Storage stability of freeze-dried colostral whey powders with different additives

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Abstract: Effects of different additives (sucrose and maltodextrin) on storage stability of colostral whey (CW) powders packaged in aluminium-laminated polyethylene pouches were investigated under different storage conditions (4℃ and 40%-70% relative humidity (RH), 25℃ and 50% RH, and 50℃ and 20%-60% RH). All the samples stored under 50℃ and 20%-60% RH showed the highest levels of lipid oxidation, Maillard reaction, proteolysis, and color difference, and the lowest immunoglobulin G (IgG) retention. Moisture contents showed an increase trend with increasing RH. Addition of sucrose into CW powders increased water adsorption capacity and Maillard reaction, whereas addition of maltodextrin showed the opposite effects. Maltodextrin as drying aids was suitable for keeping quality during storage. Sucrose did not clearly play any roles in protecting denaturation of IgG during 90-day storage. The low storage temperature and RH were helpful for keeping storage stability of CW powders with different additives.

Keywords: colostral whey (CW) powders, storage stability, freeze drying, sucrose, maltodextrin

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1 Introduction

Bovine colostrum is the initial milk secreted by cow during the first four days after calving[1]. In addition to established nutritional components, bovine colostrum is also rich in abundant bioactive components, including growth factors and immunoglobulins (Igs), and so on[2]. Oral administration of immunoglobulin preparations from bovine colostrum has been found to be effective against some pathogens and bacteria, including rotavirus, Salmonella enteriditis, enterotoxigenic E.coli in animal experiments as well as in human clinical trials[3-6]. Recently, adding Igs to infant formula and other foods has been industrially employed in Australia, New Zealand, USA, Europe and China. Colostrum-based products are commercially available as a health food supplement in those areas, and are marketed as a general "health promoting" product, particularly suitable for athletes. Immunoglobulin preparations from bovine colostrum designed for farm animals are also commercially available[7,8].

Bovine colostrum, milk and whey are considered as important sources for isolating Igs to prepare hyper-immune products[9]. Thermal treatments, including pasteurization, evaporation-concentration and dehydration, are the common methods of milk processing[10]. In order to inhibit protein denaturation during those thermal treatments, sugars and polyols as

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protectants, especially sucrose, are added into colostrum-based products. In addition, lactose, starch, maltodextrins, and sucrose as supplementary energy resources have commonly added into infant foods[11]. Powders of sugar-rich foods cannot be easily handling due to the development of stickiness. Therefore, drying aids, such as maltodextrin, are usually added into these products before drying[12]. For practical usage of colostral whey (CW) powders with different additives (sucrose and maltodextrin) as supplements to foods, their stability under different food processing and storage conditions should be determined. Until now, several reports have been published about effects of protectants like sugars, amino acid, or polyols on the stability of Igs during thermal treatments and other processing methods[10,13]. However, little information on the effects of protectants on the stability of Igs during storage has been reported.

While additives such as carbohydrate may be employed to improve thermal and dehydration stability of some products, such additives should not strongly change physical and chemical properties of original products. In this context, research about effects of different additives on storage properties of CW powders appears to be greatly valuable. Storage stability of CW powders with different additives was more or less changed due to change of product components. Addition of sugars in CW powders may change adsorption characteristics of original products, which will impact the storage ability of the products. For example, the phenomena that most sugars can spontaneously adsorb amounts of water vapor from storage environment have been reported in pharmaceutical industry[14,15]. During storage, additives in CW powders could react with components in original products or accelerate the rate of certain reactions, such as Maillard reaction[16], which is undesirable in these products. Storage stability of CW powders with different additives during different storage conditions receives more attention from producers and end users.

In the present work, effects of different additives (sucrose and maltodextrin) on physicochemical and biochemical properties of CW powders packaged in aluminium-laminated polyethylene (ALPE) pouches were investigated under different storage conditions (25℃ and 50% RH, 50℃ and 20%-60% RH, and 4℃ and 40%-70% RH).

2 Materials and methods

2.1 Materials and samples handling

Fresh bovine colostrums were provided by dairy farm of Bright Dairy & Food Co., Ltd, Shanghai, China, and then transferred to our lab and stored at minus 20℃ for 2-4 weeks prior to use in this study. Frozen bovine colostrums were thawed at room temperature, filtrated to remove non-milk ingredients by medical gauzes, defatted by SE 02.0V centrifugal separator (SEITAL S.r.l., Via delle Prese, Santorso (VI, Italy). Defatted colostrums were added with 1 mg/mL rennet solution (1 mL per liter milk) and incubated in water bath at 37℃ for 30 min to precipitate caseins, then centrifuged at 3 000 g for 30 min at 4℃. The CW obtained was homogenized by APV-1000 homogenizer (APV Manufacturing Poland Sp. z o. o, Grunwaldzka, Bydgoszcz, Poland), added with different additives, pasteurized at 63℃ for 30 min with immediate cooling in ice-water bath, and then stored in the refrigerator at 4℃. The CW was employed with a constant total solids mass concentration of (6.51±0.01)% (w.b.), containing fat of (0.74±0.02)%, protein of (2.41±0.01)%, lactose of (2.41±0.01)%, and ash of (0.956±0.002)%.

CW powders with different additives were obtained by freeze-drying CW, CW with 6% (w.b.) maltodextrin (DE10-15) (CWM), CW with 6% (w.b.) sucrose (CWS) and CW with 3% (w.b.) maltodextrin (DE10-15) and 3% (w.b.) sucrose (CWMS) in a freeze dryer (Shanghai Yucheng Dryer Equipment Co., Ltd, Shanghai, China). The plate temperature was maintained at 35℃ with a vacuum of 70-130 Pa in the chamber, while condensing plate temperature was kept at minus 60℃, and drying continued up to 72 h. All the samples were dried until moisture contents of the samples decreased below 6% (w.b.) according to national food safety standard of China[17] and transformed into powder and stored in a desiccator with silica gel until used. For storage experiment, the ALPE pouches were purchased from local market.

Four kinds of CW samples (12±0.5) g were placed in 100 μm ALPE, 12 μm PET, 8 μm Al, and 80 μm PE pouches (155 mm×135 mm) and were closed by heat sealing, avoiding any air space but without vacuum application. Every six samples of ALPE pouches were placed in a Binder constant climate chamber (constant climate chamber KBF series, Binder GmbH, Tuttlingen, Germany) setting the temperature at 25℃ and 50% RH (storage condition 1), in a Binder constant climate chamber (constant climate chamber KBF series, Binder GmbH, Tuttlingen, Germany) setting the temperature at 50℃ and 20%-60% RH (storage condition 2) and in a refrigerator (Haier BCD-196F refrigerator, Haier Group, Qingdao, China) setting the temperature at 4℃ and 40%-70% RH (storage condition 3), respectively. Storage procedure was carried out for 90 days. Every three samples of ALPE pouches under different storage conditions were taken out from the chambers at the time of 15 d, 30 d, 45 d, 60 d, 75 d, and 90 d, respectively, and analysed for physicochemical and biochemical changes.

2.2 Physicochemical and biochemical tests

2.2.1 Moisture content

The moisture contents of CW powders with different additives at different stages were determined using AOAC method 927.05[18] and the results were expressed as percentage (on dry basis).

2.2.2 Lipid oxidation

Lipid oxidation was evaluated spectrophotometrically using the thiobarbituric acid (TBA) test as described by King[19] and the results were expressed as optical density (OD) measured at 532 nm.

2.2.3 Hydroxymethylfurfural (HMF)

The total HMF content was determined following the method prescribed by Keeney and Bassette[20] and the results were expressed as micromole of HMF per kilogram powders (on dry basis).

2.2.4 Protein hydrolysis assay

The protein hydrolysis assay was carried out spectrophotometrically according to the method of Hull[21] and expressed as milligram free tyrosine per gram powders (on dry basis).

2.2.5 Color change

The color values of powder samples were measured as CIE parameters L, a, and b using a color difference meter (Model WSC-S, Shanghai precision & scientific instrument Co., Ltd, Shanghai, China). The L, a, and b values of the stored powder were measured and the net (total) color difference (ΔE) was calculated using Equation (1) using CW powders with different additives at the initial day of the storage period as a reference, respectively:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

2.2.6 Single radial immunodiffusion (SRID) test

Samples (1±0.1) g of CW powders with different additives were assayed for IgG concentration by the SRID procedure[22], and the results were expressed as percentage (on dry basis). The samples were prepared by acid coagulation procedure[23].

2.3 Statistical analysis

The results from three separate experiments were given as mean ± standard deviation (SD) values. Data for physicochemical and biochemical properties were subjected to the analysis of variance (ANOVA) using the SAS 9.2 software program (SAS Institute Inc., Cary, NC, USA). The factors included in the models were storage conditions (25℃ and 50% RH, 4℃ and 40%-70% RH, 50℃ and 20%-60% RH), different additives (none, maltodextrin, sucrose and maltodextrin combined with sucrose) and storage time (15, 30, 45, 60, 75, and 90 days). $P<0.05$ was regarded as statistically significant.

3 Results and discussion

3.1 Moisture content

Changes of moisture contents of CW powders with different additives packaged in ALPE pouches under different storage conditions were illustrated in Figure 1. It clearly showed an increase trend in moisture contents of all the samples with time increasing in three storage conditions, which was attributed to water adsorption of low-moisture-content food powders from storage environment. In addition, a steady increase in moisture content under condition 1 was found due to stable temperature and RH, while fluctuations of moisture contents appeared under conditions 2 and 3 because of fluctuant levels of RH. Similar results were found by Kumar and Misha[24] and Koç et al.[25], who reported that

mango soy fortified yoghurt powder under accelerated storage and spray-dried yoghurt powder under 25℃ and 50% RH increased slightly in 49 days and 90 days, respectively. It can also be found that at the end of storage period, moisture contents of all the samples under condition 3 were the highest, followed by those under conditions 1 and 2, which indicated that the amount of water vapor uptake during storage was positively related to levels of RH of storage environment. Comparing changes of water moisture in CW powders with different additives during 90 days storage under condition 1, increment of water moisture in CWS powders appeared to be the highest, ranging from 5.95% (dry basis, water moisture in the following is referred to as dry basis) to 9.36%, followed by CWMS powders, ranging from 6.16% to 9.20%, CW powders, ranging from 5.82% to 8.23%, and CWM powders, ranging from 6.08% to 6.84%. These results meant that adding sucrose and maltodextrin into CW powders significantly changed adsorption capacity of CW powders (P<0.01) (Table 2). Furthermore, it seemed that addition of sucrose increased adsorption capacity of CW powders. Similarly, sugars as pharmaceutical excipients showed relatively high levels of water adsorption capacity[14,15]. On the contrary, adding maltodextrin into CW powders could decrease water adsorption capacity. Therefore, maltodextrin has been commonly employed in food industry in order to reduce the stickiness of sticky food products and to produce free flowing powders[26].

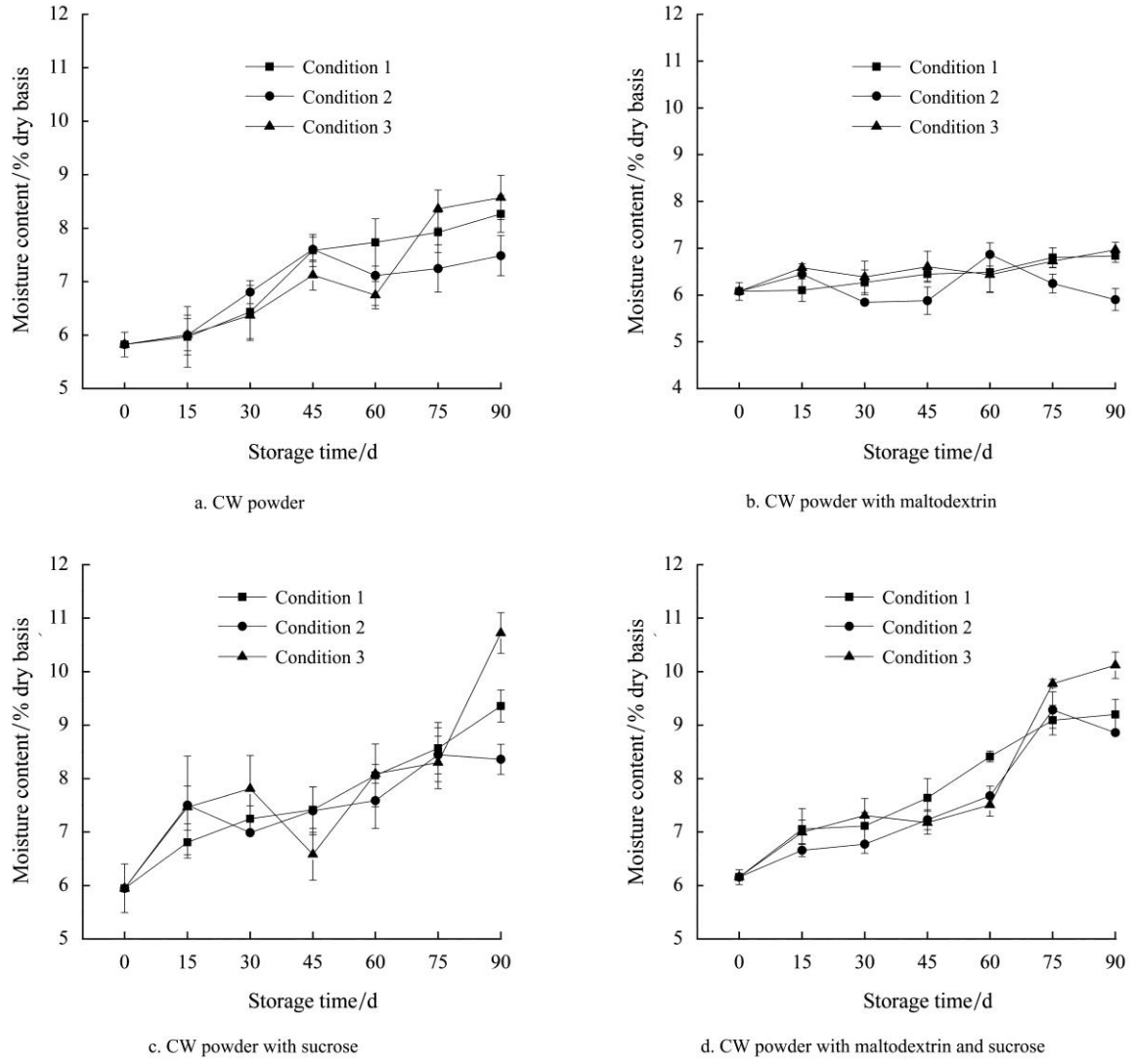

Figure 1 Changes of moisture contents of colostral whey (CW) powders with different additives under different storage conditions. Condition 1 means 25℃ and 50% RH, condition 2 means 50℃ and 20%-60% RH, condition 3 means 4℃ and 40%-70% RH.

3.2 Lipid oxidation

Secondary lipid oxidation products during long-term storage are quantified by the 2-thiobarbituric (TBA) method, and this method has been commonly employed for measuring extent of lipid oxidation in milk powders by many researchers[24,25,27,28]. Optical density (OD) values of TBA for CW powders with different additives during storage were illustrated in Figure 2. It was obvious that OD values of TBA for all the samples under storage condition 2 were higher than those under storage conditions 1 and 3 (P<0.01), which was attributed to high storage temperature favoring oxidation[28]. In addition, OD values of TBA for all the samples under storage condition 1 were close to those under storage condition 3, and statistical analysis showed that there was no significant difference between them (P>0.05). Initial OD value of TBA for CW powders was 0.042 and then increased to 0.118 after 90-day storage under storage condition 1, which was lower than that obtained by Kumar and Mishra[24], due to its low fat content. Meanwhile, OD values of TBA for CWM powders and CWMS powders under storage condition 1 ranged from 0.098 to 0.228 and from 0.068 to 0.288, respectively. The highest increment, ranging from 0.071 to 0.47, was found in CWS powders under storage condition 1. Moreover, there was no significant difference in increments of OD values of TBA between CW and CWM powders, indicating that maltodextrin as drying aid did not significantly increase lipid oxidation in CWM powders during storage. The reasons why the addition of sucrose increased the extent of lipid oxidation were unclear and needed to be further investigated.

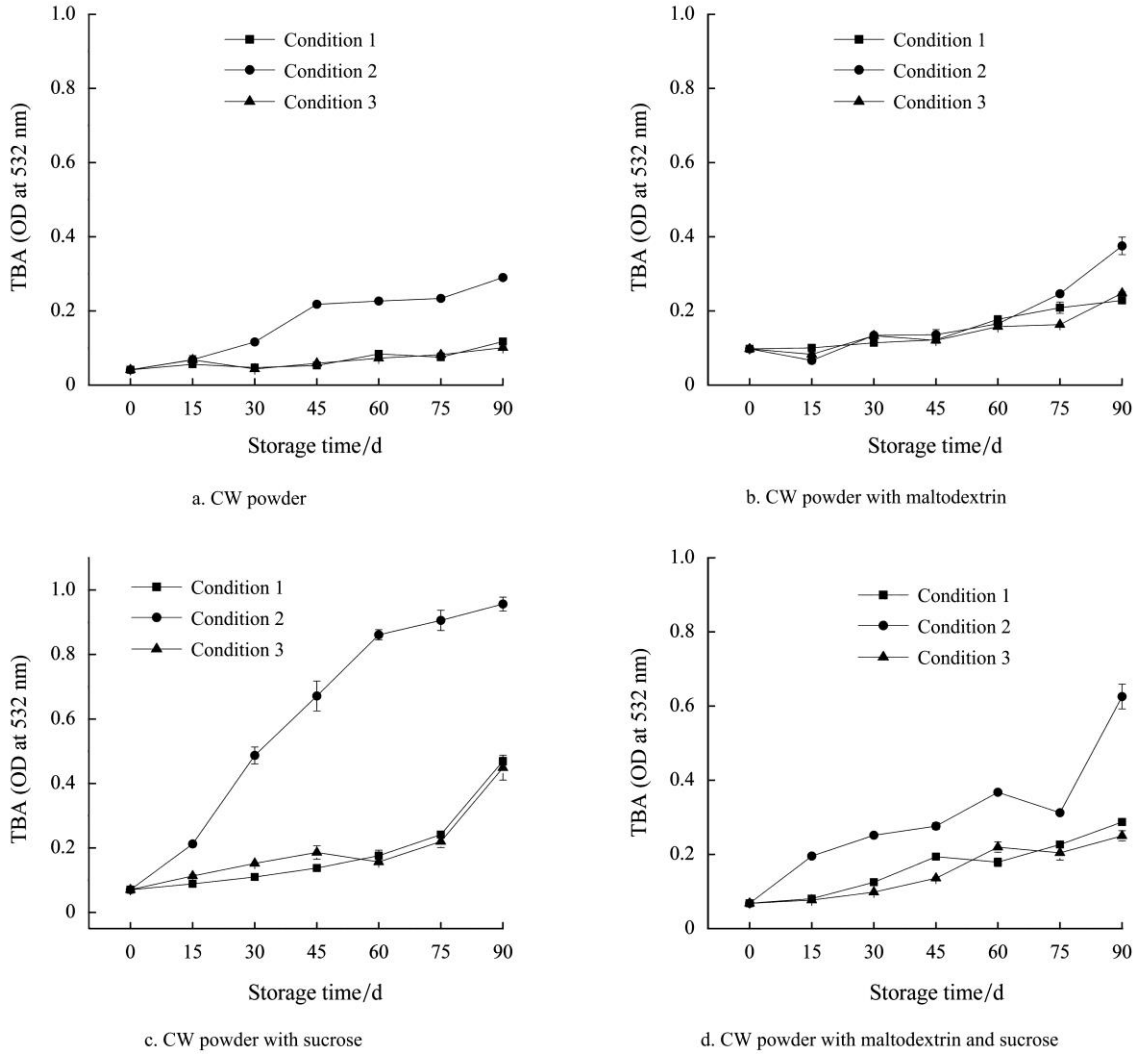

Figure 2 Lipid oxidation of colostral whey (CW) powders with different additives under different storage conditions. Condition 1 means 25℃ and 50% RH, condition 2 means 50℃ and 20%-60% RH, condition 3 means 4℃ and 40%-70% RH.

3.3 Maillard reaction

During storage of powdered dairy products, Maillard reaction, a frequent reaction between proteins and reducing sugars, is the most undesired but inevitable reaction. HMF, the compound produced during a late stage of Maillard degradation, has been commonly used as a measurement of the extent of browning in dairy products by many researchers[24,29]. Increase of HMF levels in all the samples with storage time was presented in Figure 3. The initial HMF levels were 61.21 μmol/kg in CW powders, 149.73 μmol/kg in CWM powders, 4 295.97 μmol/kg in CWS powders, and 2 900 μmol/kg in CWMS powders. The high HMF levels in CWS and CWMS powders were mainly attributed to hydrolysis of some sucrose during thermal pre-treatment. It clearly showed that the biggest increment of HMF levels in all the samples occurred under storage condition 2, mainly due to its high storage temperature and partly due to the increasing water content[30,31]. Statistical analysis also revealed that HMF levels were significantly affected by storage conditions (Table 1). Similarly, Zbikowski et al.[29] asserted that the formation of HMF was temperature dependent since milk powders produced more HMF at 30℃ than that at 20℃ after 11 months storage. It was obvious that HMF levels of CWS and CWMS powders under condition 2 increased rapidly, while HMF levels of CWS and CWMS powders increased relatively slightly under conditions 1 and 3. It can be explained that sucrose, belonging to non-reducing sugars, does not react with amino compounds through Maillard reactions under mild processing conditions, whereas, sucrose may become a major reactant in Maillard reactions due to hydrolysis of the disaccharide at high temperature[16]. In general, when the concentrations

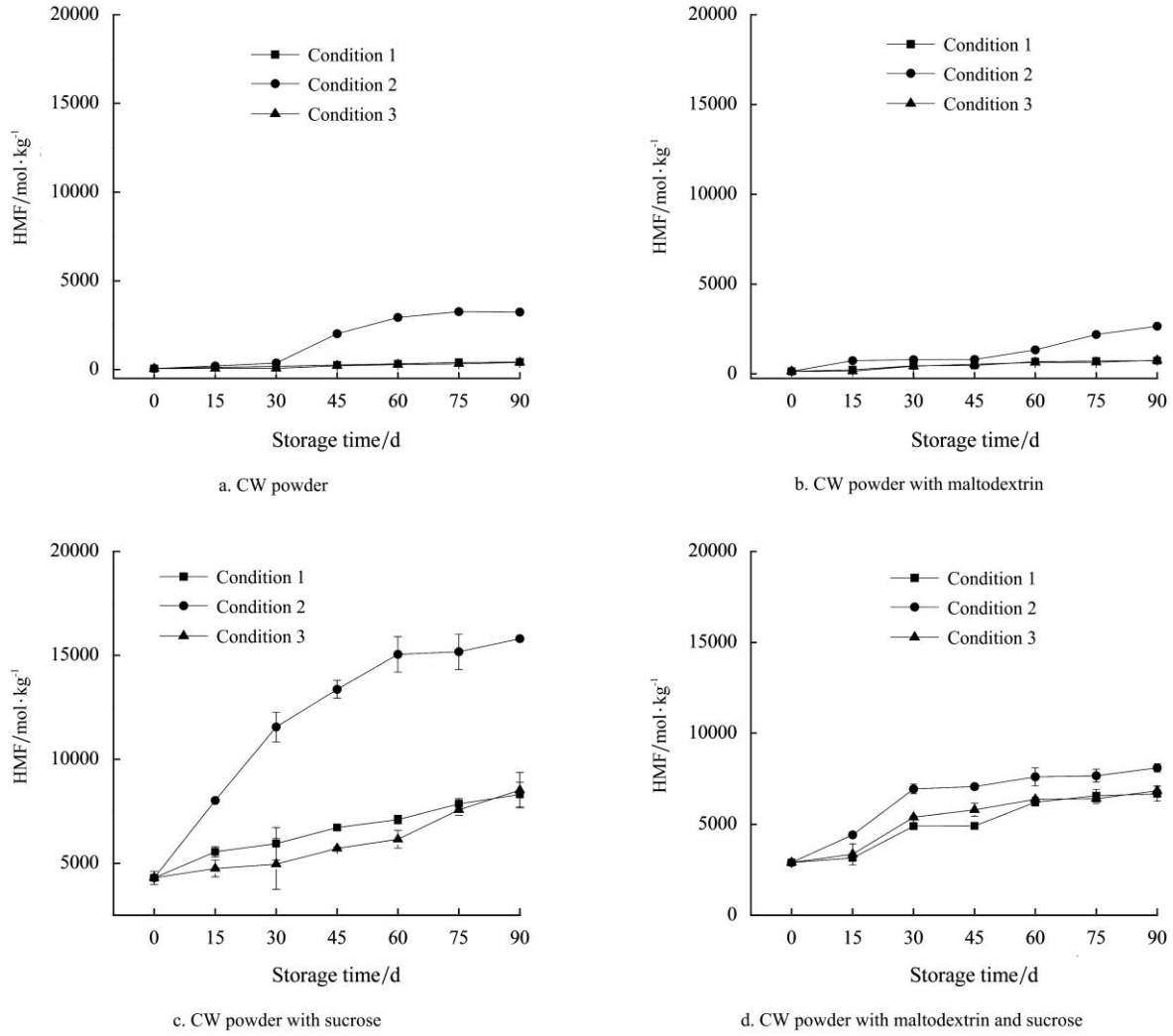

Figure 3 Changes of HMF of colostral whey (CW) powders with different additives under different storage conditions. Condition 1 means 25℃ and 50% RH, condition 2 means 50℃ and 20%-60% RH, condition 3 means 4℃ and 40%-70% RH.

Table 1 ANOVA for physicochemical and biochemical properties of CW powders with different additives under different storage conditions

Samples[c]	Source of variation	df	Management statistics subsystem					
			Moisture content	Colour difference (ΔE)	TBA	HMF	Proteolysis	IgG concentration
CW powder	Storage period	5	1.92[a]	5.57	0.00479	915423.39	0.141	0.1969[a]
	Storage condition	2	0.113	187.34[a]	0.0291[a]	6120929.45[a]	0.538[b]	0.6029[a]
	Error	10	0.179	14.08	0.00155	545858.15	0.0974	0.0252
CWM powder	Storage period	5	0.0949	22.28	0.0153[a]	464578.881	0.152	0.1749[a]
	Storage condition	2	0.278	277.35[a]	0.00221	1543754.62[a]	1.25[a]	0.0515[a]
	Error	10	0.0986	9.38	0.0013	148912.121	0.112	0.00273
CWS powder	Storage period	5	2.48[a]	300.93	0.0857[b]	9401416.1[a]	0.267	NA
	Storage condition	2	0.302	6028.55[a]	0.0449[a]	86839033.3[a]	7.88[a]	NA
	Error	10	0.351	316.6	0.016	1337677.1	0.533	NA
CWMS powder	Storage period	5	3.82[a]	17.04	0.0266[a]	5042184.72[a]	0.195	0.1234[a]
	Storage condition	2	0.279	321.35[a]	0.0551[a]	4165292.31[a]	3.49[a]	0.269[a]
	Error	10	0.138	15.95	0.00344	57418.1	0.129	0.01002

Note: [a] means significant at $P<0.01$; [b] means significant at $P<0.05$; NA means not available.

[c] CW means CW; CWM means CW with maltodextrin; CWS means CW with sucrose; CWMS means CW with sucrose and maltodextrin.

of reactants were constant, Maillard reaction was mainly affected by temperature[32]. Statistical analysis demonstrated that the conditions 1 and 3 had significant effects on HMF levels of CW and CWS powders ($P<0.01$), while they showed no significant impact on HMF levels in CWM and CWMS powders. The results suggested that the addition of maltodextrin could effectively reduce Maillard reaction rate within the temperature range investigated in this experiment. The results were in agreement with Chronakis[33], who reported that maltodextrin had the capacity to participate in Maillard reactions and can be used as non-browning carriers for drying sensitive products. The similar phenomena were found in infant formulas with addition of sugars[11]. In one word, choosing relative low storage temperature and adding maltodextrin were possibly helpful for inhibiting the rate of Maillard reaction.

3.4 Proteolysis

As proteinases remained stable in milk powder upon storage and presence of water in milk powder[34], it was valuable to evaluate extent of proteolysis of CW powders with different additives during storage. The levels of free tyrosine, which was indicative of proteolysis, of CW powders with different additives were presented in Figure 4. The data obtained for protein hydrolysis were inconsistent, and it was hard to establish a clear trend for the samples stored under conditions 1 and 3, but the levels of free tyrosine in all the samples stored under condition 2 clearly showed an increase trend. Moreover, the levels of free tyrosine in all the samples stored under condition 2 were higher than those stored under conditions 1 and 3, partly due to high temperature accelerating rate of proteolysis. Statistical analysis showed that extent of protein hydrolysis was significantly affected by storage conditions and types of additives (Tables 1 and 2).

3.5 Color difference

Color is one of the most important factors influencing consumer acceptance of dairy products. As it is shown in Figure 5, color difference (ΔE) in CW powders with different additives under different storage conditions showed an increase trend with storage time. It was obvious that ΔE of all the samples under condition 2 was the highest, regardless of types of additives, which was mainly attributed to many pigments produced by lipid oxidation and Maillard reaction at high temperature[31]. In addition, CWS showed a dramatic increment in ΔE under condition 2, due to high temperature combined with addition of sucrose accelerating the rate of Maillard reaction. On the contrary, the addition of maltodextrin seemed to reduce the rate of Maillard reaction, and it was further verified by the results that there was no significant difference in ΔE among CW, CWM and CWMS powders ($P>0.05$). Furthermore, there was a significant

difference in ΔE between CWS powders and the others under condition 2 (Table 2). Statistical analysis also indicated that there was no significant difference in ΔE among all the samples under conditions 1 and 3 (Table 2), indicating that conditions 1 and 3 were relative suitable for keeping stable appearance (color) in CW powders with additives (sucrose and maltodextrin).

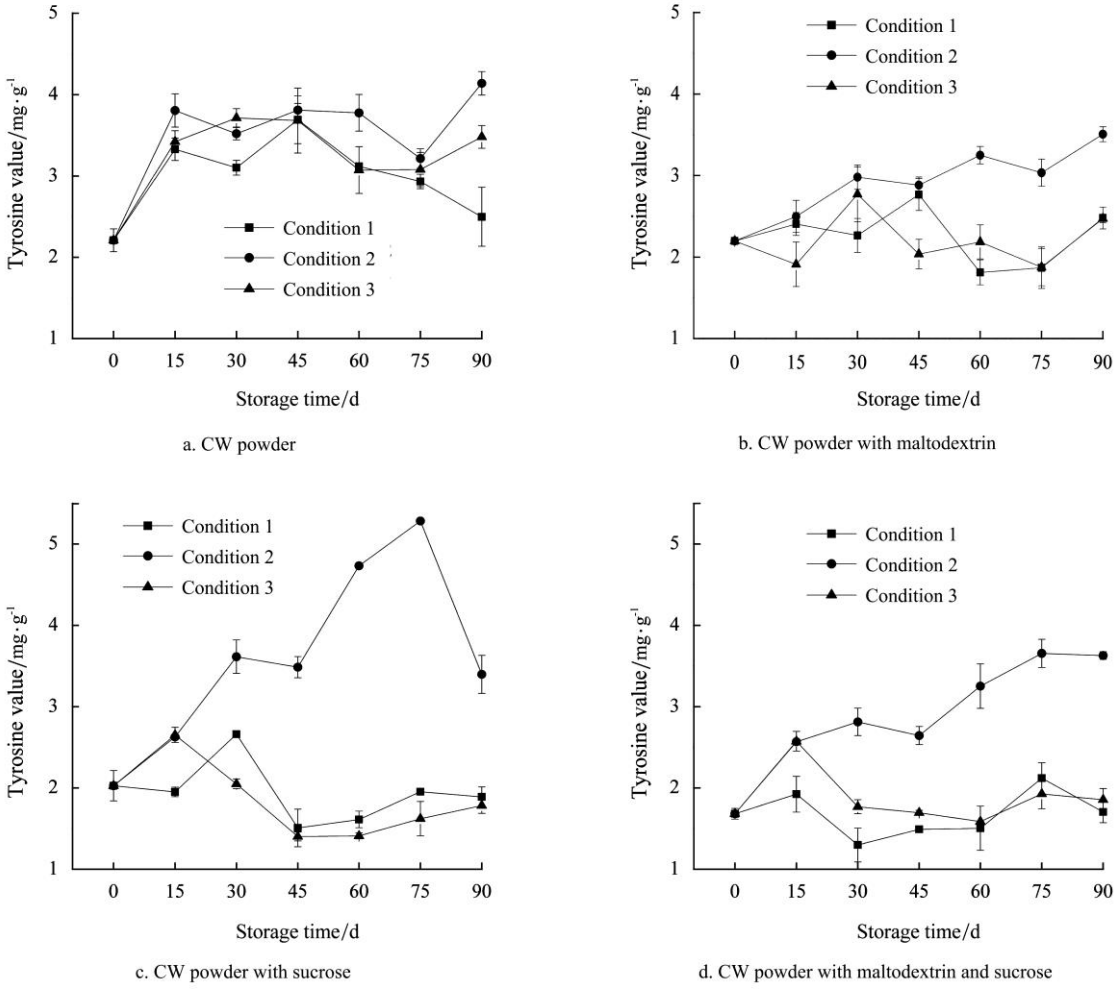

Figure 4 Rate of proteolysis of colostral whey (CW) powders with different additives under different storage conditions. Condition 1 means 25℃ and 50% RH, condition 2 means 50℃ and 20%-60% RH, condition 3 means 4℃ and 40%-70% RH.

Table 2 ANOVA for physicochemical and biochemical properties of CW powders with different additives under the same storage conditions

Storage conditions[c]	Source of variation	df	Management statistics subsystem					
			Moisture content	Colour difference(ΔE)	TBA	HMF	Proteolysis	IgG concentration
Condition 1	Storage period	5	2.26[a]	4.99	0.02[a]	1819419.2[b]	0.0894	0.022[a]
	Different additives	3	15.27[a]	9.41	0.02[a]	68082374.4[a]	2.36[a]	0.0058[a]
	Error	15	0.148	6.37	0.00293	406410.1	0.0154	0.000698
Condition 2	Storage period	5	1.01b	219.67	0.0884[a]	9576546.9[a]	0.555	N.A.
	Different additives	3	16.08[a]	2615.84[b]	0.324[a]	178010996.1[a]	1.08[b]	N.A.
	Error	15	0.34	264.81	0.0122	1179262.3	0.28	N.A.
Condition 3	Storage period	5	3.58[a]	9.13	0.0156[a]	2149034.3[b]	0.231	0.0119[a]
	Different additives	3	17.2[a]	5.39	0.0208[a]	63150543.9[a]	3.24[a]	0.00874[a]
	Error	15	0.498	3.5	0.0025	567105.5	0.106	0.000682

Note: [a] means significant at $P<0.01$; [b] means significant at $P<0.05$; NA means not available.

[c] condition 1 means 25℃ and 50% RH; condition 2 means 50℃ and 20%-60% RH; condition 3 means 4℃ and 40%-70% RH.

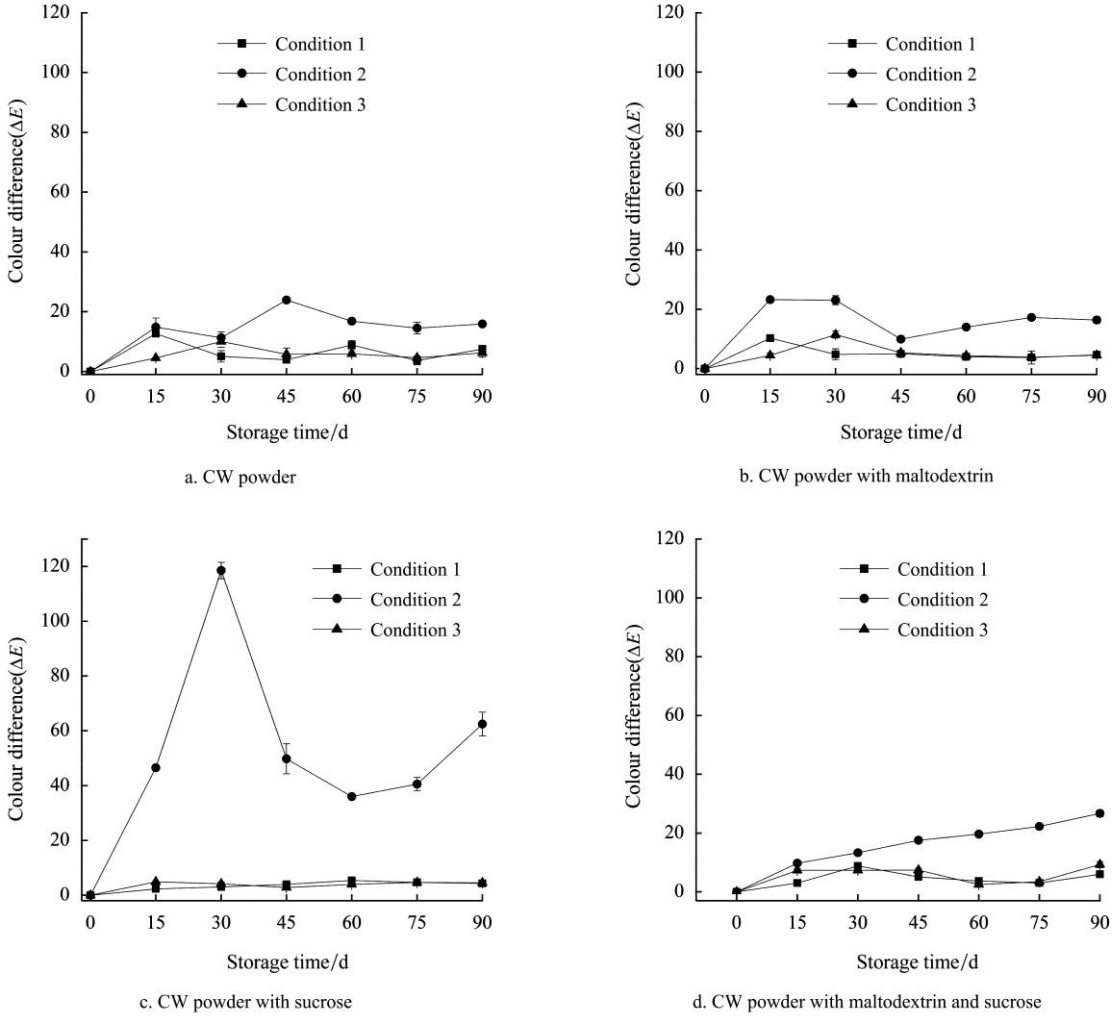

Figure 5 Changes of color difference (ΔE) in colostral whey (CW) powders with different additives under different storage conditions. Condition 1 means 25℃ and 50% RH, condition 2 means 50℃ and 20%-60% RH, condition 3 means 4℃ and 40%-70% RH.

3.6 IgG retention

The initial IgG concentrations of CW, CWM, CWS and CWMS powders were 5.34%, 2.80%, 2.24% and 2.35%, respectively. A significant decrease of IgG retention in CW powders with different additives during storage was presented in Figure 6. In general, denaturation of IgG protein was mainly affected by temperature and pH[10,13]. As it was shown from Figure 6 and Table 1, it was significant (P<0.01) that IgG retention in all the samples decreased the fastest under condition 2, followed by that under conditions 1 and 3, indicating that choosing relative low storage temperature was helpful for inhibiting denaturation of IgG protein. In addition, there appeared to be no significant difference (P>0.05) in decrease of IgG concentration in all the samples between conditions 1 and 3. At the end of storage stage, IgG retention of CW powders under condition 1 decreased to 85.8 %, which was higher than that of the other samples, partly suggesting that additives (maltodextrin and sucrose) at concentration investigated in this experiment did not play any role in delaying denaturation of IgG during storage. The similar results were also found under conditions 2 and 3. As been verified, sugars and polyols are able to protect proteins against heat denaturation because of their effects on the structure of hydroxyl groups in water[10]. Increased hydrophobic interactions in the protein molecules and changes in preferential salvation of protein molecules in sugar solution facilitate the stabilization of proteins during thermal treatment[13,35]. On the contrary, experimental data showed negative effects of additive (sucrose) on IgG retention, which was possibly attributed to high extent of the Maillard reaction caused by addition of sucrose. Statistical analysis also showed that IgG

retention was significantly affected by additives under conditions 1 and 3 (Table 2). The results obtained suggested that choosing low storage temperature (4℃) was helpful for inhibiting denaturation of protein.

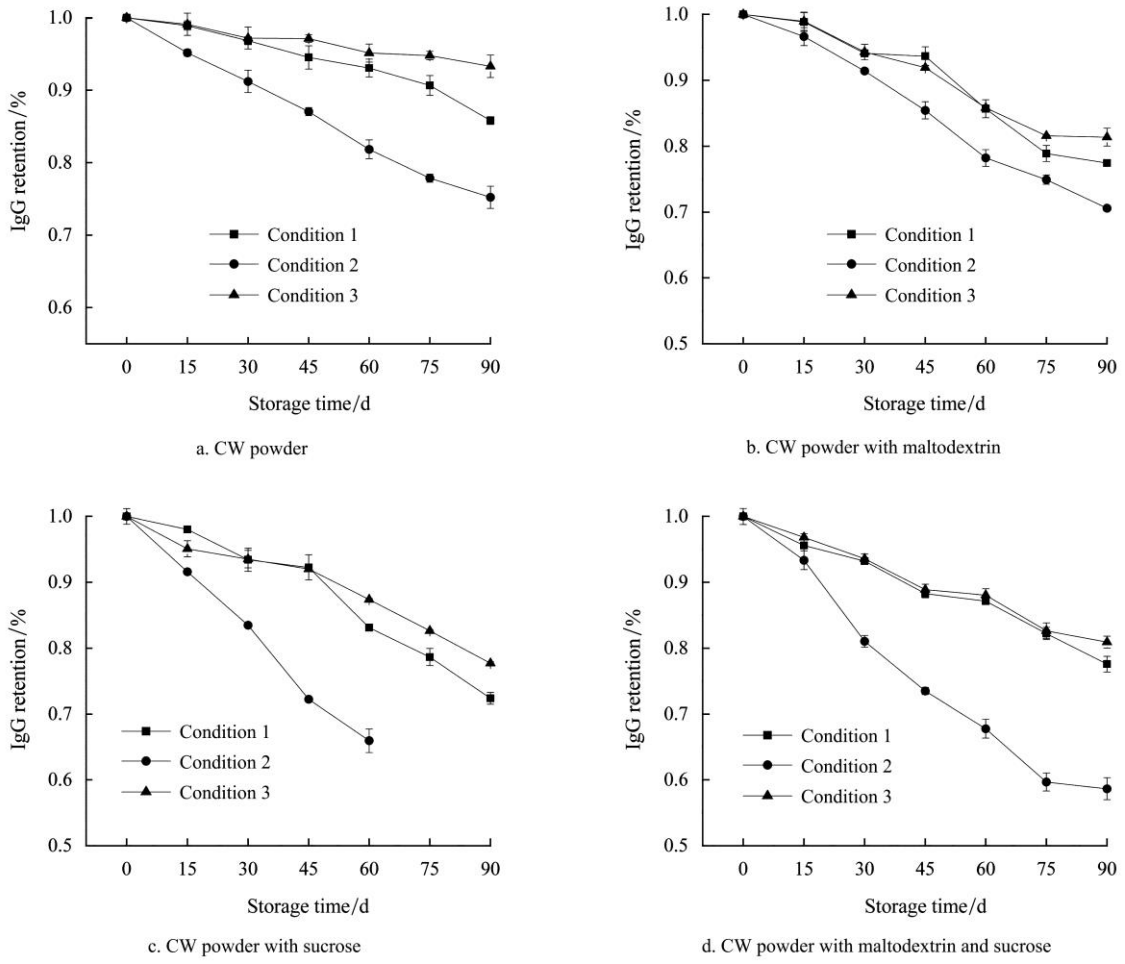

Figure 6 IgG retention of colostral whey (CW) powders with different additives under different storage conditions. Condition 1 means 25℃ and 50% RH, condition 2 means 50℃ and 20%-60% RH, condition 3 means 4℃ and 40%-70% RH.

4 Conclusions

The highest levels of lipid oxidation, Maillard reaction, proteolysis, color difference, and the lowest IgG retention in CW powders with different additives occurred under 50℃ and 20%-60% RH. With increasing temperature, the levels of Maillard reaction increased and IgG retention decreased. Moisture contents of all the samples stored under three storage conditions were greatly related to levels of RH of storage environment, and showed an increased trend with increasing levels of RH. Addition of sucrose into CW powders increased water adsorption capacity, extent of lipid oxidation and Maillard reaction of all the samples. On the contrary, addition of maltodextrin into CW powders decreased water adsorption capacity, and reduced Maillard reaction. Sucrose at the concentration investigated in this experiment did not clearly play any role in protecting denaturation of IgG during 90-day storage. Maltodextrin as drying aid was stable and did not negatively affect physicochemical properties of CW powders during 90-day storage. Choosing low storage temperature and low RH was helpful for keeping storage stability of CW powders with different additives.

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