3a	115.1 $\pm$ 0.4a	2.4 $\pm$ 0.1b
Stored crust								
Control	52.1 $\pm$ 0.1	58.7 $\pm$ 0.2	65.3 $\pm$ 0.4	2.7 $\pm$ 0.2c <sup>*</sup>	91.9 $\pm$ 0.3b	100.7 $\pm$ 0.2b	104.1 $\pm$ 0.3c	1.1 $\pm$ 0.1e
$\beta$ -CD	54.3 $\pm$ 0.2	59.3 $\pm$ 0.3	66.1 $\pm$ 0.2	2.1 $\pm$ 0.1d	102.0 $\pm$ 0.2a	107.9 $\pm$ 0.4a	114.7 $\pm$ 0.1a	1.8 $\pm$ 0.2c
Fresh crumb								
Control	57.3 $\pm$ 0.2	62.1 $\pm$ 0.1	69.3 $\pm$ 0.3	2.1 $\pm$ 0.2d	91.4 $\pm$ 0.3b	100.7 $\pm$ 0.4b	103.8 $\pm$ 0.2c	1.7 $\pm$ 0.2c
$\beta$ -CD	57.6 $\pm$ 0.3	62.7 $\pm$ 0.2	70.5 $\pm$ 0.4	1.5 $\pm$ 0.1e	101.7 $\pm$ 0.4a	107.5 $\pm$ 0.3a	114.6 $\pm$ 0.1a	2.8 $\pm$ 0.2a
Stored crumb								
Control	53.2 $\pm$ 0.2	60.7 $\pm$ 0.3	69.8 $\pm$ 0.2	6.5 $\pm$ 0.3a	91.1 $\pm$ 0.4b	100.4 $\pm$ 0.1b	102.9 $\pm$ 0.4d	1.5 $\pm$ 0.1d
$\beta$ -CD	54.1 $\pm$ 0.1	62.4 $\pm$ 0.4	70.3 $\pm$ 0.2	3.8 $\pm$ 0.1b	101.3 $\pm$ 0.2a	107.5 $\pm$ 0.4a	114.2 $\pm$ 0.3a	2.3 $\pm$ 0.2b

<sup>\*</sup> Samples means with different lower case letters in the same column are significantly different at  $P \leq 0.05$ .

retrogradation enthalpy of the first peak. This decrease indicated that the retardation of  $\beta$ -CD was more pronounced, probably as the formation of amylose–lipid– $\beta$ -CD complex improved the properties of the surroundings of starch granule and occurred a slow rate in recrystallization of crust. For fresh crumb, the  $T_0$ ,  $T_p$  and  $T_c$  of the first transition were higher than those of the stored crust,

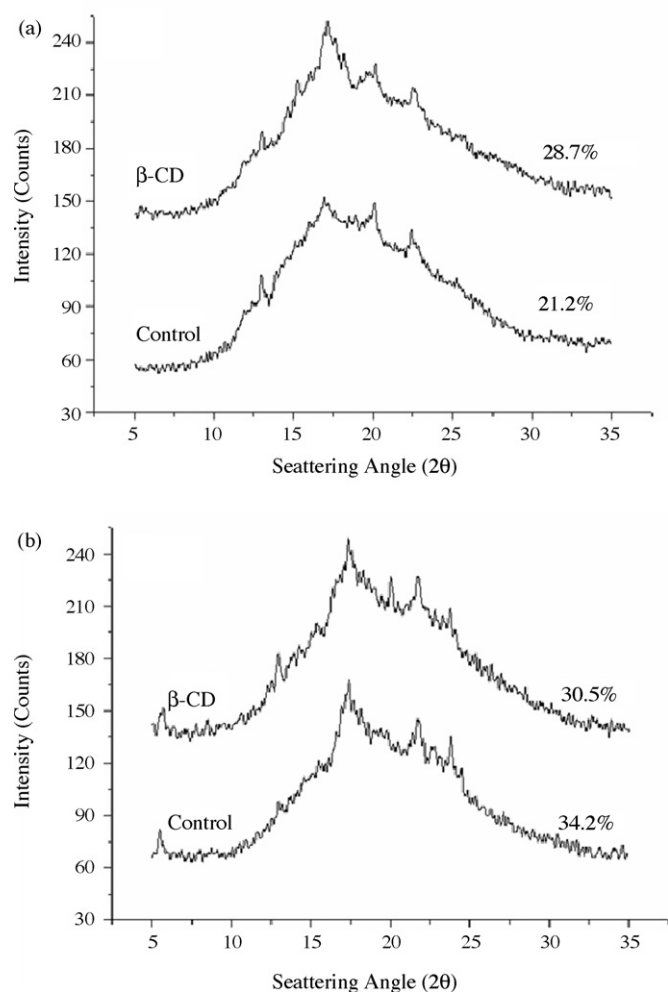
suggesting that the starch crystal in fresh crumb did not gelatinize, but it was completely gelatinized in fresh crust according to the absence of the first peak from the liquated amylopectin. Meanwhile, the addition of  $\beta$ -CD decreased the enthalpy of the amylopectin dissociation and increased the enthalpy of the second phase transition. The result further demonstrated that amylose–lipid– $\beta$ -CD was formed in crumb as it was in crust. For the stored crumb, the addition of  $\beta$ -CD significantly decreased ( $P \leq 0.05$ ) the retrogradation enthalpy by 2.7 J/g and increased the enthalpy of the novel complex dissociation, thus offering the crucial role on bread staling. These results indicated that the retardation of  $\beta$ -CD on crumb was preferable than that on crust; this seemed partially in agreement with previous RC value analyzed by X-ray diffraction. The discrepancy was attributed to the different water moisture and distribution in the crust and crumb, affecting the interaction between  $\beta$ -CD and starch lipid since the extent of gelatinization of starch was governed by temperature history and moisture content [24]. In general, the considerable water in crumb was benefit for the interaction of  $\beta$ -CD and starch since the probable electrostatic and hydrogen bond forces were formed.

#### 4. Conclusions

This work demonstrated that  $\beta$ -CD has significant impact on the staling of crust and crumb. The retarding effect of  $\beta$ -CD was strongly supported by the less changes of hardness, cohesiveness and springiness, and the decrease in retrogradation enthalpy of crust and crumb. The addition of  $\beta$ -CD significantly lowered the retrogradation  $k$  of bread crumb and transformed the crystalline type from instantaneous nucleation to rod-like growth of crystals. This could retard the crystalline change from V- to A-pattern in crust and A- to B-pattern in crumb. This transformation assigned to amylose–lipid– $\beta$ -CD complex formation was observed by DSC, thus resulting in the retardation effect on transformation of crystalline pattern in crumb. Furthermore, the retardation of  $\beta$ -CD on crumb was found more significant than that on crust due to the difference in moisture and water distribution.

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**Fig. 2.** Effect of 1.5%  $\beta$ -CD on XRD pattern of fresh crumb (a) and stored crumb (b) in the absence (control) or presence of  $\beta$ -CD.

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