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ORIGINAL PAPER

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DEVELOPMENT AND STABILITY OPTIMISATION OF COMPOSITE CEREAL-BASED MEAL REPLACEMENT

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ABSTRACT

Background. Maize is one of the traditional food crops with rich nutritional value and high dietary fiber content, but the coarse taste of maize limits consumption. The purpose of this study was to develop a composite cereal meal replacement (CCMR) containing corn, rice, millet, skimmed milk powder and oats with good taste and uniform organization, along with a stabilizer formulation.
Materials and methods. Corn, rice, and millet were purchased and ground. The formulation of the composite cereal meal replacement was optimized by an orthogonal method based on sensory scores. The stabilizer formulation was optimized using a Response Surface Method (RSM) Box-Behnken model based on centrifugal sedimentation rate (CSR).
Results. The results showed that the optimal raw material formulation for the CCMR was 55% corn, 10% rice, 10% millet, 5% skimmed milk powder, and 15% oats, with a sucralose addition amount of 0.009% and an optimal composite stabilizer formulation of 1.5% microcrystalline cellulose (MCC), 0.6% sodium carboxymethyl cellulose (CMC), 0.6% carrageenan, 0.4% xanthan gum, 2% mono-and di-glycerol of fatty acids (MFA) and 0.2% calcium hydrogen phosphate. The formulation showed the lowest CSR, the best stability and the highest CCMR sensory score. The soluble solids content was 7.466 g/100 g, the dietary fiber content was 0.378 g/100 g, the pH was 6.41, and the reducing sugar content was 2.6493 mg/g. An accelerated stability experiment predicted that the storage time of the CCMR at 20°C was 142–155 days.
Conclusion. The combination of meal replacement and cereal supports people's pursuit of nutrition and health while achieving low-fat satiety and improving quality of life, and therefore has great market potential.

Keywords: cereal, meal replacement, formulation, stabilizer, shelf life

INTRODUCTION

The development of cereal beverages has given people a new understanding of cereals as a traditional food and completely changed the perception of cereals as 'coarse grains' (Pasqualone and Summo, 2021). Compared with traditional beverages, cereal beverages focus on the concept of naturalness and health, satisfying consumers' desire for nutritious food and psychological need for a healthy diet. They can also be consumed as meal replacements. Cereal beverages not only conform to traditional diets, but also meet the current nutritional supplementation requirements of people's fast-paced lives and have meal replacement functions (Xiong et al., 2022). The cereal content can be increased to make cereal concentrates, which can

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be made into cereal meal replacements and can improve satiety due to their high solids content. Consistent long-term consumption can control the occurrence of chronic diseases such as diabetes and cardiovascular disease while contributing to weight control (Kim et al., 2020). Therefore, improving dietary structure by increasing the intake of cereals while reducing the intake of animal foods has the potential to greatly improve the health of the nation.

Corn is one of the major grain crops, and as a grain with high dietary fiber content and satiety, it is nutritious but has a coarse texture and limited edibility (Ekpa et al., 2019). Grinding maize into flour while retaining its unique taste can improve its palatability. The amino acids in millet protein are complete and the pattern values of the eight essential amino acids, except lysine, are close to those of humans (Annor et al., 2017). It is a good raw material for the production of healthy (diet) foods (Tumwine et al., 2019). Rice is rich in rice protein (Xinkang et al., 2023), low in fat, has the unsaturated fatty acids required by the body and is cholesterol free; it also contains minerals, fiber, vitamin B, vitamin E and many other trace elements. Moreover, its amino acid composition is complete and easily absorbed by the body (Al--Doury et al., 2018). The special element it contains, glutamic acid, is called the 'beauty factor' and has the effect of inhibiting melanin (Swaminathan and Guha, 2018). The β-glucan in oats has hypoglycemic, hypolipidemic, serum cholesterol-lowering, and antioxidant effects (Angelov et al., 2018), which is useful in preventing cardiovascular disease caused by hyperlipidemia, controlling diabetes, and anti-aging. The high-quality protein in skimmed milk is easily absorbed by the body (Tumwine et al., 2019) and is a good source of nutrition but lacks vitamins, while cereals are a rich source of dietary fiber, which has the effect of promoting intestinal motility and preventing many cardiovascular diseases. When skimmed milk powder is added to the traditional method of grinding and cooking porridge, the combination of cereals and milk has a synergistic effect, neutralizing the roughness of other whole grains and giving a more mellow taste to composite cereal meal replacement (CCMR). It also provides better nutrition, ultimately producing a value-added functional product. The combination of coarse grains such as corn and rice meets the scientific dietary requirements of the Chinese Dietary Guidelines for nutritional balance and combination of coarse and fine grains. Sucralose, as a non-caloric sugar substitute, was added to the CCMR. It is different from sucrose, which is decomposed into glucose by the human body and adds a burden to the human body (Magnuson et al., 2017). Sucralose is 600 times sweeter than sucrose, with a pure and consistent sweetness, while its various sweetness characteristics are extremely similar to those of sucrose but without the calories, making it an ideal alternative (Zheng et al., 2022).

Research reports have shown that many food colloids, such as xanthan gum, carrageenan and sodium carboxymethyl cellulose (CMC), can perform the function of dietary fiber. Their high viscosity fills the stomach, prolongs the retention of food in the gastric cavity, and achieves the effect of satiety, reducing energy intake and controlling body weight. CMC has stabilizing and suspending effects in cereal beverages, which can evenly distribute cereal grains and prevent precipitation (Bampidis et al., 2020). Carrageenan can form a uniform three-dimensional network structure or gel with casein, which can effectively prevent the coagulation and precipitation of dairy cereal beverages (Michna et al., 2021). Xanthan gum is a thickening agent that can give beverages refreshing properties and protect proteins in cereal beverages (Kumar et al., 2018). Mono-and di-glycerols of fatty acids (MFA) are a common emulsifier in food additives (Ferreira et al., 2021), which can make cereal beverages finely organized, improve their stability and prevent precipitation. The use of stabilizers can reduce the delamination and sedimentation problems of cereal beverages and extend their shelf life (Kumar et al., 2023). A single stabilizer cannot achieve the desired effect, and most experiments use a mixture of stabilizers, which can often produce a 1+1>2 effect when the dosage is small (Seisun and Zalesny, 2021).

A new cereal beverage was developed in this study. The combination of whole grains with fine grains and milk was used to meet the dietary needs of the population while providing a delicate taste and higher nutritional value. The development of a composite stabilizer further alleviates the stability problem of the cereal beverage and extends the shelf life of the product.

MATERIALS AND METHODS

Materials and reagents

Corn, rice, millet and oats were purchased in September 2021 from the tomato supermarket in Jilin City (Northeast China, 125°40′-127°56′ E, 42°31′-44°40′ N) Jilin Province. Skimmed milk powder came from Nestle, Netherlands. Sucralose, microcrystalline cellulose, sodium carboxymethyl cellulose, xanthan gum, carrageenan, mono-and diglycerol fatty acid esters, and calcium hydrogen phosphate were purchased from Xinyuan Biotechnology Corporation of China. The reagents, includingα-amylase, glycosylase, papain (Shanghai Maclean Biochemical Reagent Co., Ltd., China), glucose (Tianjin Fuchen Chemical Reagent Co., Ltd., CAS No:14431-43-7, China), potassium sodium tartrate (Tianjin Fuchen Chemical Reagent Co. Ltd., CAS No:609-99-4, China), NaOH and phenol (Tianjin Yongda Chemical Reagent Co., Ltd., China), were all analytically pure.

Pre-preparation of raw materials

The corn, rice, millet and oats were cleansed of impurities. Clean water was selected to remove impurities such as rice bran and dust from the surface. The grains were then dried at 80°C for 2 hours in an electric thermostatic blast drying oven (XMTD-8222, Shanghai Jinghong Experimental Equipment Co., Ltd., China), and the corn, rice and millet were crushed (Shredder, LINGSHENG, Yongkang Red Sun Electromechanical Co., Ltd., China) and then passed through an 80-mesh sieve. The coarsely crushed product was put into ultrafine grinding equipment (Ultra Micro Crusher, KC-18, Kang Yuan Xin Pharmaceutical Machinery Co., Ltd., China) and ground to 200 mesh.

Preparation of beverages

The raw materials corn, rice, millet, skimmed milk powder, 0.009% sucralose and stabilizer were mixed well with 20 mL of distilled water. The raw materials and stabilizer were poured into a liquid heater (DZG22A, Royalster Electronic & Electrical Co., Ltd, China) with 360 mL of boiling water, mixed and stirred well, then boiled with continuous stirring for 30 seconds. The stabilizer was stirred using a 90-2 Digital High Temperature Magnetic Stirrer (XMTD-702, Changzhou Yuexin Instrument Manufacturing Co., Ltd., China) until it was completely dissolved and the solution became a paste. The ingredients were mixed and boiled by hand until the stabilizer and the ingredients were evenly mixed without particles. The homogenization condition was 20 MPa, 10 min with Homogenizer (THF500-G, TUOHE Instrument Co., Ltd., China), and the sterilization condition was 121°C, 15 min with Sterilizer (LSH-12B, Li-Chen Instrument Co., Ltd., China).

Sensory evaluation of the CCMR with different raw material additions

Sensory scoring of the CCMR was performed for different raw material additions. The 10 panel members (5 male, 5 female, 20–30 years old) were selected from the faculty and students and trained according to GB/T 16291.1 (2012). The samples were labelled with a random number and provided to the testers at random (Hou et al., 2019). Each sample was assessed twice (Liu et al., 2020); the sensory evaluations were carried out between 9 am and 11 am and 2 pm and 4 pm, and the evaluators were advised to rinse their mouths out with water between tasting samples (Zhang, 2021). The sensory testing was carried out in a sensory analysis laboratory equipped with individual compartments designed according to GB/T 13868 (2009) of China (room temperature 24°C, relative humidity 50%, combined artificial light with a color temperature of 6,500K).The sensory criteria were divided into four categories: taste, color, aroma and state. The sensory scores and criteria are shown in Table 1.

Determination of centrifugal sedimentation rate (CSR)

The CSR is commonly used to assess the stability of beverages, and it is negatively correlated with beverage stability. The lower the CSR, the better the stability of the beverage. 15 mL of CCMR was placed in a centrifuge tube at 5,000 r/min and centrifuged for 10 min. After standing for 10 min, the supernatant was removed and the mass of the precipitate in the tube was measured (Wu et al., 2020). Each sample was measured three times in parallel, and the average value was taken to calculate CSR using the formula (1).

$$\text{CSR (\%)} = [M_1/M_2] \times 100\% \quad (1)$$

where:

M_1 is the mass of the precipitate after centrifugation

M_2 is the mass of the sample solution before centrifugation.

Table 1. Sensory evaluation criteria

Index	Perfect (21–25)	Commonplace (16–20)	Terrible (<15)
Taste (25)	The taste is delicate and moderate, the grain flavor is mellow, and the flavor is coordinated.	The taste of grains is light and slightly rough or is not harmonious.	The taste is poor, there is basically no grain flavor.
Smell (25)	The smell of corn is prominent and pleasantly clean, without undesirable odor.	Lighter clear flavor of grains, no corn odor, general smell.	A bad odor, without the fresh smell of grains.
Color (25)	The color is light yellow or yellowish and uniform.	The color is off-white, lack of uniformity.	The color is unevenly distributed.
State (25)	The state is homogeneous and there are no visible impurities and agglomerates.	The state is more viscous, lumpy, or thinner, with a slight delamination.	The state is too viscous or too thin, with naked-eye impurities and obvious stratification.

Selection of the optimal formulation of raw materials

Corn, rice, millet and skimmed milk powder were the main ingredients. Oats were added directly as pellets to increase chewiness. Sensory scores were used as evaluation criteria to determine the best formulation of the ingredients in single-factor and orthogonal experiments.

Table 2. Factor levels of orthogonal experiment

Level	Factors			
	A, %	B, %	C, %	D, %
1	50	5	10	4
2	55	10	15	5
3	60	15	20	6

Single-factor experimental design

Four factors were selected, including five levels each of corn addition (45, 50, 55, 60 and 65%), rice addition (5, 10, 15, 20 and 25%), millet addition (5, 10, 15, 20 and 25%) and skimmed milk powder addition (3, 4, 5, 6 and 7%). In each group of experiments, one factor was changed as a variable while the other factors remained stable. Each set of experiments was repeated three times, and the mean was taken as the result.

Orthogonal experimental design of CCMR sensory score

The orthogonal experiment parameters were determined based on the results of the single-factor design. With the addition of corn (A), rice (B), millet (C) and skimmed milk powder (D) as the main influencing factors, the L_9 (3^4) orthogonal test was carried out, and the sensory score was used as the evaluation index. The orthogonal test factors and level design are shown in Table 2.

Selection of stabilizer formula

According to the study on the stability of beverages by Krempel (Krempel et al., 2019) and Jiang Guangyang (Jiang et al., 2018), CMC, carrageenan and xanthan Gum, which are commonly used in beverages, were selected to make the beverage yellowish in color and excellent and stable in quality. Carrageenan was also selected because it has a certain tendency to reduce the occurrence of degumming. MFA can increase satiety, while calcium hydrogen phosphate and MCC have certain buffering and lubricating effects. The overall quality and stability of the CCMR was studied mainly by determining the amount of various single stabilizers added.

Single-factor experimental design of stabilizer formulation

Four factors were selected with five levels each, including CMC addition (0.2, 0.4, 0.6, 0.8, and 1%), carrageenan addition (0.2, 0.4, 0.6, 0.8, and 1%), xanthan gum addition (0.2, 0.4, 0.6, 0.8, and 1%) and MFA addition (1, 1.5, 2, 2.5 and 3%). In each group of experiments, one factor was changed as a variable while the other factors remained stable. Each group of experiments was performed three times, and the mean value was taken as the result.

RSM optimization of stabilizer formulations

Based on the results of single-factor experiments, a four-factor, three-level RSM Box-Behnken model was selected to optimize the process conditions of stabilizers for CCMR. In this optimization process, the additions of CMC (A), carrageenan (B), xanthan gum (C) and MFA (D) were selected as independent variables, and CSR (Y) was used as an evaluation index to design experimental reactions to obtain the optimal process parameters. A total of 29 sets of experiments were conducted with three replications at the center point to evaluate the pure error, and at the end of the experiments, the response variable CSR was fitted to a second-order model to determine the correlation of the response variable with the independent variable. The general form of the second-order polynomial equation is as follows:

$$Y = \beta_0 + \sum_{i=1}^{4}\beta_i X_i + \sum_{i=1}^{4}\beta_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{4}\beta_{ij} X_i X_j \quad (2)$$

where:

- Y is the predicted response
- X_i and X_j are the input variables affecting the response variable Y
- β_0 is a constant
- β_i is a linear coefficient
- β_{ii} is a quadratic coefficient
- β_{ij} ($i \neq j$) is the linear-linear interaction between X_i and X_j.

The test variables were transformed to a range between –1, 0 and 1 for the evaluation factors. The factor and level design of the response surface test is shown in Table 3.

Table 3. Factors and levels of RSM

Factor	Level		
	–1	0	1
A	0.6%	0.8%	1%
B	0.4%	0.6%	0.8%
C	0.2%	0.4%	0.6%
D	1.5%	2%	2.5%

MEASUREMENT OF EVALUATION INDICATORS

Determination of soluble solids content

The determination of the soluble solids content was carried out according to the national standard (QB/T 4221-2011-China). 10 g of CCMR was weighed in a weighing dish with a certain mass. It was steam-dried in a water bath, put into a constant-temperature drying oven and baked for 2 h at 101–105°C before being taken out and moved into a desiccator to cool for 30 min. Then the dried sample was weighed again.

$$X = \frac{M_2 - M_1}{M} \times 100 \quad (3)$$

where:

- X is the content of total solids in CCMR (g/100 g)
- M is the mass of CCMR (g)
- M_1 is the mass of sea sand and weighing dish (g)
- M_2 is the mass of the dried sample plus sea sand and weighing dish (g).

Determination of total dietary fiber content

The total dietary fiber content was determined according to the method of reference with appropriate modifications (Wang et al., 2023). 5 g of CCMR, was mixed well with a material-to-liquid ratio of 1:20, 0.5% (w/v) heat-stable α-amylase was added, enzyme digestion proceeded in hot water at 95°C for 1 hour, 0.5% glycosylase and 0.5% papain were added, and the sample was mixed well and hydrolyzed in hot water at 60°C for 2 hours, heated at 100°C for 15 min to inactivate the enzyme and centrifuged. The supernatant was removed, alcohol precipitated, and the CCMR was dried in the oven to a constant mass and weighed.

The precipitate was washed with anhydrous ethanol after centrifugation and then washed with water to neutral, and it was then dried in the oven to a constant mass and weighed. The total dietary fiber content is the sum of soluble dietary fiber and insoluble dietary fiber.

Determination of reducing sugars

The determination of reducing sugar content was measured by the 3.5-dinitrosalicylic acid method with appropriate modifications according to the reference (Lam et al., 2021), and glucose was used as the standard. 2 g of CCMR was weighed and 20 ml of deionized water were added. The CCMR was placed in a boiling water bath for 15 min, and then kept in a constant temperature water bath at 50°C for 20 min to leach the reducing sugar. It was placed in a 4,000 r/min centrifuge for 15 min, and the supernatant was removed as the reducing sugar solution to be measured. 1 mL of supernatant, 1 mL of distilled water and 1.5 mL of DNS reagent were added to a 25 mL volumetric flask, shaken well and heated in a boiling water bath for 5 min. They were then cooled to room temperature, the volume was fixed with distilled water to 25 mL, the absorbance was measured at 540 nm, and distilled water was used as a blank control.

ASLT accelerated stability experiment

The stability of CCMR was predicted by the ASLT accelerated stability experiment (Li et al., 2023), which was designed to assess the variation of CCMR at storage temperatures of 4°C, 27°C and 37°C, where CCMR was tested for sensory and colony counts at regular intervals. The CCMR was tested at 37°C every 3 days. Q_{10} indicates the sensitivity of temperature to the reaction. CCMR is classified as a canned food, for which values of Q_{10} range from 1.5 to 4. Q_{10} was temporarily set to 2, which indicates a 2-fold increase in reaction rate for every 10°C rise in temperature according to the ASLT formula:

$$f_2 = f_1 Q_{10}^{\Delta T/10} \quad (4)$$

where

- f_1 is the time interval between each measurement at the higher test temperature T_1
- f_2 is the time interval between each measurement at the lower test temperature T_2
- ΔT is the temperature difference between T_1 and T_2.

From the experimental conditions it was clear that f_1 (27°C) is about 6. Therefore, the CCMR with storage conditions of 27°C was tested every 6 days. The organoleptic indexes were observed from the color, aroma, taste and whether it was stratified. Sensory indicators should satisfy the following conditions: light creamy yellow liquid with corn and grain fragrance; uniform state; slightly sweet taste. The stability experiment was set to end when the sensory score went below 70 points. The detection of total colony number was carried out according to GB 4789.2-2022-China, the detection of *Escherichia coli* group number was carried out according to GB 4789.3-2016-China and the detection of the total amount of mold was carried out according to GB 4789.15-2016-China.

Data analysis

SPSS 20 statistical software was used for data analysis, and origin 2020 was used to draw tables. The significance level was $p < 0.05$. Three parallel experiments were conducted for each group, and the average value was selected as the final result.

RESULTS AND ANALYSIS

Single-factor experiment sensory scores

The results of the single-factor experiments showed that the amount of corn addition had a significant effect on the sensory scores of CCMR. The sensory score of CCMR increased significantly when the amount of corn addition was increased from 45% to 55%, and the sensory score reached its highest value of 83 when the amount of corn addition reached 55%. At this level, the CCMR had a natural, uniform color and the unique aroma of corn. The flavor was rich and pure with no off-flavor and fine texture. After that, the sensory score gradually decreased as more corn was added. Therefore, corn addition of 55% was selected as the optimal level (Fig. 1A).

The amount of rice addition had a certain influence on the taste and state of the CCMR, and the sensory score of the CCMR increased significantly when the amount of rice addition was increased from 5% to 25%. When the rice addition was set to 15%, the taste, color and state of the CCMR reached the best and the highest sensory score of 85, and the CCMR was light yellow, with uniform color, aroma of rice, suitable taste and no roughness. After that, the sensory score decreased gradually as more rice was added. Therefore, rice addition of 10% was selected as the optimal level (Fig. 1B).

The results of the single-factor test showed that the amount of millet addition had an effect on the taste of the CCMR. When the amount of millet addition was increased from 5% to 15%, the sensory score of the CCMR increased significantly, and when the millet addition was 15%, the structure of the CCMR was

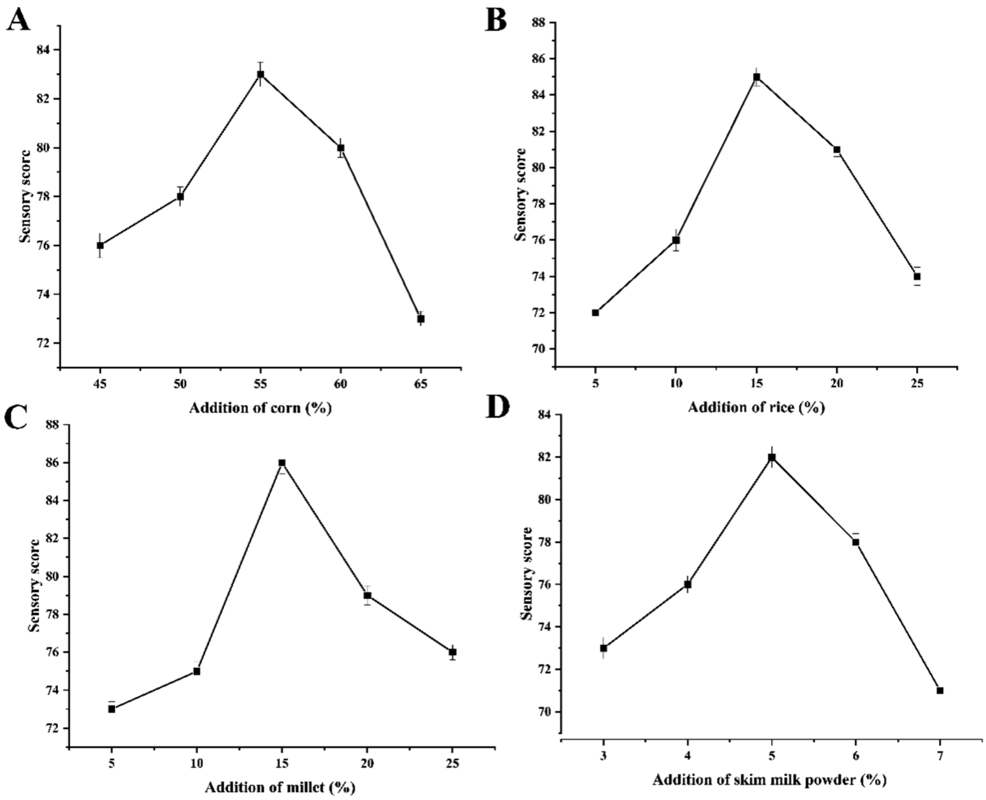

Fig. 1. Effect of single factor on sensory scores

uniform, the color was well-proportioned, and there was no off-flavor. At the same time, the roughness of corn was neutralized to achieve the best taste and texture, after which the sensory scores gradually decreased with further millet addition. Therefore, millet addition of 15% was selected as the optimal level (Fig. 1C).

The addition of skimmed milk powder gave the CCMR a milky flavor. When the addition of skimmed milk powder increased from 3% to 7%, the sensory score of the CCMR increased significantly, and when the addition of skimmed milk powder was 5%, the product was creamy yellow with a soft and refreshing taste, moderate milk flavor, harmonious flavor and the highest sensory score, 82. Thereafter, the sensory score gradually decreased with the further addition of skimmed milk powder. Therefore, 5% skimmed milk powder was selected as the optimal level (Fig. 1D).

Overall, the optimal formulation of the ingredients from the single-factor experiment was 55% for corn, 15% for rice, 15% for millet, and 5% for skimmed milk powder. The formulations were compounded three times and the resulting RSD value of the sensory score was 1.25%.

Orthogonal experimental results and analysis of raw materials

The orthogonal test results of raw materials are shown in Table 4. The closer the sensory score is to 100, the better the product tastes.

From the R-value, the addition of corn had the greatest effect on the sensory scores of the CCMR, followed by the addition of millet, the addition of skimmed milk powder, and the addition of rice. The order of influence of the factors was A > C > D > B. The best CCMR formulation obtained from the orthogonal experiment was $A_2B_2C_1D_2$, in which corn, rice, millet and skimmed milk powder were added at 55%, 10%, 10%, and 5%, respectively. Meanwhile, the highest sensory score was obtained for group 6 formulation $A_2B_3C_2D_2$ in the experiment. After validation, the

Table 4. Raw material orthogonal experiment results

Test	A	B	C	D	Sensory score
1	1	1	1	1	74
2	1	2	2	2	76
3	1	3	3	3	73
4	2	1	2	3	82
5	2	2	3	1	86
6	2	3	1	2	91
7	3	1	3	2	82
8	3	2	1	3	84
9	3	3	2	1	78
k_1	74.333	79.333	83.000	79.333	
k_2	86.333	82.000	78.667	83.000	
k_3	81.333	80.667	80.333	79.667	
R	12.000	2.667	4.333	3.667	

sensory score of formulation $A_2B_2C_1D_2$ was 95 with an RSD value of 2.08% and the sensory score of formulation $A_2B_3C_2D_2$ was 92 with an RSD value of 1.11%. In conclusion, the optimal formulation of the CCMR is $A_2B_2C_1D_2$. To increase the chewiness of the oats, 15% oats were added directly to the CCMR after the other ingredients had been mixed well. The optimal formulation of the CCMR had a light-yellow color, uniform organization, a fine taste and an obvious corn flavor.

Single-factor experiment results for the formulation of stabilizers

The results of the single-factor experiments showed that when the addition amount of CMC was increased from 0.2% to 0.8%, the texture of the CCMR was fine, the CSR was the smallest (0.442) and the stability was the best, after which the centrifugal sedimentation rate gradually increased with the further addition of CMC. Therefore, 0.8% of CMC was selected as the best addition amount of CMC (Fig. 2A)

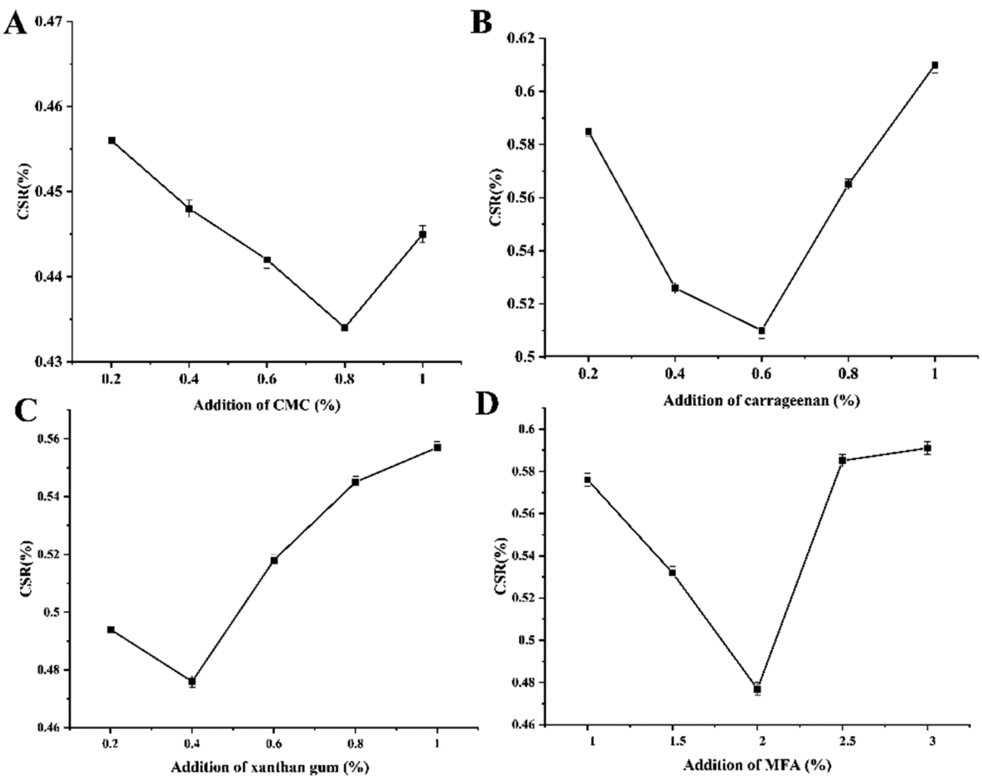

Fig. 2. Results of single factor experiment for stabilizer

When the addition amount of carrageenan was increased from 0.2% to 0.6%, the product had no additive taste, a uniform state, a minimal centrifugal sedimentation rate and the best stability. With further carrageenan addition, the brewability of CCMR changed and the tissue became viscous, causing the CSR to gradually increase. Therefore, 0.6% carrageenan was chosen as the best addition amount of carrageenan (Fig. 2B).

When the amount of xanthan gum addition was increased from 0.2% to 0.4%, the texture and grain flavor were the best and the centrifugal sedimentation rate was the lowest. After that, the fluidity of the CCMR decreased with further xanthan gum addition, and the overall viscosity and stability of the CCMR also decreased, resulting in a gradual increase of CSR. Therefore, 0.4% of xanthan gum was selected as the best addition amount of xanthan gum (Fig. 2C).

When the addition amount of MFA was increased from 1% to 2%, the CCMR was uniformly organized, without agglomeration, with the best stability and the lowest CSR. With the increase of MFA addition, CCMR developed agglomeration, sticky tissue and poor fluidity, which led to a gradual increase of CSR. Therefore, 2% MFA was selected as the best addition amount of MFA (Fig. 2D).

RSM experiment results for the formulation of stabilizers

According to the results of one-way experiments, addition of CMC (A), carrageenan (B), xanthan gum (C) and MFA (D) were used as independent variables, and CSR was used as the response value. A four-factor, three-level design was used to establish 29 sets of experiments. The results are shown in Table 5. The quadratic regression equation of CSR in CCMR was obtained by regression fitting the data in Table 5. The experimental data were fitted by quadratic multiple regression. The response Y (CSR) can be predicted by the following second-order polynomial equation (2):

$$Y = 0.36 + 0.014A - 0.018B - 0.045C + 0.017D + 0.053AB + 0.040AC - 0.025AD - 2.500E\text{-}003BC - 0.013BD - 0.038CD + 0.069A^2 + 0.094B^2 + 0.11C^2 + 0.068D^2$$

Table 5 shows the effect of four factors on the response values, CSR and interactions, where the contours are elliptical and the response surface curves are relatively steep, significantly affecting the CSR of the CCMR. An analysis of variance (ANOVA) was performed on the above regression model, and the high F-value (144.98) and low p-value (<0.0001) indicate that the model effectively predicted the data. The coefficient

Table 5. Results of response surface experiments

Text	A	B	C	D	CSR%
1	0	0	0	0	0.36
2	0	–1	–1	0	0.62
3	0	1	0	1	0.5
4	1	0	1	0	0.54
5	–1	0	0	1	0.51
6	–1	1	0	0	0.45
7	0	1	1	0	0.5
8	1	1	0	0	0.57
9	1	0	0	1	0.51
10	0	0	0	0	0.36
11	–1	0	–1	0	0.61
12	1	–1	0	0	0.5
13	1	0	0	–1	0.53
14	0	0	–1	–1	0.53
15	–1	–1	0	0	0.59
16	0	0	1	–1	0.51
17	0	0	0	0	0.36
18	1	0	–1	0	0.55
19	0	0	0	0	0.36
20	0	–1	1	0	0.54
21	–1	0	0	–1	0.43
22	0	0	1	1	0.48
23	0	0	–1	1	0.65
24	0	1	1	0	0.59
25	0	–1	0	1	0.56
26	0	0	0	0	0.36
27	0	1	0	–1	0.5
28	–1	0	1	0	0.44
29	0	–1	–1	–1	0.51

of determination (R^2) was 0.9931, indicating that the model has good reliability and reflects 99.31% of the variation in response values. The precision value (39.794 > 4) indicates that the model has good accuracy.

The linear coefficients (A, B, C, D, AB, AC, AD, CD, A^2, B^2, C^2, D^2) had a highly significant effect when $p < 0.05$, and BC and BD had no significant effect on each other. Therefore, the interaction between the factors was significant. The effects of stabilizer additions on the CSR of the CCMR were most pronounced for xanthan gum addition (C), followed by carrageenan addition (B), MFA addition (D) and finally CMC addition (A). The contour plots and three-dimensional response surfaces obtained from the experiment indicated the interaction between two independent factors and the optimal values of each experimental variable.

The contour plots and 3D response surfaces obtained from the experiment show the interaction between the two independent factors and the optimal values of each experimental variable. The interaction between the amount of CMC added and the amount of carrageenan added is shown through the 3D response surfaces and contour plots in Figure 3 (a). In the initial stage of the reaction, increasing the amount of CMC addition and carrageenan addition decreases the CSR of the CCMR. After that, it stops decreasing after reaching a certain level. Figures 3 (b)–(g) show the relationship between CMC addition and xanthan gum addition, CMC addition and MFA addition, carrageenan addition and xanthan gum addition, carrageenan addition and MFA addition, and xanthan gum addition and MFA addition, respectively.

The compound stabilizer formulation obtained with Design Expert 8.0.6.1 is as follows: 0.6% of CMC, 0.6% of carrageenan, 0.4% of xanthan gum, 2% of MFA, 1.5% of MCC. Three trials were carried out to assess the accuracy of the model. The actual CSR of the CCMR under optimal process conditions was 36.24% ± 0.02%, and the predicted yield was 35.81%. The experimental results were in good agreement with the predicted results, with an error of 0.43%.

Physical and chemical analysis

The CCMR produced by the optimal process has obvious cereal aroma, soft taste, uniform color, delicate state, no delamination, and good fluidity. The soluble solids content is an important indicator for evaluating the quality of food products (Montanuci et al., 2016), and can affect the taste and quality of beverages; it also correlates with beverage viscosity (Liguori et al., 2021). The soluble solids content of CCMR was 7.466 g/100 g > 6 g/100 g, and the dietary fiber content was 0.378 g/100 g > 0.1 g/100 g. The pH value of the product was 6.41, and the CCMR met the green food-cereal beverage requirements (NY-T 3901-2021 China) for a compound cereal beverage.

Reducing sugars are sugars with reducing properties that play an important role in the body's metabolism (Sustriawan et al., 2021). Excessive intake of reducing sugars can increase the burden on blood sugar and insulin, increasing the risk of diabetes and other metabolic diseases (Deliza et al., 2021). The glucose standard curve was $Y = 0.3895X + 0.056$, and the reducing sugar content was 2.6493 mg/g. Physical and chemical experiments showed that the CCMR had a nutritional value and palatability that ensured proper energy intake and had the potential to be reasonably healthy for weight loss.

Result of ASLT accelerated stability experiment

At storage temperatures of 4°C, 27°C and 37°C, the accelerated stability experiment ended when the sensory score of CCMR reached 70. The total number of bacteria, *Escherichia coli*, and the total amount of mold in the samples were tested according to GB 4789.2 (2022), GB 4789.3 (2016) and GB 4789.15 (2016). *E. coli* and mold were at concentrations below 100 CFU/ml, so the samples all met the requirements of the Chinese beverage microbiological limit standard GB 7101 (2015) before the accelerated stability experiment ended. The changes in sensory scores of the CCMR during the stability experiments are shown in Figure 4.

The sensory scores of the CCMR gradually decreased over time, and storage times at 27°C and 4°C are greater than storage times at 37°C ($p < 0.05$). As shown in Fig. 4, when the sensory score reaches 70, the end point of storage at 37°C is day 40, and the end point of storage at 27°C is day 66. According to the formula, the results from the stability experiments of 66d and 40d obtained at 27°C and 37°C respectively are substituted into the formula to find Q_{10}, that is:

$$Q_{10} = \frac{\text{Storage period at 27°C}}{\text{Storage period at 37°C}} = \frac{66}{40} = 1.65 \text{ or } \frac{66}{42} = 1.57$$

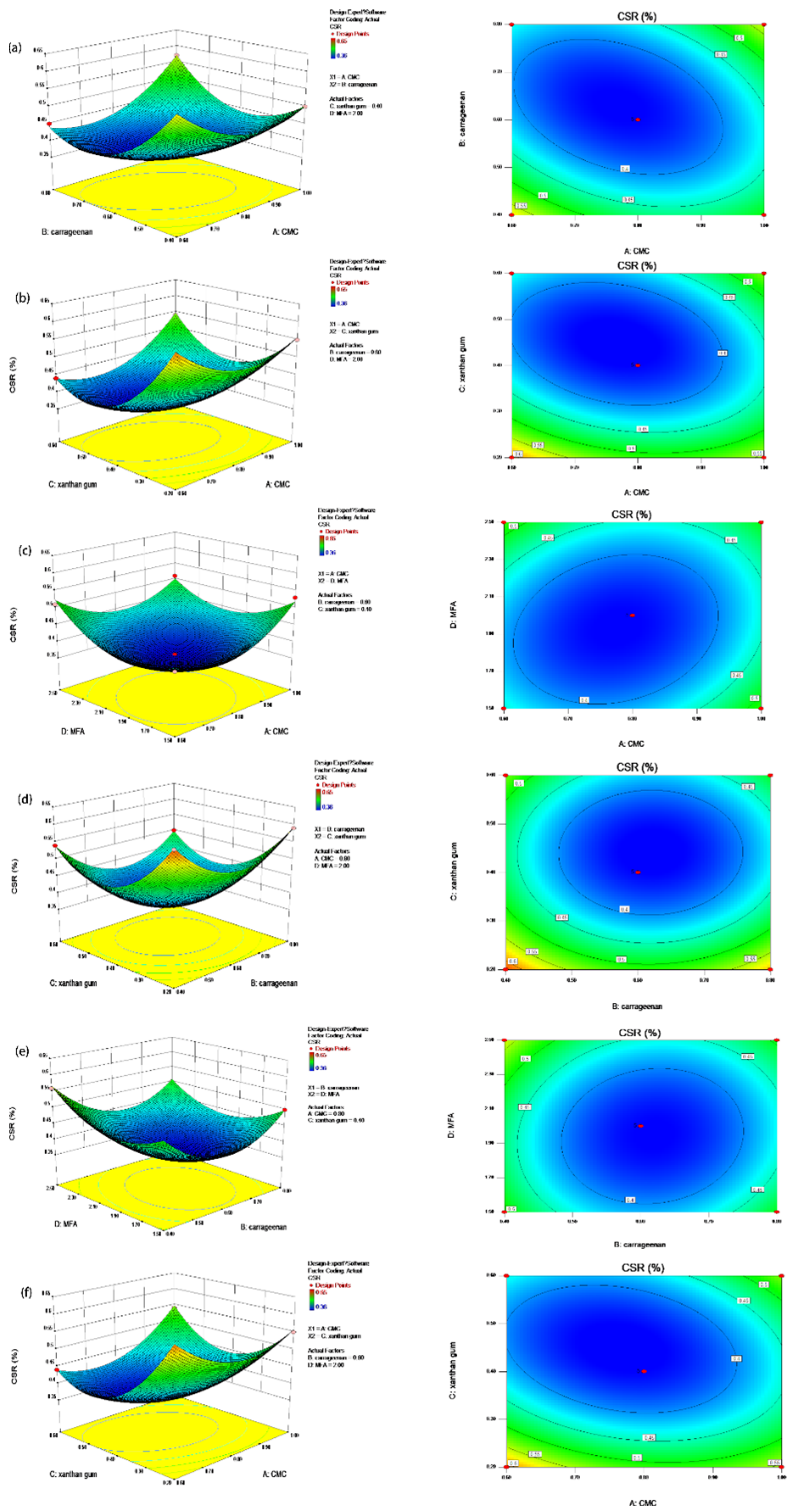

Fig. 3. 3D response surface plots of the effects of corn addition, rice addition, millet addition and skimmed milk powder addition on the CSR of the CCMR

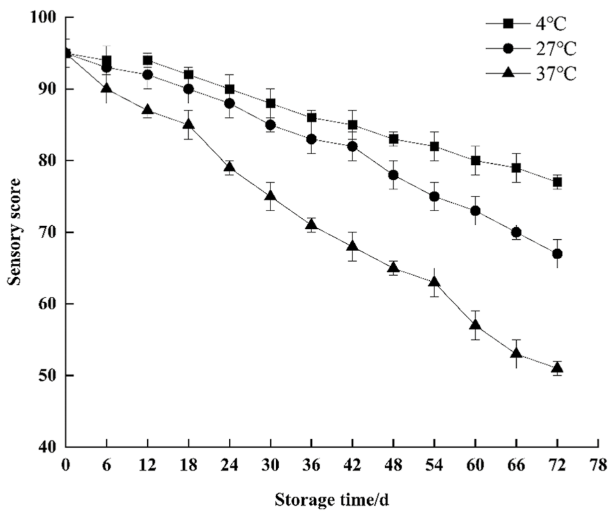

Fig. 4. Changes in sensory scores of the CCMR during storage

Therefore, the formula $Q_{10}^{\Delta T/10} = Qs_{(T1)}/Qs_{(T2)}$ can be calculated for the CCMR at a commercial storage temperature of 20°C, giving the result: 142–155 days. Because skimmed milk powder is rich in nutrients, it is susceptible to environmental influences that can lead to nutrient decay and thus affect the stability of CCMR. The storage period for related products of the same type containing dairy products on the market is between 21 days and 3 months at 20°C, with an extended storage period for CCMR. The reason for this may be that the addition of complex stabilizers improved the stratification of the CCMR without affecting its organoleptic score and increased its stability and shelf life. Properly added stabilizers not only enhance the stability of the product but also retain the active ingredients (Wani et al., 2021). The results indicate that the CCMR is more suitable for storage at room temperature and that high temperatures will break down its taste and shorten storage time.

CONCLUSION

In this study, the optimal formulations of CCMR raw materials were selected by single-factor and orthogonal experiments. They were: 55% corn, 10% rice, 10% millet, 5% skimmed milk powder and 15% oats. The stabilizer formulations of CCMR were optimized by RSM with CMC, carrageenan, xanthan gum and MFA as response variables and CSR as response values. The optimal formulation of CCMR was optimized by a regression model and was calculated to be 1.5% of MCC, 0.6% of CMC, 0.6% of carrageenan, 0.4% of xanthan gum, 2% of MFA and 0.2% of calcium hydrogen phosphate. The CCMR developed in this paper has a light-yellow color, uniform organization, good stability, unique aromatic cereal flavor, harmonious taste and rich nutrition. The sensory score was significantly affected by temperature in the stability experiment ($p < 0.05$). The CCMR would provide consumers with a cereal beverage with excellent taste and a corresponding stabilizer formulation and increased maize consumption. These results provide a theoretical basis for further research and development of cereal beverages and stability studies and demonstrate a method for the processing and utilization of cereals which could promote the future development of the cereal industry.

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