Carbohydrate Polymers 86 (2011) 566–573

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Extraction of water-soluble polysaccharides (WSPS) from Chinese truffle and its application in frozen yogurt

Yu-Zhi Miao, Qiang Lin, Yu Cao, Gui-Hua He, Dai-Rong Qiao, Yi Cao*

Microbiology and Metabolic Engineering Key Laboratory of Sichuan Province, College of Life Sciences, Sichuan University, Chengdu 610064, China

ARTICLE INFO

Article history:
Received 14 February 2011
Received in revised form 28 March 2011
Accepted 30 April 2011
Available online 10 May 2011

Keywords:
Chinese truffle
Water-soluble polysaccharides
Extraction
Application
Frozen yogurt

ABSTRACT

WSPS from truffles are very valuable source which can be used in the areas of foods as natural functional ingredient. In this work, WSPS were extracted from Chinese truffle by RSM combined with single-factor screening, and applied into milk to make a frozen yogurt. Optimum conditions were: temperature 98.96–100 °C, time 4.94–5.37 h, particle size 155.91–164.77 mesh, water to truffle ratio 46.66–49.3:1 and pH value at 7.0. These optimum conditions yielded WSPS of 12.89–13.03%, which is well matched with the predictive yield. WSPS prepared contained 86.37% carbohydrates, 3.15% proteins, 7.54% moisture and 2.88% ash. WSPS consisted of glucose, mannose, galactose, and lyxopyranose. Based on the experiments of the addition, the presence of WSPS reduced significantly the syneresis, improved the viscosity and titratable acidity and contributed better flavour in the FYP yogurt in comparison to the FYC yogurt, which provided important insight in the potential commercialization of WSPS as a functional component of yogurt.

1. Introduction

The food industry is experiencing a constantly growing demand for new versatile functional ingredients or biologically active components from natural sources because they are widely applied to make functional foods which are believed to protect against a wide range of diseases. This demand has drawn researchers' interest in extracting these ingredients from bio-materialsm, such as medicinal mushroom (Hou & Chen, 2008; Wang, Li, Li, & Han, 2010) and developing functional foods (Coisson, Travaglia, Piana, Capasso, & Arlorio, 2005).

Truffles are hypogeous medicinal mushroom, belonging to Pezizales, a large group of ectomycorrhizal fungi growing in symbiosis with roots of several vascular plant species. The known natural truffles have about 100 species, but only *Tuber* taxa is edible (Pomarico, Figliuolo, & Rana, 2007). Their geographic distribution mainly covers the temperate zones of the northern hemisphere, with at least three differentiation areas: Europe, South-East Asia and North America. In addition, semi-artificial plantations (Hall, Wang, & Amicucci, 2003; Olivier, 2000) and the submerged fermentation process (Liu, Li, Li, & Tang, 2008; Tang et al., 2008) of Chinese truffle were being developed for the production of truffles and truffle polysaccharides, respectively. Truffle polysaccharides isolated from the fruiting-body of truffles are water-soluble, and have immunomodulating, anticancer and antitumor activity (Hu, Li, Lin, Hang, & Guo, 1994). In the past several years, many medicinal mushroom polysaccharides have been widely studied for their extractions, chemical properties and biological activities (Dey et al., 2010; Pramanik, Mondal, Chakraborty, Rout, & Syed, 2005; Wong, Connie, Lai, & Cheung, 2011). However, little information is available about the water-soluble polysaccharides (WSPS) from truffles. Aqueous extractions are the most common methods used for the extraction of WSPS. Preliminary tests in our laboratory showed that extraction temperature, extraction time, water to truffle ratio and pH had significant influence on the yield of WSPS. Thus, it is important to optimize the extraction process in order to obtain high yields.

In extraction processes of WSPS, multiple independent factors might interact and influence on the yields. The classical method of studying single factor at a time fails to consider the combined effects of several factors involved. However, Response surface methodology (RSM) is an optimization method that can determine all the factors as well as the possible interactions among different independent variables, so that a set of experimental conditions can be optimized (Cui, Mazza, Oomah, & Biliaderis, 1994). RSM is widely applied in the food industry to determine the effects of several variables and optimize conditions (Levigne, Ralet, & Thibault, 2002; Tanyildizi, Ozer, & Elibol, 2005). In this research, as many factors can influence the extraction yield, RSM is applied to fit and exploit a mathematical model representing the relationship between the responses (extraction yield) and variables (i.e. temperature, time, particle size and ratio of water to truffle).

* Corresponding author. Tel.: +86 28 85412842; fax: +86 28 85412842.
E-mail address: geneium@scu.edu.cn (Y. Cao).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.04.071

Table 1
The range of independent variables and their corresponding levels.

Name	Coded symbol	Coded factor level				
		−2	−1	0	1	2
Extraction temperature (°C)	X_1	85	90	95	100	105
Extraction time (h)	X_2	3	4	5	6	7
Particle size (mesh)	X_3	120	140	160	180	200
Water to truffle ratio (mL/g)	X_4	30	35	40	45	50

Health has been popular selling concept in the dairy and juice categories. Among these, fermented milk products, like frozen yogurt by itself has been recognized as a healthy food, due to the positive healthy image resulting from the beneficial action of their viable bacteria (Sendra et al., 2008), which has gained wide-spread consumer acceptance all around the world and its consumption has increased significantly over the past few year. Functional yogurt involving positive bio-active ingredients or the presence of natural healthy bio-active molecules is being developing (Ramirez-Santiago et al., 2010) because it can satisfactorily demonstrate to affect beneficially one or more target functions in the body. Thus, frozen yogurt is good candidates to be incorporated with WSPS of truffles.

The objective of the present work is twofold: (1) to explore the extraction of WSPS from Chinese truffle and to optimize the conditions of the extraction by RSM. (2) To apply WSPS as functional ingredients in frozen yogurt, to analyze and compare the syneresis, titratable acidity, viscosity and sensory quality with and without WSPS. Results in this line should provide important insight in the potential commercialization of WSPS as a functional component of dairy products.

2. Materials and methods

2.1. Materials

The dried fruit bodies of Chinese truffle were kindly provided by Sichuan Hexie Foodstuff Co., Ltd, China (origin: Huidong county of Yunnan Province, China). Fresh raw cow's milk was obtained from the dairy farm (Sichuan Agricultural University, China), and skim milk powder was purchased from local market located in Ya'an. *Lactobacillus bulgaricus*, *Streptococcus thermophilus* were kept in our laboratory. All chemicals used in the experiment were of analytical grade, and additives were food grade.

2.2. Water-soluble polysaccharides (WSPS) extraction

Samples were ground in a high speed disintegrator (Model SF-2000, Chinese Traditional Medine Machine Works, Shanghai, China) to obtain a fine powder. Then the powder obtained was sieved (particle size: 40–200 mesh size screen). The other operations were carried out by the methods described in Wu, Cui, and Tang (2007). The extraction yields (Y) of polysaccharides were calculated as a percentage of the weight of the truffle dry powder, as follows:

$$Y \quad (\%, \quad \mathrm{w/w}) = \frac{\mathrm{We}}{\mathrm{Wp}} \times 100$$

where We is defined as weight of extracted polysaccharides whereas Wp is defined as weight of dried truffle used.

2.3. Optimization of WSPS extraction

2.3.1. Screening of important conditions

To determine which variables significantly affect WSPS yield, single-factor tests were used. Five variables were screened with their levels as follows: extraction temperature from 50 to 100 °C (within ±1 °C); extraction time ranging from 1 to 10 h; particle size of 40–200 mesh; water to truffle ratio of 20:1 to 60:1 (mL/g). When above levels of each extraction parameter was investigated, the pH was set at 4.0, 5.5, 7.0 and 8.5 (within ±0.1, adjusting the suspension pH by 0.1 mol/L NaOH or HCl).

2.3.2. Optimization of screened conditions

The optimization of extraction conditions for WSPS production from Chinese truffle were carried out in the following steps.

The extraction parameters were optimized by RSM. A central composite design (CCD) was employed in this regard. Extraction temperature (X_1), extraction time (X_2), particle size (X_3) and water to truffle ratio (X_4) were chosen for independent variables. The range and center point values of four independent variables presented in Table 1 were based on the results of single-factor screening.

The quadratic model for predicting the optimal point was expressed according to:

$$Y_k = \beta_{k_0} + \sum_{i=1}^{4} \beta_{k_i} x_i + \sum_{i=1}^{4} \beta_{k_{ii}} x_i^2 + \sum_{i<j=2}^{4} \beta_{k_{ji}} x_i x_j$$

where Y_k is the dependent variables (extraction yield), β_{k0} is the model constant, β_{ki}, β_{kii} and β_{kij} are the model coefficients. They represent the linear, quadratic and interaction effects of the variables respectively. Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (trial version 8.0, USA). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design.

2.4. Application of WSPS in frozen yogurt

2.4.1. Procedure for manufacturing functional yogurt

WSPS as a functional factor were applied for manufacturing functional yogurt. According to the report of Hu et al. (1994), health-promoting bio-active dose of WSPS to body is 25 mg/kg. Setting the average weight of normal adult body is 60 kg, and daily consumption to yogurt is 100 mL. Therefore, the amount of WSPS applied in yogurt is determined for 1.5%.

Three replicate trials were carried out in the manufacture of frozen yogurt using fresh raw cow's milk. Two variations were manufactured: one without WSPS used as control (FYC) and another containing 1.5 g of WSPS per 100 mL (FYP). After standardizing the milk with 1.5% WSPS (control 0%), 6% sugar and skim milk powder, the mixtures were separately homogenized using an Ultra Turrax blender (Shanghai Donghua, China) at 10,000 rpm until all ingredients were dissolved. Then, the homogenates were pasteurized at 85 ± 1 °C for 15 min, cooled to 43 ± 2 °C, and inoculated with the freeze-dried starter culture at a ratio of 3% (w/v), dispersed into a 150 mL cylindrica glass container, ca. 100 mL, and pre-fermentation process was carried out at 41 ± 1 °C for approximately 6 h. Samples were then cooled to 4 °C, and stored at 4 °C for post-fermentation during 20 days.

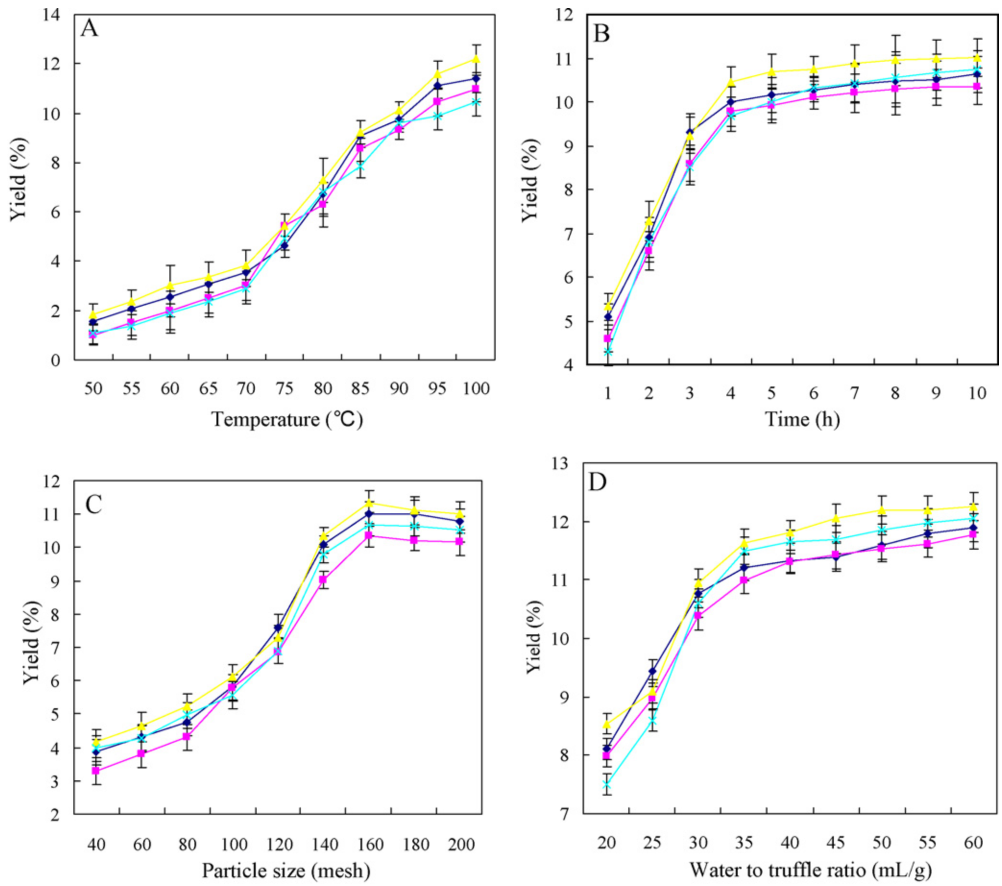

Fig. 1. Effect of different parameters on WSPS yield at different pH values of 4.0, 5.5, 7.0 and 8.5. (A) Extraction temperature, (B) extraction time, (C) particle size, (D) water to truffle ratio. Symbols for different pH values: (◆) 4, (■) 5.5, (▲) 7, and (✕) 8.5. The error bars in the figure indicated the standard deviations from three independent samples.

2.4.2. Titratable acidity and pH analysis

Samples (10 mL) were titrated using NaOH (0.1 N). The amount of NaOH used (expressed in milliliter) was multiplied by 10, and titratable acidity was thus obtained in Dornic degrees (°D) or in gram of lactic acid per litre. The pH value of samples was determined at room temperature with a pHS-3C$^+$-meter and combined glass electrode (Fangzhou Instruments Chengdu, China) standardized with pH 4.01, 6.86 and 9.18 standard buffer solutions.

2.4.3. Viscosity analysis

Viscosity of the samples was tested at +4 °C with a spindle (No. 4) rotation of 60 rpm using a Hengping Viscometer (model SNB-1, Hengping Instrument, Shanghai, China).

2.4.4. Syneresis analysis

The release of whey in the yogurt samples was measured by inverting a 30 g sample at 8 °C on a fine cheese cloth placed on top of a funnel. The quantity of whey collected in a graduated cylinder after 2 h of drainage was used as an index of syneresis.

2.4.5. Sensory evaluation

A triangle test to evaluate the differences of appearance, consistency, odour and flavour between FYP and FYC variations was carried out by the 11 expert panel members from the Key Laboratory of Microbiology and Metabolic Engineering of Sichuan province (5 males and 6 females). The other operation was carried out as described previously (Ramirez-Santiago et al., 2010).

3. Results and discussion

3.1. Definition of important conditions

Fig. 1A clearly demonstrated that the polysaccharides yield was obviously affected by the temperature in extraction process. The experimental results indicated that the polysaccharides yield constantly increased with an increase of the temperature within the range as investigated. The maximum polysaccharides yield was 12.19 ± 0.28% obtained at temperature 100 °C, pH 7.0, which was 3.2 folds of the lowest yield (3.85 ± 0.15%) at 70 °C, pH 7.0. it is reasonable to anticipate that increasing extraction temperature will increase the solubility of the extracted WSPS. Also, the diffusion coefficient will increase and thus improves the rate of diffusion (Masmoudi et al., 2008).

Fig. 1B was the pattern of extractions yield under various extraction time. It indicated that the polysaccharides yield increased quickly with an increase of extraction time at the begin stage of extraction time (from 1 to 4 h). Then, yield increased slowly with an increase of time until the end of extraction process. This might be due to the time requirement of the exposure of the polysaccharide to the release medium (distilled deionised water) where the liquid penetrated into the dried powdered pod, dissolved the polysaccharide and subsequently diffused out from the pod (Gan, Abdul Manaf, & Latiff, 2010). The highest polysaccharide yield of 11.02 ± 0.42% was obtained at 10 h, pH 7.0. While, the high-performance effect of extraction time on yield was observed before the extraction of time 5 h.

Table 2
CCD with the observed responses and predicted values for yield (%).

Run order	Coded variable levels				Yield (%)	
	Temperature, X_1 (temperature)	Time, X_2	Particle size, X_3	Water to truffle ratio, X_4	Observed[a]	Predicted
1	−1	−1	−1	−1	3.159	3.738
2	1	−1	−1	−1	8.374	8.266
3	−1	1	−1	−1	8.201	7.978
4	1	1	−1	−1	11.411	10.602
5	−1	−1	1	−1	9.79	8.466
6	1	−1	1	−1	11.106	11.354
7	−1	1	1	−1	9.517	10.226
8	1	1	1	−1	10.219	11.21
9	−1	−1	−1	1	7.054	6.594
10	1	−1	−1	1	11.934	10.942
11	−1	1	−1	1	10.717	10.186
12	1	1	−1	1	10.778	12.63
13	−1	−1	1	1	8.565	9.09
14	1	−1	1	1	11.046	11.798
15	−1	1	1	1	9.563	10.202
16	1	1	1	1	11.867	11.006
17	−2	0	0	0	7.581	7.749
18	2	0	0	0	13.494	13.081
19	0	−2	0	0	7.283	7.795
20	0	2	0	0	12.006	11.243
21	0	0	−2	0	7.055	7.523
22	0	0	2	0	11.339	10.627
23	0	0	0	−2	8.325	8.237
24	0	0	0	2	11.048	10.889
25	0	0	0	0	11.846	11.827
26	0	0	0	0	11.742	11.827
27	0	0	0	0	11.856	11.827
28	0	0	0	0	11.566	11.827
29	0	0	0	0	12.003	11.827
30	0	0	0	0	11.95	11.827

[a] Mean of triplicate determination.

Fig. 1C the variation pattern of yield was quite different under particle size. The positive effect of the yield was observed when particle size was in the range of 100–160 mesh. Then, yield decreased slowly with a diminish of particle size. The reason for this was that a smaller amount of the polysaccharides could be transported easily from the interior of smaller truffle particles to the bulk of liquid extract than from the larger ones, due to a smaller resistance and short path to the polysaccharides diffusion. However, when the particle size continually reduced, mass transfer resistance started to become large. The greater mass transfer resistance between solid-phase and liquid-phase may become a large bottle-neck to raise extraction yield of the polysaccharides (Hou & Chen, 2008). The maximum yield was 10.99 ± 0.38% obtained at particle size of 160 mesh, pH 7.0.

Fig. 1D was the pattern of polysaccharides yield under various water to truffle ratio. It indicated that the polysaccharides yield increased quickly with an increase of water to truffle ratio in the range of 20:1 to 40:1. Ever since then, yield increased slightly with an increase of water to truffle ratio. This might be due to the increase of the driving force for the mass transfer of the polysaccharides with the increase of water to truffle ratio. However, the driving force between solid-phase and liquid-phase no longer changed when the ratio continued to increase, so that the extraction yields no longer changed (Bendahou, Dufresne, Kaddami, & Habibi, 2007). The highest polysaccharide yield of 12.26 ± 0.24% was obtained at water to truffle ratio of 60:1, pH 7.0.

In addition, Fig. 1A–D also indicated that different pH conditions gave different yield of the extracts, and the maximum yield

Table 3
Test of significance for regression coefficient.

Source	Sum of squares	df	Mean square	F-value	p-value, Prob > F
Model	127.234	14	9.088	10.365	<0.0001[**]
X_1	42.653	1	42.653	48.647	<0.0001[**]
X_2	17.838	1	17.838	20.345	0.0004[**]
X_3	14.435	1	14.435	16.463	0.0010[**]
X_4	9.618	1	9.618	10.969	0.0047[**]
X_1X_2	3.624	1	3.624	4.134	0.0601
X_1X_3	2.692	1	2.692	3.070	0.1001
X_1X_4	0.0321	1	0.032	0.037	0.8508
X_2X_3	6.159	1	6.159	7.025	0.0182[*]
X_2X_4	0.420	1	0.420	0.479	0.4993
X_3X_4	4.983	1	4.983	5.683	0.0308[*]
X_1^2	3.423	1	3.423	3.904	0.0669
X_2^2	9.116	1	9.116	10.397	0.0057[**]
X_3^2	12.997	1	12.998	14.824	0.0016[**]
X_4^2	8.787	1	8.787	10.022	0.0064[**]

R^2 0.903; Adj R^2 0.818; Pred R^2 0.464; Adeq precision 14.065.
[*] Significant at <0.05 level.
[**] Very significant at <0.01 level.

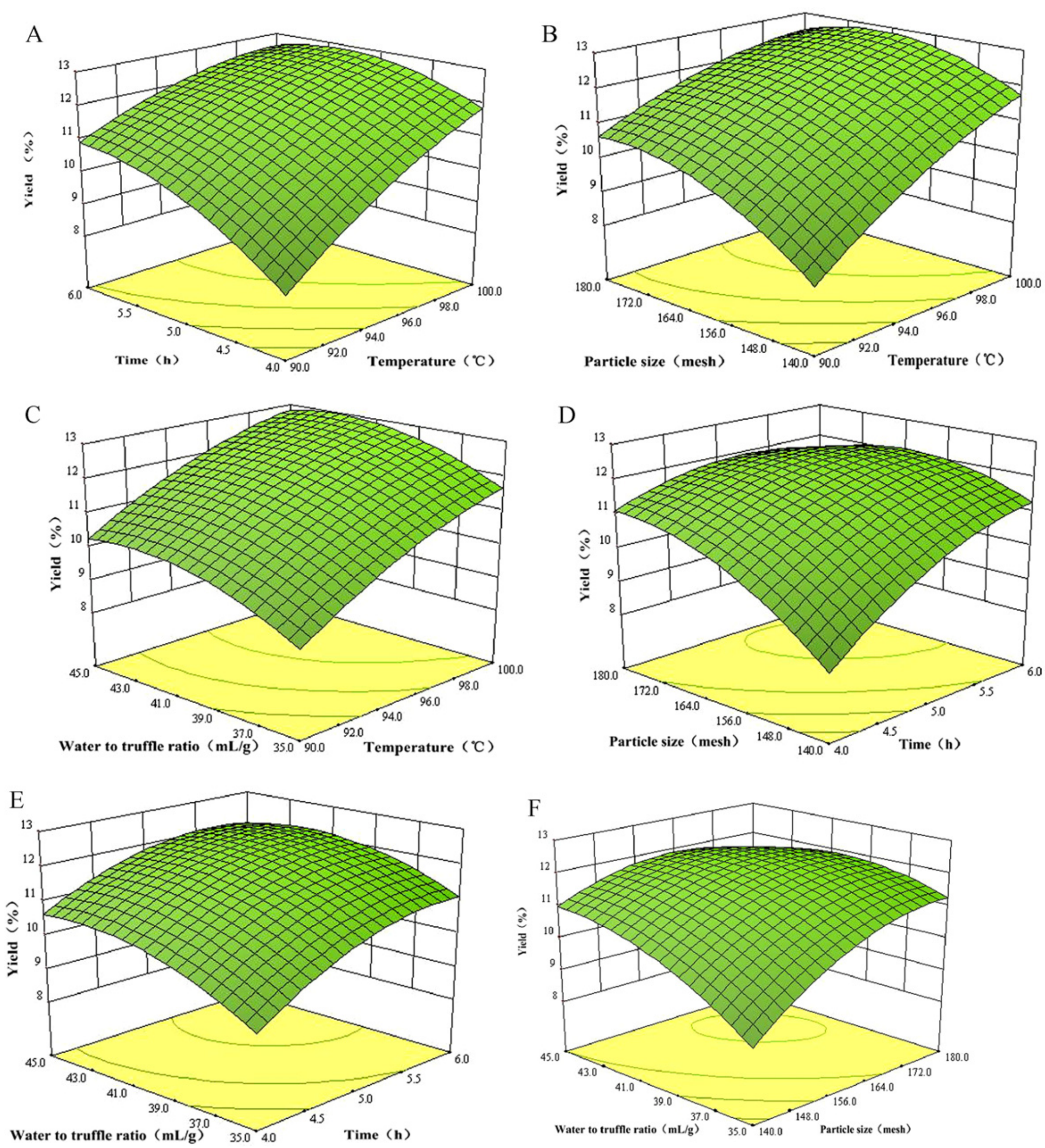

Fig. 2. (A–F) Three-dimensional plot showing the effect of the four process variables on extraction yield. (A) $Y = f(X1, X2)$, (B) $Y = f(X1, X3)$, (C) $Y = f(X1, X4)$, (D) $Y = f(X2, X3)$, (E) $Y = f(X2, X4)$, (F) $Y = f(X3, X4)$.

condition was pH value at 7.0. This was explained that higher pH condition would induce cell wall disruption, protopectin hydrolysis and solubilization which disrupt the ester linkages and hydrogen bonds between polysaccharide and cell wall (Renard, Crépeau, & Thibault, 1995).

3.2. Optimization of the screened variables

Based on the single-factor screening, pH value was held constantly at 7.0, and the temperature (X_1), time (X_2), particle size (X_3) and water to truffle ratio (X_4) were determined as four process variables. The range and center point values of four process variables were determined (Table 1). The results of 30 runs using CCD design were presented in Table 2 that include the design, mean observed responses and the predicted values.

The regression equations obtained after the analysis of variance (ANOVA) provided the levels of WSPS yield as a function of the values of four process variables. The extraction of WSPS could be predicted by the model:

$$\begin{aligned} Y = {} & 11.827 + 1.333X_1 + 0.862X_2 + 0.776X_3 + 0.663X_4 \\ & - 0.476X_1X_2 - 0.410X_1X_3 - 0.045X_1X_4 - 0.62X_2X_3 \\ & - 0.162X_2X_4 - 0.558X_3X_4 - 0.353X_1^2 - 0.577X_2^2 \\ & - 0.688X_3^2 - 0.566X_4^2 \end{aligned}$$

The effect of treatment variables as the linear, quadratic and interaction terms were tested for adequacy and fitness by analysis of variance (ANOVA) (Table 3). The coefficient of determination (R^2) was calculated to be 0.903 for polysaccharide production. This implied that 90.3% of experimental data of the polysaccharide production was compatible with the data predicted by the model. However, Pred R^2 of 0.464 was not as close to the Adj R^2 of 0.818, probably indicating a large block effect. The 'adequate precision' measures signal to noise ratio and a value >4 was desirable. The 'adequate precision' value of 14.065 for polysaccharide production indicated that the model can be used to navigate the design space.

The significance of each coefficient was determined using the *F*-test and *p*-value in Table 3. It can be seen that the variable with very significant ($p < 0.01$) influence on extractions yield was linear term of temperature (X_1), time (X_2), particle size (X_3) and water to truffle ratio (X_4), quadratic term of particle size (X_3^2), time (X_2^2), water to truffle ratio (X_4^2). Interaction terms of X_2X_3, X_3X_4 had significant ($p < 0.05$) effect on extractions yield. However, the other terms were found insignificant ($p > 0.05$). The Model *F*-value of 10.365 ($p < 0.01$) implied the model was very significant.

The response surface curves were plotted to understand the interaction of the variables and to determine the optimum level of each variable for maximum response. In the plots two continuous variables were developed for extraction yield responses, while the other two variables were held constant at their respective zero level (Fig. 2A–F). It can be seen that the extraction temperature and time demonstrated an obvious increase in the response. While, particle size and water to truffle ratio increase at begin stage, then slightly decrease at a range of the experiment. The independent variables and maximum predicted values from the figures correspond with the optimum values of the responses obtained by the model. The optimal ranges of the four variables were extraction temperature of 98.96–100 °C, extraction time of 4.94–5.37 h, particle size of 155.91–164.77 mesh and water to solid ratio of 46.66–49.3:1, at pH value 7.0. These optimum conditions yielded polysaccharides of 12.89–13.03%.

3.3. Verification of results

To take into consideration of the feasibility of the experiment, the suitability of the model equations for predicting optimum response values was tested by using the optimal conditions: extraction temperature 100 °C, time 5.3 h, particle size 160 mesh, water to solid ratio 47.6:1 and pH value 7.0. Under this set of combination, the predicted and experimental values of yield were 13.013% and 12.95 ± 0.12%, respectively. Only small deviations were found between the actual values and predicted values. Thus, the model can be used to optimize the process of WSPS extraction from Chinese truffle. WSPS extracted under the optimum conditions were further analyzed for chemical and monosaccharide compositions, respectively. WSPS contained 86.37 ± 1.81% carbohydrates, 3.15 ± 0.73% proteins, 7.54 ± 0.81% moisture and 2.88 ± 0.31% ash. WSPS consisted of glucose, mannose, galactose, and lyxopyranose.

3.4. Comparative analysis of FYC and FYP yogurt

3.4.1. Changes in viscosity

Viscosity values at stage of the different fermentation were reported in Fig. 3. Significant differences were noted between the samples FYC and FYP. At first, the sample containing WSPS composite had higher viscosity than control at 0 h, which indicated WSPS can contribute to high viscosity. It can be used as filler in partial stead of viscosity gum. Then, viscosity values increased with fermentation time, and the yogurts with WSPS composite had significantly higher viscosities compared to the control throughout fermentation. The highest levels of viscosity were obtained at 20 days. For example, the values of FYC and FYP were 13,600 and 16,500 cp, respectively. Viscosity values were steady for two treatments during 15–20 days. The results showed that the addition of WSPS composite changed the fermentation efficacy of lactic acid bacteria, significantly added to the viscosity, and eventually would help to develop a better mouthfeel.

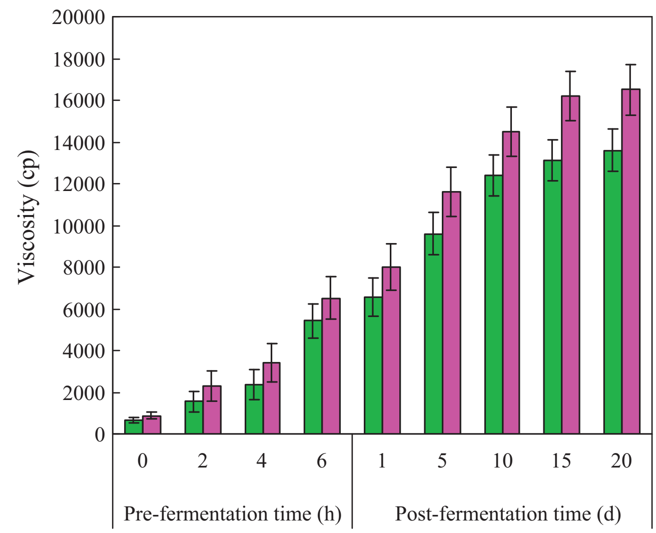

Fig. 3. Changes in viscosity (cp) values of yogurts made with or without WSPS composite during fermentation. Symbols for FYC yogurt ( ), FYP yogurt ( ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.4.2. Changes in titratable acidity and pH

These studies were conducted to see if WSPS addition to milk affected the development of titratable acidity and pH during fermentation. As seen in Fig. 4, similar changes of pH values and titratable acidity development were observed during two samples fermentation. This process was almost completed in the 0–6 h time frame during pre-fermentation, only slight changes were found in stage of post-fermentation. Furthermore, pH and acidity development were steady for two treatments from 15 to 20 days. However, addition of WSPS had obvious effect on the ability of pH values and acidity development. Significant differences were noted between FYC and FYP yogurt samples at any fermentation stages. This result indicated that the addition of WSPS component supported lactic acid production by *S. thermophilus* and *L. bulgaricus* at this stage.

3.4.3. Changes in syneresis

Whey separation is an important defect in frozen yogurt and can be defined as the appearance of whey on the gel surface of yogurts. Syneresis is the shrinkage of the gel, which then leads to whey separation (Lucey, 2004). Fig. 5 shows the changes in syneresis during 20 days of post-fermentation. The amount of syneresis was significantly higher at 1–10 days of storage compared with that at 15–20

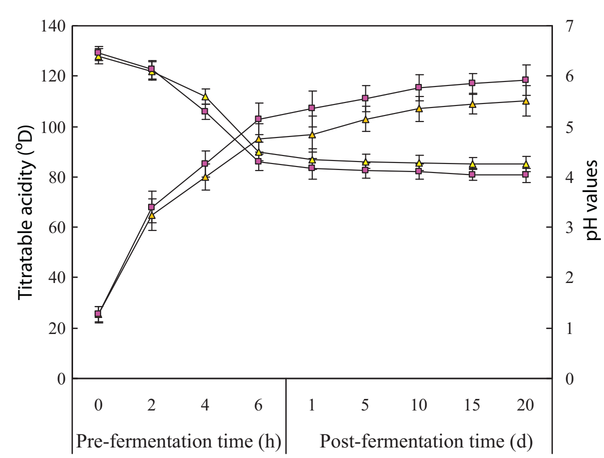

Fig. 4. Acidity and pH values evolution of the yogurt during fermentation. ( ) FYC yogurt, ( ) FYP yogurt.

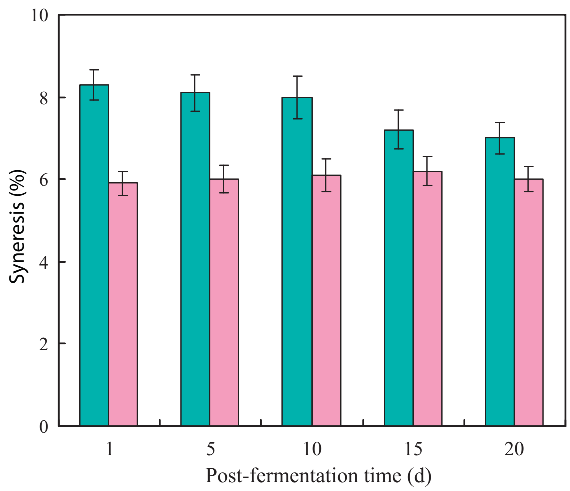

Fig. 5. Changes in whey separation values of yogurts made with or without WSPS composite during post-fermentation. ( ) FYC yogurt, ( ) FYP yogurt. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

days in the FYC yogurt, and syneresis decreased significantly in all time during storage, agreeing with the observations by Tamime and Robinson (1999), Guzel-Seydim, Sezgin, and Seydim (2005) and Isleten and Karagul-Yuceer (2006). However, syneresis was not different in the FYP. WSPS composite-added yogurts had obvious lower syneresis values than Control sample, and a slight decrease of the amount of whey separated was observed at all storage periods. This result showed that the addition of WSPS composite caused a decrease in syneresis values of FYP yogurt. This decreased syneresis probably can be ascribed to the improved water holding capacity of WSPS added to the milk to make the healthy yogurts.

3.4.4. Changes in sensory properties

The triangle tests applied to 20 days old frozen yogurt variations showed that overall expert panel members were able to distinguish between FYC and FYP yogurt. Nine members indicated that FYP yogurt had as distinctive attribute a higher acidity and more likeable palatability (mouthfeel) than FYC yogurt. The acidity perception agrees with the experimental acidity levels and apparent viscosity variations displayed by the yogurts after 20 days of storage. Hashim, Khalil, and Afifi (2009) reported that sensory ratings and acceptability of yogurt decreased significantly when yogurt incorporated with 4.5 g of date dietary fiber per 100 g of yogurt. In this study, addition only 1.5 g of WSPS composite per 100 g to frozen yogurt not only satisfied the demand as functional ingredient, but also improved the mouthfeel of frozen yogurt.

4. Conclusions

The extraction conditions have very significant effects on the yield of WSPS. Based on the RSM analysis, the optimal conditions were: temperature 98.96–100 °C, time 4.94–5.37 h, particle size 155.91–164.77 mesh and water to solid ratio 46.66–49.3:1, pH at 7.0. These optimum conditions yielded polysaccharide of 12.89–13.03%. Through the analysis of chemical and monosaccharide compositions, WSPS contained small amount of non-carbohydrate materials. It is necessary to purify further in order to elucidate the structure and function of WSPS from truffle.

The frozen yogurt with 1.5% of WSPS displayed lower syneresis percent, higher viscosity, higher acidity (lower pH values) and better mouthfeel than the control frozen yogurt without added WSPS, providing important insight in the potential commercialization of WSPS as a functional component of yogurt.

Acknowledgements

The financial supports from National Special Basic Research Projects of China (SB2007FY400-4), Technology Pillar Program during the Eleventh Five-Year Plan Period (2008GZ0020, 2008GZ0021) are gratefully acknowledged.

References

Bendahou, A., Dufresne, A., Kaddami, H., & Habibi, Y. (2007). Isolation and structural characterization of hemicelluloses from palm of Phoenix dactylifera L. *Carbohydrate Polymers*, *68*, 601–608.

Coisson, J. D., Travaglia, F., Piana, G., Capasso, M., & Arlorio, M. (2005). Euterpe oleracea juice as a functional pigment for yogurt. *Food Research International*, *38*, 893–897.

Cui, W., Mazza, G., Oomah, B. D., & Biliaderis, C. G. (1994). Optimization of an aqueous extraction process for flaxseed gum by response surface methodology. *Food Science and Technology, Lebensmittel-wissenschaft and Technologie*, *27*, 363–369.

Dey, B., Bhunia, S. K., Maity, K. K., Patra, S., Mandal, S., Maiti, S., et al. (2010). Chemical analysis of an immunoenhancing water-soluble polysaccharide of an edible mushroom, *Pleurotus florida* blue variant. *Carbohydrate Research*, *345*, 2736–2741.

Gan, C. Y., Abdul Manaf, N. H., & Latiff, A. A. (2010). Optimization of alcohol insoluble polysaccharides (AIPS) extraction from the *Parkia speciosa* pod using response surface methodology (RSM). *Carbohydrate Polymers*, *79*, 825–831.

Guzel-Seydim, Z. B., Sezgin, E., & Seydim, A. C. (2005). Influences of exopolysaccharide producing cultures on the quality of plain set type yogurt. *Food Control*, *16*, 205–209.

Hashim, I. B., Khalil, A. H., & Afifi, H. S. (2009). Quality characteristics and consumer acceptance of yogurt fortified with date fiber. *Journal of Dairy Science*, *92*, 5403–5407.

Hall, I. R., Wang, Y., & Amicucci, A. (2003). Cultivation of edible ectomycorrhizal mushrooms. *Trends in Biotechnology*, *21*, 433–438.

Hou, X. J., & Chen, W. (2008). Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology. *Carbohydrate Polymers*, *72*, 67–74.

Hu, H. J., Li, P. Z., Lin, T., Hang, B. Q., & Guo, Y. W. (1994). Effects of polysaccharide on Tuber sinica on tumor and immune system of mice. *Journal of China Pharmaceutical University*, *l25*, 289–292.

Isleten, M., & Karagul-Yuceer, Y. (2006). Effects of dried dairy ingredients on physical and sensory properties of nonfat yogurt. *Journal of Dairy Science*, *89*, 2865–2872.

Liu, R. S., Li, D. S., Li, H. M., & Tang, Y. J. (2008). Response surface modeling the significance of nitrogen source on the cell growth and Tuber polysaccharides production by submerged cultivation of Chinese truffle *Tuber sinense*. *Process Biochemistry*, *43*, 868–876.

Levigne, S., Ralet, M. C., & Thibault, J. F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydrate Polymers*, *49*, 145–153.

Lucey, J. A. (2004). Cultured dairy products: An overview of their gelation and texture properties. *International Journal of Dairy Technology*, *57*, 77–84.

Masmoudi, M., Besbes, S., Chaabouni, M., Robert, C., Paquot, M., Blecker, C., et al. (2008). Optimization of pectin extraction from lemon by-product with acidified date juice using response surface methodology. *Carbohydrate Polymers*, *74*, 185–192.

Olivier, J. M. (2000). Progress in the cultivation of truffles. In *Science and Cultivation of Edible Fungi. Proceedings of the 15th International Congress on the Science and Cultivation of Edible Fungi* Maastricht, The Netherlands, (pp. 937–942).

Pomarico, M., Figliuolo, G., & Rana, G. L. (2007). Tuber spp. biodiversity in one of the southernmost European distribution areas. *Biodiversity and Conservation*, *16*, 3447–3461.

Pramanik, M., Mondal, S., Chakraborty, I., Rout, D., & Syed, S. I. (2005). Structural investigation of a polysaccharide (Fr. II) isolated from the aqueous extract of an edible mushroom, *Pleurotus sajor-caju*. *Carbohydrate Research*, *340*, 629–636.

Ramirez-Santiago, C., Ramos-Solis, L., Lobato-Calleros, C., Peña-Valdivia, C., Vernon-Carter, E. J., & Alvarez-Ramírez, J. (2010). Enrichment of stirred yogurt with soluble dietary fiber from Pachyrhizus erosus L. Urban: Effect on syneresis, microstructure and rheological properties. *Journal of Food Engineering*, *101*, 229–235.

Renard, C. M. G. C, Crépeau, M. J., & Thibault, J. F. (1995). Structure of repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins. *Carbohydrate Research*, *275*, 155–165.

Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barberá, E., & érez-Alvarez, J. A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiology*, *25*, 13–21.

Tang, Y. J., Zhu, Li. L., Liu, R. S., Li, H. M., Li, D. S., & Mi, Z. Y. (2008). Quantitative response of cell growth and Tuber polysaccharides biosynthesis by medicinal mushroom Chinese truffle Tuber sinense to metal ion in culture medium. *Bioresource Technology*, *99*, 7606–7615.

Tanyildizi, M. S., Ozer, D., & Elibol, M. (2005). Optimization of a amylase production by Bacillus sp. using response surface methodology. *Process Biochemistry*, *40*, 2291–2296.

Tamime, A. Y., & Robinson, R. K. (1999). *Yoghurt: Science and technology*. Woodhead Publishing: Cambridge.

Wang, Q., Li, B. B., Li, H., & Han, J. R. (2010). Yield, dry matter and polysaccharides content of the mushroom Agaricus blazei produced on asparagus straw substrate. *Scientia Horticulturae*, *125*, 16–18.

Wong, K. H., Connie, K. M., Lai, P., & Cheung, C. K. (2011). Immunomodulatory activities of mushroom sclerotial polysaccharides. *Food Hydrocolloid*, *25*, 150–158.

Wu, Y., Cui, S. W., & Tang, J. (2007). Optimization of extraction process of crude polysaccharides from boat-fruited sterculia seeds by response surface methodology. *Food Chemistry*, *105*, 599–1605.