The effect of process parameters and microstructural changes on a new convenience food – quick-frozen paste-coated mushrooms (*Agaricus bisporus*)

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Abstract The technology of quick-freezing paste-coated mushrooms (*Agaricus bisporus*) was studied and optimized. The best microwave pretreatment condition for 1 cm slices, regarding color protection, was 5.4 W/g, for 55, 55–60 and 60 s for mushrooms with 3, 4 and 5 cm diameter caps respectively. For a batch of paste (668.2–1034.6 g), the process parameters considered were oil content (46.6–63.4 g), water content (381–562.6 g) and flour content (166–334 g) with a constant additional content of 30 g starch, 9 g baking powder, 2.6 g carrageenan, 30 g salt and 3 g pepper. These parameters were investigated using response surface methodology (RSM) with a central composite design. The optimal levels of the major paste components were 300 g flour, 432.5 g water and 50 g oil. The freezing time and sensory acceptability for paste-coated *Agaricus bisporus* (PCAB) under the optimized conditions were 7.49 min and 6.2 respectively. The freezing curves of PCAB were established at different temperatures and the freezing rates were calculated to find the freezing characteristics. In addition, the cell structure of PCAB, frozen at -75°C , the lowest freezing temperature, and studied using transmission electron microscopy, was similar in quality to that of fresh *Agaricus bisporus*. The results suggested that *Agaricus bisporus* can be quick-frozen with a paste coating to produce an acceptable and nutritious convenience food.

Keywords Paste coating · *Agaricus bisporus* · Microwave · Response surface methodology (RSM) · Quick-freezing · Microstructure

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Introduction

Agaricus bisporus, known as the common mushroom or table mushroom and belonging to the *Agaricaceae* family, is the most widely cultivated species of edible mushroom, comprising about 32 % of world production (Tsai et al. 2007; Wani et al. 2009). This kind of mushroom is rich in nutrients with a white color and crisp texture. It is a good source of high quality proteins, amino acids, carbohydrates, vitamins and minerals, such as iron, calcium, phosphorus, potassium, selenium and dietary fiber (Beelman and Edwards 1989; Matilla et al. 2002; Vaidya et al. 2008). As a macrofungus, *Agaricus bisporus* is perishable when stored at room temperature because of its high physiological activity (Lee 1999; Taghizadeh et al. 2010). It has a short shelf life of 3–5 days at 2°C and around 1–2 days under ambient conditions ($25\pm 2^{\circ}\text{C}$, RH 70 %). The short shelf life of white button mushrooms is an impediment to the distribution and marketing of the fresh produce (Brennan and Gormley 1998; Wani et al. 2009). Currently, processing methods for edible mushrooms include quick freezing, canning, salting and drying. During canning or salting, the color of *Agaricus bisporus* products darkens and the flavor changes because of enzymatic reactions and pigment replacement (Brennan et al. 2000; Buckow et al. 2009; Ismaya et al. 2011; Son et al. 2001; Urbany and Horti 1999). Quick freezing is a suitable method for preserving *Agaricus bisporus* as the mushrooms retain their color, flavor and nutrients (Santerre et al. 1988).

Coating raw materials with a paste or batter, then frying them, gives the products a crisp texture. The combination of flour, starch, baking powder and oil directly affect the quality of the paste used for coating. Zhou et al. (2010) have studied the blends of ingredients for coating pastes, but few papers have been found that investigate the technology of quick freezing paste-coated food products.

In the present study, we aim to optimize a method of deactivating enzymes using microwaves, to study the quick

freezing technology for *Agaricus bisporus* and to improve the quality of the coating paste. This study will also study the freezing rate of the pileus (mushroom cap) of different sizes and thicknesses under various quick freezing conditions to provide useful data for large-scale industrial production. Transmission electron microscopy is a valuable tool to provide data on the microstructure of mushrooms, which is necessary for a thorough understanding of the changes that occur during food processing (Nath and Chattopadhyay 2008). This can offer quality assurance for food manufacturers and ensure that PCAB is a convenient food and efficient for consumers to enjoy by frying or microwave processing without thawing.

Materials and methods

Material preparation

Fresh raw samples of *Agaricus bisporus* were purchased at a market (Beijing, China). The mushrooms were selected by visual inspection according to their homogeneity in size, shape and ripeness. Ripeness was evaluated by inspection of the hyphae with open cap or veil samples being rejected. The *Agaricus bisporus* samples selected had caps of 3–5 cm diameter with a fine color and free of pests, disease and mechanical damage. The initial moisture content was 90.23 ± 0.5 kg H₂O kg⁻¹. The samples were washed three times in flowing water then cut into 1 cm thick slices. For color protection, the samples were immersed in 0.06 % sodium pyrosulfite solution (w/w), then rinsed several times. The wheat flour, corn starch, soybean salad oil, iodized salt and baking powder for the coating paste were procured from the same local market.

The reagents used, 95 % ethanol, sodium pyrosulfite, catechol, guaiacol, Na₂HPO₄, NaH₂PO₄ and hydrogen peroxide, were all analytical grade, and gelatin food grade (Yongda reagents Co., Tianjin, China).

Treatment for enzyme inactivation

Forty groups (the slices of each group were 200 g) of *Agaricus bisporus*, after treatment for color protection, were heated by microwave (Sanle Co. Ltd, Nanjing, China) at 9.0, 7.2, 5.4, 3.6 and 1.8 W/g for 40, 45, 50, 55, 60, 65, 70 and 75 s. Then the *Agaricus bisporus* samples were quickly cooled in water to below 5 °C (Jaworska et al. 2008; Main et al. 1986). Polyphenoloxidase (PPO) and peroxidase (POD) were extracted from the cooled *Agaricus bisporus* samples after being ground then activity curves were plotted for each microwave condition. The methods for extracting enzyme solutions and measuring their PPO and POD activity were the pyrocatechol and guaiacol colorimetric

methods, respectively (Long and Alben 1969; Vamos-Vigyazo 1981).

Experimental design for optimizing paste composition and data analysis

To make up a 668.2–1034.6 g batch of coating paste, oil content (46.6–63.4 g), water content (381–562.6 g) and flour content (166–334 g), 30 g starch, 9 g baking powder, 2.6 g carrageenan, 30 g salt and 3 g pepper were mixed. Next, the *Agaricus bisporus* slices were coated in the paste, they were put on a metal dish for quick-freezing in a static freezer (liquid nitrogen freezer, Sanyo, Co., Ltd, Japan). The schematic flow diagram for the preparation of the PCAB is summarized in Fig. 1.

The central composite design for the three independent variables was performed. The independent variables considered were oil content (x_1), water content (x_2) and flour content (x_3). A five-level central composite design was used (Table 1), resulting in 20 runs, and the results were analyzed using response surface methodology (RSM). The level of each variable was established according to literature data and preliminary trials (Hernández-Díaz et al. 2007; Rossella et al. 2010; Seog et al. 2008). The freezing time and sensory evaluation scores of PCAB were considered as product responses. RSM was applied to the experimental data using a commercial statistical package, Design Expert version 7.0.0 (Statease Inc., Minneapolis, MN, USA), for the generation of response surface plots. The results were analyzed by a multiple linear regression method which describes the effects of variables in second order polynomial models. The experimental data were fitted to the selected models and regression coefficients obtained. The statistical significance of the terms in the regression equations was examined by analysis of variance (ANOVA) for each response. Duncan's multiple range tests were performed for sensory and freezing rate data to determine difference between treatments by using SPSS (IBM SPSS Statistics, IBM Corp., Version 17.0). All analyses were performed in triplicate including sensory evaluation and freezing process data. The data are shown as means $\pm$ standard deviation (SD).

Sensory evaluation

Product acceptability was assessed by 40 consumer panelists (20 males, 20 females between the ages of 18 and 45) selected from the staff and graduating class students of the Food Engineering Department (Hebei Normal University of Science and Technology, Qinhuangdao, China). Panelists evaluated the PCAB samples for color, flavor, appearance, texture and overall acceptability using a 7-point hedonic scale (from 1=dislike extremely to 7=like extremely). After orientation, sample products coded with three digit numbers were

Fig. 1 Flow chart for the production of quick-frozen paste-coated mushrooms

given in random order to the panelists (Altan et al. 2008; Gajula et al. 2008; Michael et al. 2011).

Freezing process

After the *Agaricus bisporus* were coated with paste, they were put on a metal dish for quick-freezing. The temperature was recorded at the center of the PCAB every 0.5 min using an electronic thermometer (Sanbo, Co., Zhengzhou, China) to obtain the freezing curves at freezing temperatures of -75°C , -65°C , -55°C and -45°C .

To describe the experimental data, the freezing rate was calculated as:

$$r_f = \frac{T_f - T_F}{t_f} \quad (1)$$

Where r_f =freezing rate ($^{\circ}\text{C}/\text{h}$); T_f =temperature before freezing ($^{\circ}\text{C}$); T_F =temperature after freezing ($^{\circ}\text{C}$); t_f =freezing time (h).

Table 1 Experimental range and levels of independent process variables

		Variable level codes				
	Code	−1.682	−1	0	+1	+1.682
Oil (g)	x_1	46.6	50	55	60	63.4
Water (g)	x_2	381	415	465	515	562.6
Wheat flour (g)	x_3	166	200	250	300	334

Transmission electron microscopy

After optimization of the PCAB freezing process, the structure and quality of PCAB and fresh *Agaricus bisporus* samples were studied with a transmission electron microscope (Hitachi Co. Ltd, Japan) to observe any changes caused by the different treatment processes.

Results and discussion

Effect of microwave power on enzyme activity

Microwave processing can be used to partially destroy micro-organisms, thus limiting PPO and POD activity and therefore enzymatic browning. Figure 2 shows the changes in PPO and POD activity in *Agaricus bisporus* with 4 cm diameter caps related to the microwave power and processing time used.

Results showed that a microwave power of 9.0 and 7.2 W/g killed enzymes quickly, but a 40 s processing time caused *Agaricus bisporus* to darken with a 45 % water loss. Microwave power levels of 3.6 and 1.8 W/g killed enzymes slowly, but could not prevent the samples from browning. Bottcher (1975) indicated that vegetables would have better quality if a little POD activity still existed after they were hot-processed and that no residual POD activity meant excessive hot-processing. Therefore, the processing technique for 1 cm thick *Agaricus bisporus* slices was 5.4 W/g microwave power for 55 s, so that the PPO activity remained at 8.6 % and the POD activity at 3.2 %. Using this microwave power level of

Fig. 2 Effects of microwave treatment on PPO and POD activity for *Agaricus bisporus* with 4 cm diameter. Each value is the mean of three replicates; vertical bars indicate the standard errors. Microwave action power: black diamond 9.0 W/g; black square 7.2 W/g; black triangle 5.4 W/g; white square 1.8 W/g

5.4 W/g killed enzymes quickly while retaining an appropriate amount of water.

Effect of cap size on microwave processing

Figure 3 shows changes in PPO and POD activities for 3, 4 and 5 cm cap *Agaricus bisporus* (slices 1 cm thick) when treated with 5.4 W/g microwave power during processing.

Figure 3 indicates that PPO activity in *Agaricus bisporus* slices with different cap sizes was deactivated when microwave treated for 60 or 65 s. For *Agaricus bisporus* with a 3 cm cap, PPO activity decreased from 460.3 U/g at the beginning to 87.7 U/g when microwave treated for 40 s. PPO activity remained at 4.8 % for 55 s of treatment, but declined to 0 % after 55 s. The changes for *Agaricus bisporus* with a 4 cm cap were almost the same as for the 3 cm cap, but the PPO deactivated completely after treatment for 60 s. For *Agaricus bisporus* with a 5 cm cap, PPO activity decreased faster when microwave treated for 55–60 s, from 157 U/g to 24.7 U/g. PPO activity remained at 5.4 % after 60 s and disappeared completely after 65 s. Figures 2 and 3 also indicate that POD activity was lower than PPO activity in *Agaricus bisporus*, and deactivated simultaneously with PPO during microwave processing. Usually POD in vegetables is more heat-resistant than PPO, but this is not the case for *Agaricus bisporus*. In addition, PPO activity in *Agaricus bisporus* was high but changed considerably with increasing microwave treatment

Fig. 3 Effects of microwave treatment on PPO and POD activity of *Agaricus bisporus* with 1 cm slice thickness using 5.4 W/g microwave power. Each value is the mean of three replicates; vertical bars indicate the standard errors. Pileus diameter: black diamond 3 cm; black square 4 cm; black triangle 5 cm

time. Therefore, PPO could be considered as an indicator during microwave treatment (Long and Alben 1969; Vamos-Vigyazo 1981).

Coating paste composition optimization

The Design-Expert software was used to perform multiple regression fitting of the data shown in Table 2 to obtain two quadratic polynomial regression models to predict the freezing time Y_1 and sensory score Y_2 . The prediction models for freezing time (Y_1) and sensory score (Y_2) can be described by the following equations in terms of the coded values:

$$Y_1 = 9.00 - 0.11x_1 + 0.46x_2 - 0.54x_3 + 0.15x_1x_2 + 0.27x_1x_3 + 0.14x_2x_3 - 0.029x_1^2 + 0.031x_2^2 - 0.50x_3^2 \quad (R_1^2 = 0.9140) \quad (2)$$

$$Y_2 = 8.91 - 0.30x_1 + 0.42x_2 - 0.44x_3 + 0.63x_1x_2 - 0.84x_1x_3 + 0.49x_2x_3 - 0.14x_1^2 - 0.26x_2^2 - 0.37x_3^2 \quad (R_2^2 = 0.9226) \quad (3)$$

The significance of the coefficients of the fitted quadratic models was evaluated using the F -test and P -value. According

Table 2 Experimental design and results

Run	Coded levels			Response	
	Oil x_1	Water x_2	Flour x_3	Freezing time Y_1 (min)	Sensory evaluation scores Y_2
1	0	0	0	9.0	6.0
2	-1	+1	+1	8.3	6.5
3	0	0	0	9.0	6.6
4	0	0	0	9.3	6.2
5	0	-1.682	0	8.3	6.1
6	0	0	+1.682	6.3	5.7
7	+1	-1	-1	8.0	6.2
8	0	0	-1.682	9.0	3.6
9	-1.682	0	0	9.0	5.7
10	-1	-1	+1	7.7	6.4
11	+1.682	0	0	9.0	5.1
12	0	0	0	9.0	4.8
13	0	+1.682	0	10.0	6.3
14	+1	+1	+1	9.0	6.5
15	0	0	0	8.7	6.3
16	+1	-1	+1	7.3	5.1
17	0	0	0	9.0	6.1
18	-1	+1	-1	9.5	6.4
19	-1	-1	-1	9.0	6.2
20	+1	+1	-1	8.7	5.7

to the regression coefficients, the composition of the coating paste can influence the freezing time and the sensory evaluation scores in the following order: wheat flour (x_3)>water (x_2)>oil (x_1). The results of the analysis of variance (ANOVA) for freezing time (Y_1) and sensory evaluation (Y_2) for the two quadratic models are given in Table 3. The fit of the regression models to the experimental data for freezing time (Y_1) and sensory evaluation (Y_2) provided high indices of determination

($R_1^2=0.9140$ and $R_2^2=0.9226$ respectively). Table 3 shows that the regression F -values for Y_1 and Y_2 were significant ($P<0.01$) whereas the lack-of-fit F -values were not significant ($P>0.05$). Table 4 shows that water (x_2) and wheat flour (x_3) had highly significant effects on Y_1 and Y_2 ($P<0.01$) while oil (x_1) had a less significant effect on Y_2 ($P<0.05$) and no significant effect on Y_1 ($P>0.05$). The interactions of oil and water (x_1x_2), oil and wheat flour (x_1x_3) and water and wheat flour (x_2x_3) had a significant effect ($P<0.01$) on sensory evaluation scores (Y_2) and the oil and wheat flour interaction (x_1x_3) had a significant effect on Y_1 ($P<0.05$) but not on Y_2 . The quadratic effect of wheat flour (x_3^2) had a significant effect on both Y_1 and Y_2 ($P<0.01$) while water (x_2^2) had a significant effect ($P<0.05$) only on sensory evaluation (Y_2) (Hernández-Díaz et al. 2007; Rossella et al. 2010; Singh et al. 2008).

The response surfaces for the significant interactions are displayed in Figs. 4 and 5. When the flour content is low, freezing time decreased with increasing oil content. The maximum freezing time was obtained at a high level of wheat flour. With an increasing amount of added oil and flour content, the freezing point paste coating and freezing time decreased.

With an decreasing amount of added water and oil contents, sensory evaluation scores increased (Fig. 5a). The maximum sensory evaluation scores were obtained at low levels of water and oil possibly because when the water content is low, increasing the amount of oil will greatly increase the smoothness of the paste, making it difficult to coat the mushrooms and so reduce the sensory score. As the wheat flour and oil contents increased, the sensory evaluation scores increased (Fig. 5b). The wheat flour content was more effective at increasing sensory evaluation scores for PCAB in comparison with the oil content from regression coefficients.

As the wheat flour and water content increased, the sensory evaluation scores increased (Fig. 5c) to a peak and then became lower. The reason for this may be that at low flour levels the paste becomes thin, reducing its ability to coat the product.

Table 3 Variance analysis for regression model

Response	Source	df	Sum of squares	Mean squares	F-value	P-value
Freezing time	Regression	9	11.61	1.29	11.81	0.0003**
	Residual	10	1.09	0.11		
	Lack-of -fit	5	0.89	0.18	4.49	0.063 ^{ns}
	Pure error	5	0.2	0.04		
	Total	19	12.7			
Sensory evaluation scores	Regression	9	19.91	2.21	13.25	0.0002**
	Residual	10	1.67	0.17		
	Lack-of -fit	5	0.84	0.17	1.02	0.4930 ^{ns}
	Pure error	5	0.83	0.17		
	Total	19	21.58			

**Significant at $P<0.01$, *Significant at $P<0.05$, ^{ns} Not significant. df: degrees of freedom

Table 4 Regression coefficients and their significance

Variable	df	Sum of squares		Mean squares		F-value		P-value	
		Freezing time	Sensory evaluation scores	Freezing time	Sensory evaluation scores	Freezing time	Sensory evaluation scores	Freezing time	Sensory evaluation scores
x_1	1	0.16	1.27	0.16	1.27	1.45	7.59	0.2564 ^{ns}	0.0203*
x_2	1	2.93	2.45	2.93	2.45	26.8	14.70	0.0004**	0.0033**
x_3	1	3.93	2.64	3.93	2.64	35.96	15.82	0.0001**	0.0026**
x_1x_2	1	0.18	3.18	0.18	3.18	1.62	19.02	0.2318 ^{ns}	0.0014**
x_1x_3	1	0.57	5.71	0.57	5.71	5.19	34.22	0.0459*	0.0002**
x_2x_3	1	0.16	1.88	0.16	1.88	1.46	11.27	0.2545 ^{ns}	0.0073**
x_1^2	1	0.012	0.27	0.012	0.27	0.11	1.62	0.7448 ^{ns}	0.2318 ^{ns}
x_2^2	1	0.014	0.95	0.014	0.95	0.13	5.71	0.7294 ^{ns}	0.0380*
x_3^2	1	3.59	1.98	3.59	1.98	32.91	11.84	0.0002**	0.0063**

**Significant at $P < 0.01$, *Significant at $P < 0.05$, ^{ns} Not significant. df: degrees of freedom

Model validation

Based on using the Design Expert software optimization function over the test range, with the minimum freezing time and maximum sensory evaluation scores as constraint conditions, the optimal coating paste composition was oil code level (−1), water code level (+0.65) and wheat flour code level (+1). This leads to the values of three key factors as oil 50 g, water 432.5 g, flour 300 g with the other ingredients - starch 30 g, baking powder 9 g, carrageenan 2.6 g, salt 30 g and pepper powder 3 g. For this paste composition, the results from the model give sensory evaluation scores of 6.22 and a freezing time of 7.494 min. This trial was conducted five times using these optimal technological parameters to check the dependability of the experimental model: the results are listed in Table 5.

Table 5 indicated that the predicted values of sensory evaluation scores and freezing time were close to the true values (no significant difference, $P > 0.05$), meaning that the best technological parameters resulting from RSM were reliable and could be considered in practical applications.

Freezing temperature curves

The process of food freezing is the physical procedure of free water in food turning into crystals and is similar to that of water turning to ice because of food's high water content. The freezing temperature-time curve for a food declines continuously (Sahari et al. 2004). Freezing temperature curves for PCAB (1 cm thick) are shown in Fig. 3 for the different freezing temperatures of −75 °, −65 °, −55 ° and −45 °C.

Fig. 4 Response surface for freezing time as a function of wheat flour content and oil content when water=465 g

Fig. 5 Response surface for sensory evaluation scores as a function of wheat flour content, oil content and water content

(a) Wheat flour (250 g)

(b) Water (465 g)

(c) Oil (55 g)

Table 5 Parameters from regression model and verification results

Actual values are average of five measurements $\pm$ SD. ^{ns} Not significant

Parameter	Factor			Sensory evaluation scores		Freezing time (min)	
	Oil (g)	Water (g)	Flour (g)	Predicted value	Actual value	Predicted value	Actual value
Values	50	432.5	300	6.220	6.2 $\pm$ 0.1 ^{ns}	7.494	7.48 $\pm$ 0.08 ^{ns}

Figure 6 suggests that the freezing characteristics of PCAB are not the same as foods in general. At -75°C , the centre temperature of PCAB declined at an approximately constant speed, which means that the freezing process did not exhibit the usual constant temperature stage when liquid water is changing state to solid ice. The situation at -65°C was similar to that at -55°C exhibiting a not markedly constant temperature stage. At -45°C the constant temperature stage appeared followed by a rapid decline in temperature. In addition, the freezing curves of PCAB declined quickly in the last stage which is different from other foods. All the freezing curves for PCAB did not exhibit an overcooling stage, because of food moisture or a falling frost point at its surface (Santerre et al. 1988). Table 6 shows the changes in freezing rate of PCAB at different freezing temperatures.

The results showed that freezing rates were significantly different ($P < 0.01$) at different freezing temperatures. Our investigations revealed that mushrooms which are cut too thin or too thick can lead to a poor flavor. So choosing a 1.0 cm mushroom thickness is advisable and a freezing temperature of -75°C can improve the production efficiency.

Microstructure

Figure 7a–f show photomicrographs of treated *Agaricus bisporus* using the best technique available by transmission electron microscope (TEM). The TEM studies showed the changes inside the dense structure of PCAB frozen at different

temperatures and the results are explained below (Cole and Skellerup 1986; Zivanovic et al. 2000).

The results showed that the fresh *Agaricus bisporus* samples had intact cell walls and unbroken cytoplasmic membranes between cells with clearance and the cell interior showed dense cytoplasm. In the cytoplasm, multivesicular bodies caused by a few small vacuoles coming together and intact caryon, were clearly observed. The PCAB microstructures were in a good condition after a -75°C freezing treatment with cell walls and cytoplasmic membrane remaining intact. In addition, between cells with clearance, for cell contents without outflow, the cytoplasm was distributed with small vacuoles. All the photomicrographs showed that PCAB microstructures frozen at -75°C were similar to the fresh product. However, at -65°C , the freezing treatment showed slight deformation and intercellular gaps smaller than with the -75°C treatment. This means that cell walls began to degrade and show plasmolysis, with vacuoles larger than with the -75°C treatment, yet the overall structure remained intact. Figure 7d–f shows the photomicrographs of *Agaricus bisporus* treated at different temperatures (-55°C , -45°C) and without coating with paste. The results showed that cells were extremely deformed; cell walls had completely degraded and the organelle cavities had changed. In addition, the intercellular gap was so small that some had even joined together and the vacuoles were extremely large. If Fig. 7b and f are compared, it can be seen that the PCAB samples are significantly better than *Agaricus bisporus* without the paste coating treatment at the same freezing temperature (-75°C). The cells of *Agaricus bisporus* without paste coating were extremely deformed with completely degraded cell walls and extremely large vacuoles. So coating with paste can be very good for protecting cell structure and reducing the loss in nutrition.

Fig. 6 Freezing curves for paste-coated mushroom slices (*Agaricus bisporus*) of 1 cm thickness. Each value is the mean of three replicates; vertical bars indicate the standard errors. Freezing temperature: black diamond -75°C ; black square -65°C ; black triangle -55°C ; black circle -45°C

Table 6 Freezing rate for paste-coated *Agaricus bisporus* at different temperatures ($^{\circ}\text{C}/\text{h}$)

Freezing temperature ($^{\circ}\text{C}$)			
-75	-65	-55	-45
67.79 $\pm$ 2.25 ^a	55.75 $\pm$ 1.98 ^b	36.90 $\pm$ 1.76 ^c	28.08 $\pm$ 1.26 ^d

Results are expressed as means $\pm$ SD ($n=3$). Means with different letters are significantly different ($p < 0.05$). ANOVA and Duncan's test were used to analyze for significant differences between samples at different temperatures

Fig. 7 Microstructure of *Agaricus bisporus* – (a) fresh, (b) – (e) paste-coated with rapid freezing (-45°C , -55°C , -65°C , -75°C) and (f) with rapid freezing (-75°C) and no paste coating

A. Intercellular ($\times 1200$)

B. Vacuole ($\times 5000$)

(a) Fresh *Agaricus bisporus*

A. Intercellular ($\times 1200$)

B. Vacuole ($\times 3000$)

(b) Paste-coated *Agaricus bisporus* after rapid freezing at -75°C

A. Intercellular ($\times 1200$)

B. Vacuole ($\times 3000$)

(c) Paste-coated *Agaricus bisporus* after rapid freezing at -65°C

Fig. 7 (continued) **A. Inter-cellular(×1200)****B. Vacuole (×3000)****(d)** Paste-coated *Agaricus bisporus* after rapid freezing at -55°C **A. Inter-cellular (×1200)****B. Vacuole (×3000)****(e)** Paste-coated *Agaricus bisporus* after rapid freezing at -45°C **A. Inter-cellular (×4000)****B. Vacuole (×4000)****(f)** *Agaricus bisporus* after rapid freezing at -75°C

Conclusions

For making quick-frozen PCAB, it is better to classify materials according to slice thickness and then treat them with different microwave pretreatment conditions to get a better color and taste. The best microwave conditions for *Agaricus bisporus* slices 1 cm thick are 5.4 W/g, and 55, 55–60, 60 s treatment times for 3, 4, 5 cm diameter caps respectively.

The RSM approach was used to optimize the process parameters for making quick-frozen PCAB using freezing time and sensory evaluation scores as responses. Wheat flour, water and oil are the main coating paste ingredients for developing the convenience food, PCAB. The optimal composition for the coating paste was: 50 g oil, 432.5 g water and 300 g flour. In addition, 30 g starch, 9 g baking powder, 2.6 g carrageenan, 30 g salt and 3 g pepper were also incorporated to improve the flavor (previous investigations).

The results showed the changes in freezing rate of PCAB at different freezing temperatures to obtain the best product. The TEM studies showed that significant changes occurred in the microstructure of PCAB at different freezing temperatures. At -75°C , PCAB has a similar structure to the fresh mushroom because of the protection from the paste coating.

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