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NOTE

Changes of microbiological and physicochemical properties in Chinese infant formula caused by high heat treatment applied on concentrated milk

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Abstract In 2010, China published highly strict food safety standard for infant formula (GB 10765 2010). However, many manufacturers in China found it difficult to meet the new microbiological standard. The aim of this study was to find the appropriate processing conditions for producing Chinese infant milk with reduced microbial load. Concentrated milk was heated at 95 °C for 15 s (LHT) and 110 °C for 4 s (HHT), and their effects on the microbiological quality and physicochemical properties of the infant formula milk powder were investigated. Compared to LHT, HHT made significant effects on the bacterial, thermophilic, and aerobic spore counts. In addition, the total bacterial count of the infant formula milk powder after HHT met the requirements of the safety standard (GB 10765 2010). In terms of nutritional content, both powders met the requirement (GB 10765 2010) with no significant differences observed. There were also no significant differences observed between the two powders in terms of particle morphology, wettability, free fat content, thermal characteristics of fat, or milk fat globule membrane permeability. Although some slight differences ($P \leq 0.05$) were found in solubility, dispersibility, viscosity, particle size of the reconstituted powder, and protein characteristics, the acceptability of the product under both heat treatments was found to be similar. The knowledge derived from this work can potentially provide an effective method for processing infant formula milk powder in China.

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浓缩乳高温处理工艺对中国婴儿配方乳粉微生物和理化特性的影响

摘要 ：2010年中国颁布更严格的婴儿配方食品标准(GB 10765-2010)，致使许多中国婴儿配方乳粉企业要达到新国标中的微生物标准较为困难。本研究目的在于分析浓缩乳热杀菌工艺对婴儿配方乳粉微生物指标的作用效果。研究比较了95 °C15 s(LHT)和110 °C 4 s(HHT)两种浓缩乳加热工艺对婴儿配方乳粉产品中微生物和理化指标的影响，结果表明，HHT工艺对乳粉细菌总数，嗜热菌和需氧芽孢数指标的影响比LHT更明显，经过HHT工艺后婴儿配方粉的细菌总数能够符合新国标要求(GB 10765-2010)。在营养成分上，两种工艺的乳粉均符合新国标要求且基本无显著差别。两种工艺处理的乳粉在微粒形态、可润湿性、游离脂肪含量、乳脂热力学特性以及脂肪球膜渗透性方面也均无显著差异。尽管在溶解度、可分散性、粘度、复溶粒径以及蛋白质特性方面两者有一定的差异($P \leqslant 0.05$)，但对于产品的可接受性影响不大。本研究结果为改善中国婴儿配方粉品质提供了一个新的方法选择。

Keywords High heat treatment · Concentrated milk · Microbiological qualities · Physicochemical properties · Chinese infant formula

关键词 高温处理 · 浓缩乳 · 微生物质量 · 理化特性 · 中国婴儿乳粉

1 Introduction

Each year in China, 16–18 million babies are born, and 300,000 tons of milk powder is consumed. The quality of infant milk is very important for babies' health. Due to the melamine scandal in late 2008 and other quality problems in milk powder, China revised and gazetted a highly strict food safety standard for infant formula. The new food safety standard for aerobic plate counts in infant formula is quite stringent (acceptable aerobic plate count of 1×10^3 cfu·g^{-1} compared to the previous 1×10^4 cfu·g^{-1}) (GB 10765 2010). Many small- and medium-sized manufacturers in China have found it difficult to meet the new food safety standard.

Thermoduric bacteria, especially thermophilic bacilli, were reported to be critical contaminants in milk powders (Ronimus et al. 2003; Ruckert et al. 2004; Scott et al. 2007). The high number (>10^4 cfu·g^{-1}) of thermophilic bacilli in milk powders indicates bad hygiene during processing (Burgess et al. 2010). Many facultative thermophiles could grow at 37 °C (Ronimus et al. 2003); thus, there might be some correlations between the contamination of thermophilic bacilli and high aerobic plate counts (APC; enumerated at 37 °C, according to GB 4789.2 2010). Research in our lab showed that 3 of 22 milk powder samples (including infant formula) in China had counts of thermophilic bacilli of between 1×10^3 and 1×10^4 cfu·g^{-1}, and another two exceeded 1×10^4 cfu·g^{-1} (Yuan et al. 2012b). In most of these infant formulas, the ratios of counts of thermophilic bacilli and thermophilic aerobic spores to total bacterial counts were both over 80% (Yuan et al. 2012a). During milk powder processing, evaporator stages in particular create opportunities for contamination by thermophilic bacilli (Scott et al. 2007). The combination of heat and vacuum used to remove water from milk during production of the milk concentrate, along with the temperature gradient, supports the germination of spores, and promotes bacterial growth and colonization on the walls of the evaporator (Murphy et al. 1999). Both

can lead to the high number of thermophilic bacilli in the final product. Some industries in China wish to trial high heat treatment of concentrated milk after evaporation to reduce microbial contamination. Nowadays, the indirect heat exchanging equipment are widely used by dairy manufacturers in China. In our previous study, the UHT treatment (121 °C for 15 s) could effectively kill almost all thermophilic bacilli and spores (Yuan et al. 2012b). However, we discovered that heating concentrated milk (approximately 35% total solids) at 110 °C for 4 s is the maximum possible treatment attainable in tube heat exchanger as it would lead to wall sticking and affect heat and production efficiency when the treatment was over 110 °C for 4 s (unpublished data).

It is well known that heating conditions play a major role in determining the functional properties of milk powder, i.e., whey protein denaturation, association of denatured whey proteins with the casein micelle, protein-induced fat globule aggregation, enhanced permeability of milk fat globule membrane (MFGM), fatty oxidation, physical properties, and the loss of vitamins and minerals (Murphy et al. 1999; Oldfield et al. 2005; Singh and Ye 2010; Vignolles et al. 2007). However, there have been limited studies regarding the effects of heat treatment on the physicochemical properties of infant formula milk powder. In this study, a pilot plant was used to simulate the process of producing Chinese infant milk, and *Bacillus licheniformis*, the main strain of thermophilic bacilli in Chinese milk powders (Yuan et al. 2012b), was added to simulate the contamination of thermophilic bacilli during the production of infant formula milk powder. Heating condition of 110 °C for 4 s was chosen to compare with 95 °C for 15 s (the conventional condition used in the process of producing infant formula milk powder in China) in terms of producing infant formula milk powder with reduced microbial number and good quality. This research was also performed to establish a processing method that will reduce bacterial contamination and improve the quality of infant formula milk powder in China.

2 Materials and methods

2.1 Pilot plant and sampling

2.1.1 Concentrated milk

Concentrated milk (approximately 35% total solids) was produced by mixing ingredients. The formula (one of conventional formulas for producing infant formula milk powder in China) is 69 g raw milk, 1.6 g whole milk powder, 20 g demineralised (70%) whey powder, 7.5 g soybean oil, 2 g sucrose, 0.08 g vitamin, 0.03 g mineral, and 0.018 g choline per 100 g concentrated milk. The raw materials were mixed with high shearing (T18BS25 model, IKA Co., Germany) in a vessel for 20 min. *B. licheniformis* was then added directly (approximately 10^4 cfu·g^{-1} total solids) and mixed for a further 10 min.

The concentrated milk samples were collected from the mixtures before and after inoculation with *B. licheniformis* L1 (reserved in our lab), which was isolated from Chinese commercial milk powders and represented the most prevalent thermophilic isolates from infant formula and whole milk powders manufactured from different areas of China (Yuan et al. 2012b), then filled into sterile bottles for microbiological analyses.

2.1.2 Pilot plant of infant formula milk powder

The concentrated milk samples were preheated to 65 °C by a heat exchanger and immediately transferred to a two-stage valve homogenizer (APV Homogenizer Division, Wilmington, MA, USA). The milk samples were homogenized at a pressure of 15 MPa in the first stage and 5 MPa in the second stage. The homogenized samples were pasteurized by a tube in tube heat exchanger (Type PT-20T, Powerpoint International Ltd., Japan) separately at 95 °C for 15 s (LHT) or 110 °C for 4 s (HHT), respectively. The samples were then immediately cooled to a temperature of 55 °C in a water bath, then dried and packed. The feed rate of the milk concentrate to the dryer (Mobile minor, GEA Group, Germany) was 2 $kg{\cdot}h^{-1}$. The inlet and outlet temperatures were 180 and 80 °C, respectively.

The concentrated milk samples were collected after heat treatment and filled into sterile bottles for microbiological analyses. The powder samples were collected and packed in sterile plastic bags and stored at 4 °C for further analysis.

2.2 Microbiological analysis

The total bacterial counts (BC) were determined using Plate Count Agar (PCA) medium. Ten milliliters of concentrated milk and 10 g of powder were mixed in 90 mL of 0.1% sterile peptone water with sterile glass beads, and then agitated for 20 min in an ice bath. Serial dilutions were prepared in 0.1% sterile peptone water, and pour-plated in Petri dishes with PCA in duplicate. The aerobic spore counts (ASC) were determined similarly after a heat treatment of the 10^{-1} concentrated milk and 10^{-1} reconstituted powders (in 0.1% peptone) at 80 °C for 10 min to eliminate the vegetative cells and activate the spores. Enumeration of colonies on dishes was carried out after incubation at 37 °C for 48 h.

Determination of total thermophilic counts (TC) was the same as for the total BC. Thermophilic spore counts (TSC) were also determined after heat treatment at 100 °C for 30 min. Enumeration of colonies on dishes was carried out after incubation at 55 °C for 48 h. The APC in milk powder was enumerated according to the Chinese standard method GB 4789.2 (2010).

2.3 Compositional analysis

The basic compositions (protein, fat, and carbohydrate), vitamins (vitamin A, vitamin D, vitamin E, vitamin K_1, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, vitamin niacin and niacinamide, folic acid, pantothenic acid, vitamin C, free biotin, and choline), and minerals (calcium, iron, zinc, sodium, potassium, magnesium, copper, phosphorus, iodine, chlorine, and selenium) of infant formula milk powder were determined according to described methods in Chinese National Food Safety Standard (GB 10765 2010).

2.4 Physical measurements

2.4.1 Scanning electron microscopy

The LHT and HHT infant formula powder was examined with a Hitachi scanning electron microscopy (SEM) S4800 instrument operating at 10 kV. Samples were

spread on the surface of a sticky plastic circle fixed on a support and covered with gold particles.

2.4.2 Particle size analysis of reconstituted infant formula

The particle size distribution of the samples was measured using a computerized laser particle analyzer (Bluewave S4801 model, Mactrotrac Inc.), with a presentation code that assumed the following optical parameters: a relative refractive index between the dispersed and continuous phases of 1.59, a particle absorbance of 0.00, and a continuous phase refractive index of 1.333. The samples were dispersed in water or a mixture solution (pH 6.8) of 50 mM EDTA and 20 $g \cdot L^{-1}$ SDS to dissociate the casein micelles and any aggregation of fat globules.

2.4.3 Solubility and viscosity

Powder samples were analyzed for solubility according to the Chinese National Food Safety Standard (GB 10765 2010) methodology. Infant formula powder was mixed with water and centrifuged twice. The supernatant was removed, and the precipitate was washed into a tared dish then dried in an oven at 100 °C to a constant weight. Solubility was calculated by dividing the mass of the soluble portion by the mass of the total powder. Wettability and dispersibility were determined according to described methods in IDF 87 (1979). Viscosity measurements were performed using a digital viscometer (DV-C model, Brookfield Engineering Laboratories Inc., Middleboro, USA) and conducted with no. 0 spindle at 60 rpm. Samples were reconstituted in distilled water at 10% (*w*/*w*) and maintained at 20±0.1 °C using a temperature-controlled water bath. Results were expressed as the mean of triplicate analysis.

2.5 Protein properties analysis

2.5.1 Whey protein nitrogen index

Whey protein nitrogen index (WPNI) was determined according to ADMI (1971) method.

2.5.2 Determination of free sulfhydryl content

Changes in the free sulfhydryl (SH) content of infant formula milk powder were determined according to the method of Ellman (1959), with some modifications. In this experiment, low-turbidity protein solutions were prepared by separating the proteins from milk via acetone precipitation (Ou et al. 2004). Mixtures of 1.5 mL of pretreated samples with 1.5 mL buffer and 0.02 mL Ellman's reagent were mixed and reacted at 25 °C for 5 min, and the same mixture without samples was used as blank. The absorbance of sample mixture and blank was measured at 412 nm by UV spectrophotometer (Jinghua JH756, Jinghua Instrument Co., China). Free SH content was obtained by dividing the value of the absorbance by the molar extinction coefficient of 13,600. The results were expressed in micromoles SH per gram of powder and as the mean of triplicate analysis.

2.5.3 Determination of surface hydrophobicity

Surface hydrophobicity (H_0) of infant formula milk powder was determined according to the method of Kato and Nakai (1980), using 1-anilinonaphthalene-8-sulfonic acid as probe. Protein dispersions were diluted (0.0025–0.0125%, *w*/*v*) in 0.01 M phosphate buffer (pH 7.6). Fluorescence intensity (FI) was measured with a Cary Eclipse Luminescence Spectrophotometer (Varian, America) at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of the FI versus protein concentration plot was used as an index of H_0. Measurements were performed in triplicate.

2.5.4 SDS–PAGE analysis

Samples were reconstituted in distilled water at 10% (*w*/*w*), and micelles were separated from the milk serum in a Hitachi ultracentrifuge at 25,000×*g* for 1 h at 20 °C. This divides the milk samples into three fractions: pellet, supernatant, and fat accumulates above the supernatant. Protein composition of the supernatants and corresponding complete (i.e., not centrifuged) samples were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE; Martin et al. 2007).

SDS–PAGE was performed by the method of Laemmli (1970). Samples were diluted twofold in 50 μL loading buffer, then boiled for 5 min. Five-microliter aliquots of samples underwent SDS–PAGE in the following experiment, and the gel was run in a Mini-Protean Tetra system (Bio-Rad, Richmond, CA, USA) at 120 V using a Bio-Rad PowerPac Universal power supply unit (Model 164-5070, Bio-Rad). The protein bands were fixed and stained using a solution of Coomassie Blue R-250. After the gels had been stained and destained, the protein bands were digitally scanned using Kodak Gel Logic 212 Imaging System and ImageJ software to obtain quantitative results.

2.6 Fat properties analysis

2.6.1 Fat extraction

Free fat was extracted according to Murrieta-Pazos et al. (2012). The extraction of encapsulated fat and total fat was conducted by the Röse-Gottlieb method (IDF9C 1987) using the powder recovered after the free fat extraction and the initial powder, respectively.

2.6.2 Differential scanning calorimetry

The thermal properties of the three fat fractions (free, encapsulated, and total) were investigated by differential scanning calorimetry (DSC) with TA Q200 thermal analyzer (TA Instruments, New Castle, DE) using aluminum hermetic pans. Cell calibration was achieved with indium. About 10 mg of sample was placed in an aluminum pan manually sealed and perforated. An empty sealed crucible was used as reference. The melting profiles of fat fractions were first studied on heating from 20 to 70 °C at 10 °C·min^{-1}. Then, the overall thermal profiles were registered on cooling

from 70 to −60 °C and on subsequent heating from −60 to 50 °C at 10 °C·min^{-1}. Each sample was performed in duplicate.

2.6.3 Analysis of MFGM protein components

Milk fat globule surface material was isolated from the infant formula milk powders, as described by Ye et al. (2004). The diluted samples were centrifuged, and the top layer (cream) was removed and suspended in simulated milk ultrafiltrate (SMUF), or SMUF containing urea and EDTA. This mixture was recentrifuged, and the top layer was collected. Washing in SMUF or SMUF containing urea and EDTA was repeated twice.

The individual proteins in the washed cream were determined by SDS–PAGE, as described by Ye et al. (2007). The washed cream was dispersed (1:2, *w*/*w*) in 0.5 M Tris–HCl buffer and then heated at 95 °C for 5 min in a boiling water bath. After heating, the sample was centrifuged at 2,500×*g* for 30 min before the fat was removed by PAGE analysis. Subnatant (2 μL) was then loaded on to the SDS gel, and the later steps were the same as for Section 2.5.4.

2.7 Statistical analysis

An analysis of variance using the software PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) was performed on the data. Differences were considered to be significant at $P \leq 0.05$.

3 Results and discussion

3.1 Microbiological characteristics

The enumeration results of BC, TC, ASC, and TSC are shown in Fig.1a–d. As shown in Fig. 1a, a 6-log reduction of BC was attained after HHT, in comparison to a 5-log reduction attained after LHT. In contrast to LHT, the total bacterial count of HHT powder was below 10^3 cfu·g^{-1}, meeting the Chinese national food safety standard limits for infant formula (GB 10765 2010).

As shown in Fig. 1, after the addition of *B. licheniformis*, TC exceeded by approximately 4 log cfu·g^{-1} TS (total solids), and was reduced to 2.17 log cfu·g^{-1} powder and 3.11 log cfu·g^{-1} powder, after HHT and LHT processing in the final product, respectively (Fig. 1b). The ASC was significantly reduced ($P \leq 0.05$) after HHT (1.07-log reduction) and LHT (0.67-log reduction) processing, but then underwent growth after spray drying (Fig. 1c), possibly due to heat activation of spores (Curran and Evans 1945). However, the TSC had no marked reduction after both heat treatments (Fig. 1d). These results indicated that HHT has a better inactivation effect on BC, TC, and ASC than LHT. However, both treatments had no effect on TSC.

3.2 Composition

Results of chemical composition analysis showed that infant formula milk powder contained (g·100 g^{-1}): 13.3 proteins, 48.6 lactose, 6.5 sucrose, 27.6 fat, 9.9 linoleic

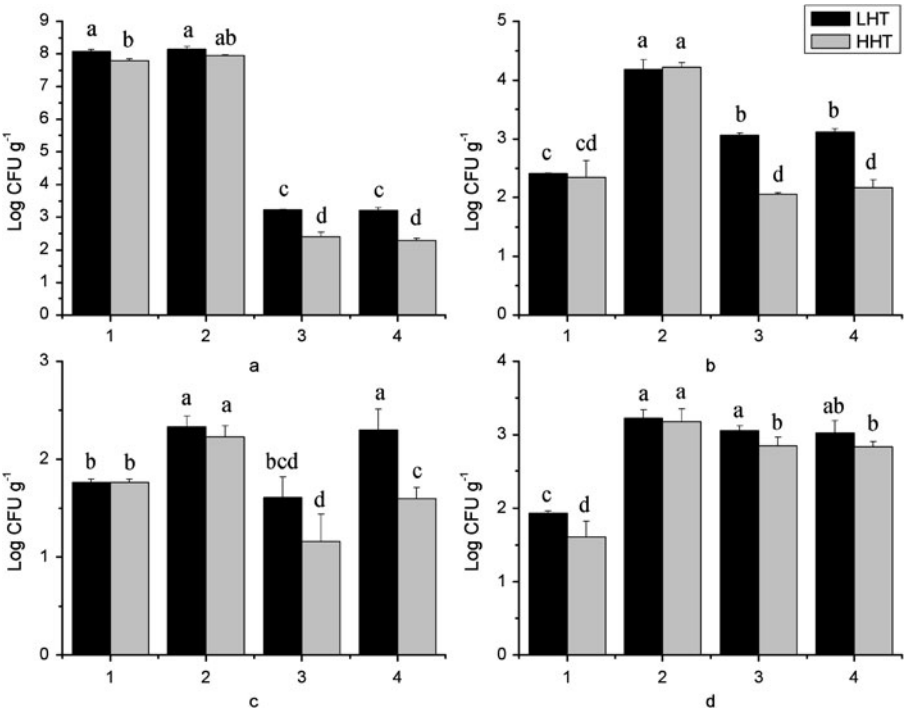

Fig. 1 Effect of heat treatment on bacterial counts in the concentrated milk (1), after addition of *B. licheniformis* (2), after heating (3) and final product (4) during the pilot plant test. **a** Total bacterial count (BC), **b** total thermophilic counts (TC), **c** aerobic spore counts (ASC), **d** thermophilic aerobic spore counts (TSC). Each *bar* represents the mean and standard deviation of triplicate counts

acid, and 1.5 α-linolenic acid. These results met the Chinese national food safety standard for infant formula (GB 10765 2010).

The vitamin and mineral contents of the infant formula samples treated by LHT and HHT were analyzed (Table 1), and both were found to have met the Chinese standard (GB 10765 2010). While Lešková et al. (2006) reported the heat sensitivity of vitamins A, E, C, B_1, B_6, and folic acid, this study showed that the vitamin content of powder treated at 110 °C for 4 s did not significantly differ from that treated at 95 °C for 15 s. Reddy and Love (1999) noted that minerals, unlike vitamins, are not destroyed by heat, which is in agreement with our results. These results indicated that the nutritional quality of the HHT sample is similar to that of LHT sample.

3.3 Physical characteristics

3.3.1 SEM and particle size

As shown in Fig. 2, no significant differences were observed in the particle size of formula powder; average size of powder particles was about 40 μm. However, HHT powder particles are slightly more shriveled (1b and 2b of Fig. 2). Shriveled particles were related to higher degree of whey denaturation when the powder was heat-treated (Millqvist-Fureby et al. 2001).

Table 1 Composition and physicochemical properties of infant formula milk powders

Powder	LHT	HHT
Vitamin A (mg·100 g^{-1})	0.46±0.02[a]	0.46±0.02[a]
Vitamin D (μg·100 g^{-1})	1.05±0.11[b]	1.34±0.13[a]
Vitamin E (mg·100 g^{-1})	5.00±0.25[a]	5.20±0.26[a]
Vitamin K_1 (μg·100 g^{-1})	22.0±2.1[a]	24.0±2.2[a]
Vitamin B_1 (mg·100 g^{-1})	0.26±0.01[a]	0.24±0.01[a]
Vitamin B_2 (mg·100 g^{-1})	1.94±0.20[a]	1.87±0.19[a]
Vitamin B_6 (mg·100 g^{-1})	0.51±0.03[a]	0.49±0.03[a]
Vitamin B_{12} (μg·100 g^{-1})	3.40±0.16[a]	3.40±0.17[a]
Niacin (mg·100 g^{-1})	3.06±0.31[a]	2.98±0.30[a]
Folic acid (mg·100 g^{-1})	0.13±0.01[a]	0.16±0.02[a]
Pantothenic acid (mg·100 g^{-1})	5.94±0.60[a]	5.61±0.55[a]
Vitamin C (mg·100 g^{-1})	58.0±6.0[a]	62.0±6.1[a]
Free biotin (μg·100 g^{-1})	46.2±4.5[a]	45.0±4.5[a]
WPNI (mg·g^{-1})	5.77±0.13[a]	5.16±0.07[b]
Free SH (μM·g^{-1})	3.66±0.16[a]	3.16±0.10[b]
H_0	132.1±0.4[a]	123.3±0.4[b]
Free fat (g·100 g^{-1})	0.28±0.01[a]	0.29±0.01[a]
Calcium (g·100 g^{-1})	0.28±0.03[a]	0.27±0.03[a]
Iron (mg·100 g^{-1})	5.90±0.60[a]	3.60±0.35[b]
Zinc (mg·100 g^{-1})	3.30±0.32[a]	3.40±0.31[a]
Sodium (g·100 g^{-1})	0.17±0.02[a]	0.17±0.02[a]
Potassium (g·100 g^{-1})	0.77±0.08[a]	0.73±0.07[a]
Magnesium (mg·100 g^{-1})	53.0±5.0[a]	52.0±5.1[a]
Copper (mg·100 g^{-1})	0.29±0.04[a]	0.33±0.05[a]
Phosphorus (g·100 g^{-1})	0.38±0.02[a]	0.33±0.02[a]
Iodine (μg·100 g^{-1})	90.0±9.0[a]	89.0±9.0[a]
Chlorine (g·100 g^{-1})	0.31±0.02[a]	0.32±0.02[a]
Selenium (μg·100 g^{-1})	19.9±2.0[a]	20.7±2.1[a]
Choline (g·100 g^{-1})	0.16±0.01[a]	0.17±0.01[a]
Solubility (g·100 g^{-1})	99.98±0.01[a]	99.95±0.01[b]
Wettability (s)	4.62±0.21[a]	4.40±0.08[a]
Dispersibility (%)	82.98±0.09[b]	88.19±0.20[a]
Viscosity (mPa·s)	1.82±0.01[b]	1.86±0.02[a]

All data are means±SD, n=3. Superscript letters indicate significant ($P \leq 0.05$) difference within the same line

As indicated by a reduction in the proportions of particles above 2 μm after the addition of dissociating buffer (Fig. 3), there was considerable fat globule clustering in LHT and HHT powder (McKenna et al. 1999). Compared to LHT, the HHT powder, when dispersed in distilled water, showed a significant increase in particle size. This size increase may be attributed to the attachment of denatured whey

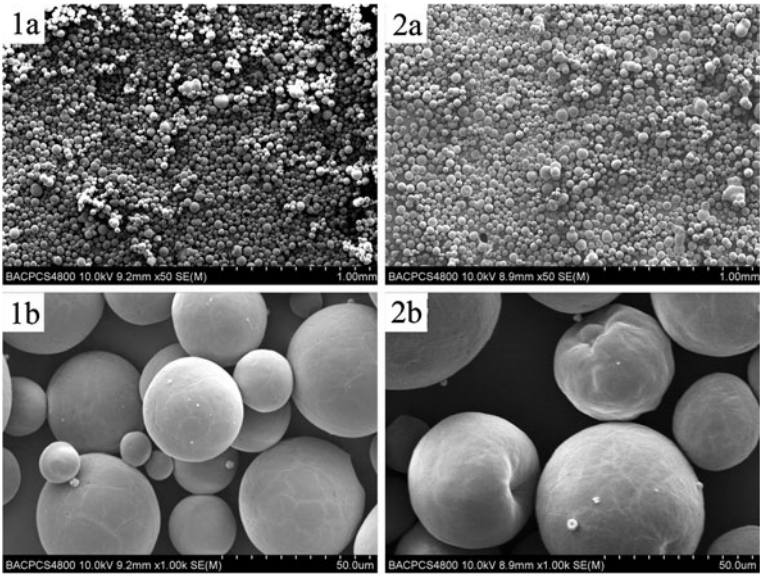

Fig. 2 Scanning electron microscopy of LHT (1) and HHT (2) infant formula milk powder. *a* ×50, *b* ×1,000

proteins onto the outside of the casein micelles, and fat globule clustering in powder (Martin et al. 2007; McKenna et al. 1999). It can therefore be assumed that HHT will result in a higher degree of modification on the protein and aggregation of the milk fat globules.

3.3.2 Solubility and viscosity

As summarized in Table 1, compared to LHT, HHT infant formula showed slightly lower solubility ($P \leq 0.05$) and wettability ($P > 0.05$), and a higher ($P \leq 0.05$)

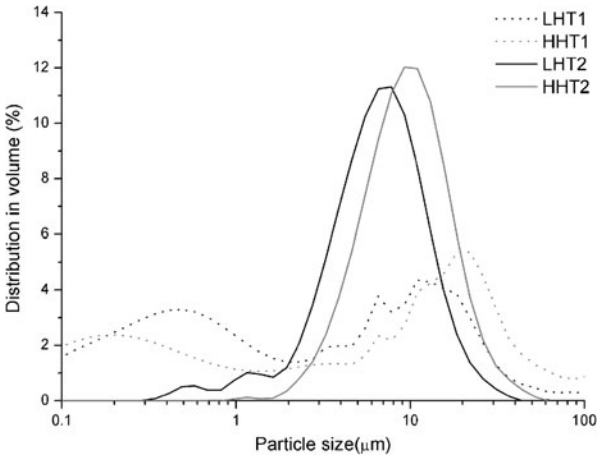

Fig. 3 Distribution of particle size obtained after reconstitution of infant formula milk powder. *LHT1* LHT powder dispersed in a dissociating buffer, *HHT1* HHT powder dispersed in a dissociating buffer, *LHT2* LHT powder dispersed in distilled water, *HHT2* HHT powder dispersed in distilled water

dispersibility. However, the fine distinction had little overall effect on their quality, especially the solubility of both powders is close to complete dissociation. These results confirmed that there is no obvious difference in the dissolution of the two milk powders.

HHT sample was found to have a slightly higher ($P \leq 0.05$) viscosity than that of the LHT sample. This result may be predominantly due to the degree of denaturation of the whey proteins, which can change rheological properties of milk powders (Xu 2008).

3.4 Protein characteristics

3.4.1 WPNI, free sulfhydryl content, and surface hydrophobicity

Compared with the LHT sample, the WPNI, free SH content, and H_0 of the HHT sample were all significantly ($P \leq 0.05$) lower (Table 1). In general, WPNI measures the proportion of undenatured whey protein that remains after heat treatment and is used for the heat classification of skimmed milk powders. In this study, WPNI was used to compare the effects of the degree of heating on infant formula. Results of WPNI showed that the HHT powders had more denatured whey protein due to more severe heating during manufacture. The degree of whey protein denaturation could have in turn affected the solubility, wettability, dispersibility, viscosity, and particle morphology of the milk powder (McKenna et al. 1999; Millqvist-Fureby et al. 2001; Westergaard 2004).

The decrease in free SH content in HHT powder may be primarily attributed to protein aggregation. Although a pasteurization temperature could promote the formation of SH groups, they react with casein and are thus not found in this free form (Westergaard 2004). The measurement of surface hydrophobicity is also an approach for studying the protein–protein interactions due to heat treatment (Sava et al. 2005). In milk protein, when β-lg is unfolded, an increase in surface hydrophobicity is expected (Sava et al. 2005). The unfolding of β-lg molecules is followed by protein aggregation leading to a decrease in surface hydrophobicity (Shimada and Cheftel 1988). Thus, these results confirmed that the HHT processing caused further association of whey proteins with micelles, which affected the particle size for the reconstituted powders as discussed in Section 3.3.1.

3.4.2 SDS–PAGE analysis

Protein compositions of the supernatants and corresponding complete samples were determined by SDS–PAGE (not provided). Through quantitative analysis, we found that the soluble nonmicellar casein (as a percentage of total casein) was slightly higher in the HHT than LHT supernatants, but there was no statistical significance ($P > 0.05$), which indicated that HHT processing did not cause further dissociation of the casein micelles. The α-la was no significantly different ($P > 0.05$), whereas the proportion of the β-lg was significantly lower ($P \leq 0.05$) in the HHT than LHT supernatants. Early studies have demonstrated that model mixtures of isolated β-lg and κ-casein yielded covalent complexes through thiol-disulphide exchanges on heat treatment (Donato and Guyomarc'H 2009). So the lower β-lg proportion confirmed that whey proteins had been pelleted along with the micelles in line with the severity of heat treatment.

3.5 Fat characteristics

3.5.1 Free fat contents

As noted by Fink and Kessler (1985), high temperature treatment can influence the MFGM permeability of the milk, which can lead to a larger free fat content. In this study, there is no significant difference ($P>0.05$) in the free fat content between HHT and LHT samples (Table 1). It confirmed the two processing methods may have a similar effect on the free fat content.

3.5.2 Thermal properties

The melting profiles of LHT and HHT samples are respectively presented in Fig. 4a and b. Three endothermic peaks (1, 2, and 3) were observed with a typical melting behavior from approximately −45 to 30 °C. However, the melting point of milk fat ranged from −40 to 40 °C (Walstra et al. 1995). This is possibly because the infant formula in this study was mixed with soybean oil, which is rich in unsaturated fatty acid and hence has a relatively low melting point of around −20 °C (Pedersen et al. 1998). Early studies showed that a similar behavior was observed in fractions of dairy powders, and the thermal behavior may present differences only when important variations in the fatty acid composition occurred (Kim et al. 2005; Murrieta-Pazos et al. 2012; Vignolles et al. 2009). It can be observed that the properties of the encapsulated and total fat were similar between the two samples (three peak temperature (1, 2, and 3) of approximately −23, 0.5, and 11 °C). However, compared with LHT (−17 °C), the first peak temperature of free fat in the HHT sample (−6 °C) increased significantly. It may be attributed to the variation in the fatty acid composition of free fat after HHT processing. Free fat losing the protection of the MFGM is more susceptible to heat. In addition, soybean oil which is prone to fat oxidation (Vignolles et al. 2007) may affect the melting behavior of free fat in infant formula. Kim et al. (2005) reported that the saturated and unsaturated fatty acid content of milk fat affected its melting behavior. However, fat oxidation of milk powder is mainly dependent on the degree of oxidation of the encapsulated fat (Hardas et al. 2002); thus, the content of free fat in this formula milk is too insignificant (1%) to have an effect on the quality of the total fat.

3.5.3 MFGM protein components

Protein compositions of the MFGM were determined by SDS–PAGE (not provided). Through quantitative analysis, we found that the proportions of total casein, β-lg, α-la, and the native MFGM proteins (as a percentage of total surface protein) were no significantly different ($P>0.05$) between the LHT and HHT samples. These results indicated that HHT processing did not cause further association of proteins (casein micelles, β-lg, and α-la) with the surface of the fat globules (Singh 2006).

The protein bands of the LHT and HHT samples, which were washed with SMUF, showed that the proportions of whey proteins and casein micelles were 43% and 36%, respectively. These results are different from that reported by Ye et al. (2007), who found the proportions of casein micelles associated with the MFGM in whole milk

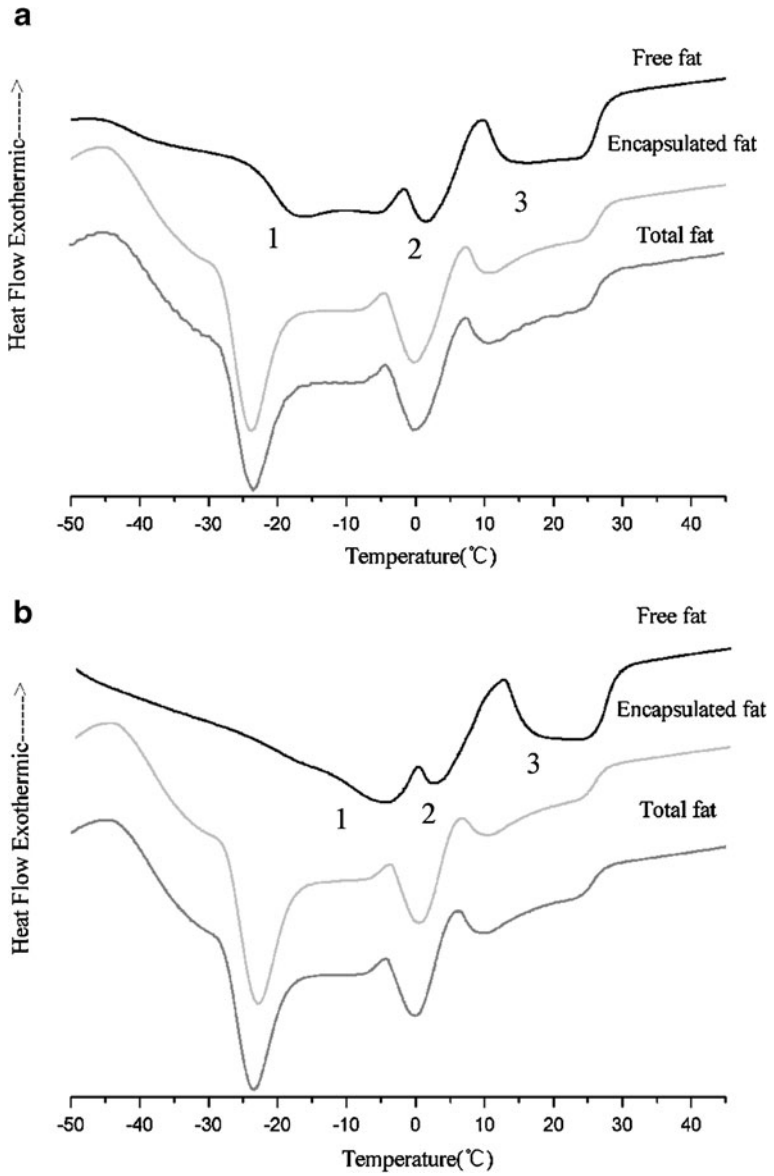

Fig. 4 DSC melting curves of three different fat fractions (free, encapsulated, and total) in the infant formula milk powder. **a** LHT, **b** HHT

powders to exceed 65%. This difference may be attributed to the higher whey protein content in infant formula milk powder.

4 Conclusions

To the best of our knowledge, this is the first time heat treatment applied on concentrated milk has been used to control the microbiological qualities of

infant formula milk powder. Heat treatment at 110 °C for 4 s was shown to improve the microbiological qualities of infant formula milk powders and make them to meet the new Chinese standard (GB 10765 2010). The powder particle morphology, wettability, free fat content, thermal characteristics of fat, and MFGM permeability after the two treatments were not significantly different. Despite some differences in solubility, dispersibility, viscosity, particle size, and protein characteristics, there was no obvious effect on the acceptability of the product. This investigation could potentially provide an effective method for processing infant formula milk powder in China.

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