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RESPONSE SURFACE OPTIMIZATION OF MANUFACTURING OF DIETARY STARCH PRODUCTS

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Background. The development of food ingredients that beneficially affect the human organism has attracted much interest recently. Especially important seems to be resistant starch i.e. starch fraction which resists hydrolysis catalysed by amylases present in the gut. Although research on starches resistant to amylolytic enzymes began in 1990s, there is still lack of cheap and easy methods of its production. The aim of the work was to optimize the process of high pressure homogenization of potato starch pastes in order to reduce their digestibility to the utmost.

Material and methods. The optimization of the homogenization process was examined by means of the commercial software STATISTICA. Homogenisation was performed for the pastes of the concentration of 5%. Digestibility of the obtained starch samples was evaluated by the amount of glucose formed after 16 h of hydrolysis with the mixture of pancreatic alpha-amylase and glucoamylase.

Results. It was found that high pressure homogenization of starch pastes provides products of digestibility reduced up to 50%. Moreover, it was proved that at low temperatures, it is necessary to apply high pressure and low number of passages. At high temperatures, it is necessary to apply low pressure and high number of passages. Medium values of all of parameters did not provide low values of digestibility.

Conclusions. The application of the response surface methodology (RSM) for development of dietary starch products allows a quick identification of important process factors (such as temperature, pressure or numbers of passages) and shows interactions between them.

Key words: starch modification, homogenization, digestibility, response surface methodology (RSM)

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INTRODUCTION

Starch is primarily considered as a source of energy and it mainly determines its nutritive importance. However, in many foodstuffs starch escapes complete digestion by amylases in the alimentary tract, which makes it possible to apply them as a dietary food product. From nutritional point of view, starch can be classified into three basic groups: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [Englyst et al. 1992]. Firstly, rapidly digested and slowly digested starch were defined by the amount of glucose released after hydrolysis at 37°C for 20 and 100 minutes respectively. Resistant starch was recognised as the starch not hydrolysed after 120 minutes of incubation [Englyst et al. 1992]. However, the development in understanding of the phenomena relating to digestion in the human gastrointestinal tract requires changes in the defining of resistant starch. Now, it is postulated that resistant starch is the fraction which does not undergo hydrolysis with the mixture of pancreatic alpha-amylase and glucoamylase at the temperature of 37°C for the time of 16 hours [Akerberg et al. 1998, Champ et al. 1999, 2001]. Moreover, recently a new category of enzyme resistant starch has been formulated, namely very resistant starch (VRS), which is not digested for a long time, up to 24 h and more [Soral-Śmietana and Wronkowska 2004].

Starch modification techniques have been developed for industrial processing to produce a wide range of potential food ingredients. However, interest in modified starches has been restricted mainly to technological aspects with little concern about the possible impact of the modification on the digestibility and fermentability of the product. Physical modification of starch is mainly applied to change the granular structure and convert native starch into cold water-soluble starch or small-crystallite starch. The major methods used in the preparation of cold water-soluble starches involve instantaneous boiling-drying of starch suspensions on heated rolls (drum-drying), puffing, continuous boiling-puffing-extruding, and spray-drying [Jarowrenko 1986]. Among the physical processes applied to starch modification, high pressure treatment has attracted special attention as an example of "minimal processing". The effect of high pressure on starch is dependent on the environmental conditions (moisture content, pressure value, temperature) and the origin of the starch [Stute et al. 1996, Bauer and Knorr 2005, Błaszczak et al. 2007, Kawai et al. 2007, Buckow et al. 2007]. As an effect of ultra high pressure treatment (above 400 MPa), starches gelatinise but show very little swelling and maintain their granular character, which results in quite different paste and gel properties of the UHP-gelatinised starches compared to the heat-gelatinised starches [Stute et al. 1996]. Our previous work has concerned a typical high pressure treatment of starch, that is homogenization of the starch pastes instead of the hydrostatic pressure treatment of the granular starch-water mixtures. It proved that the high pressure homogenization of the starch pastes could be the way for the manufacturing of the starch products which reveal decreased digestibility [Grajek et al. 2004]. However, the relationship between parameters of the processing and digestibility level is not clear. The large number of experiments necessary to establish an adequate functional relationship between the observed responses (digestibility) and the high-pressure homogenization parameters (pressure, temperature and number of passages), make the experimentation time consuming and prohibitively expensive. For that reason, response surface methodology (RSM) seemed to be the most suitable experimental design strategy. RSM is a collection of mathematical and statistical tools used to model and analyse problems whose desired responses are influenced by many variables [Montgomery 2001]. The response surface method permits to define empirical models such as linear, linear with two-factor interaction or quadratic polynomials which describe accurately how responses behave at all values of the studied variables in the experimental region. However, it should be stressed that in order to calculate quadratic polynomial model coefficients, each design variable has to be studied at three distinct levels at least. The aim of the work was to optimize the process of high pressure homogenization of potato starch pastes in order to reduce their digestibility to the utmost. In particular, the Box-Benkhen design (BBD) constructed by combining two-level factorial designs with incomplete block designs has been used to determine the optimal conditions for high pressure homogenization of starch pastes.

MATERIAL AND METHODS

Materials

Commercial potato "Superior Standard" starch (Polish product) manufactured by Potato and Starch Company WPPZ Luboń S.A. was used as a raw material.

Methods

Homogenization. Starch suspensions of the concentration of 5% were gelatinised and then sterilized at 121°C for 20 min. Homogenization was performed using the GEA Niro Soavi (Italy) homogenizer. Homogenized starch pastes were dried with Mobile Miner^{TM} 2000 (Niro A/S) spray dryer.

Experimental design. In this study, the effects of homogenization pressure (X_1), temperature (X_2) and number of passages (X_3) on digestibility of starch samples (Y) was evaluated using the Box-Behnken design. As shown in Table 1, the variable levels X_i were coded as x_i according to the following equation:

$$x_i^{(+1,0,-1)} = \frac{2X_i - X_{(min)i} - X_{(max)i}}{X_{(max)i} - X_{(min)i}} \qquad (1)$$

where x_i is the dimensionless value of an independent variable, X_i the real value of an independent variable and $X_{(min)i}$, $X_{(max)i}$ are the lower and the upper limit of the independent variable respectively. A total of 17 experiments were performed and the central point was repeated five times to estimate the experimental error variance. The order in which the experiments were performed was randomised, according to the requirement for the observations to be distributed independently and randomly, which additionally helps to avoid the influence of unknown nuisance variables. The set points were selected according to the results obtained during a preliminary study.

A multiple regression analysis of the data was carried out to obtain empirical models that define response (Y) in terms of the independent variables. For a three-factor system, the following second-order polynomial equation was then applied to the data by the multiple regression procedure (equation 2):

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (2)$$

with Y, being the predicted response; b_0, intercept; b_1, b_2 and b_3, linear coefficients; b_{11}, b_{22} and b_{33}, squared coefficients and b_{12}, b_{13} and b_{23}, interaction coefficients. The accuracy and general ability of the above polynomial model was evaluated by the adjusted coefficient of determination Adj-R^2, significance of total regress F-value and non significance of lack of fit F-value. The commercial software STATISTICA, version 6.0 PL from StatSoft, Inc. (2004) was used for regression and graphical analyses of the obtained data.

Table 1. Independent variables and their levels for the design used in the present study

Variable				Coded values		
real			coded	–1.0	0	+1.0
		unit		real values		
Pressure	X_1	MPa	x_1	4.0	22.0	40.0
Temperature	X_2	°C	x_2	50.0	67.5	85.0
Number of passages	X_3	–	x_3	5.0	10.0	15.0

Digestibility. The rate of digestion of starch was determined by its hydrolysis with the mixture of pancreatic alpha-amylase and glucoamylase at the temperature of 37°C, at particular periods of time, followed by the measurement of the released glucose using glucose oxidase. Porcine pancreatic alpha-amylase type VI-B (Sigma) as well as glucoamylase AMG 300L (Novozymes) were used for the analyses. The amount of released glucose was determined colorimetrically at the $\lambda = 500$ nm using Liquick Cor-Glucose diagnostic kit (Cormay, Poland). Four replicates were made for each probe and standard deviation was calculated.

RESULTS AND DISCUSSION

Statistical analysis

In order to find the optimum conditions for high pressure homogenization of the starch pastes experiments were performed according to the BBD experimental plan (Table 2). Analysis of variance indicated that the response surface model developed for the digestibility of starch samples was statistically significant by the probability of the F test at the level below 0.0001 (Table 3). Furthermore, the probability value of the lack-of-fit test was higher than 0.05, indicating that the regression model is in good prediction of the experimental results. As it was mentioned above, the precision of a model can be checked by the adjusted coefficient of determination Adj-R^2. It is well known that the closer the Adj-R^2 is to 1, the better the model fits the experimental data. As shown in Table 4, the value of Adj-R^2 was equal 0.9202 indicating a close agreement between the experimental results and the theoretical values predicted by the model equation, which can be proved by Figure 1.

Table 2. Box-Behnken experiments design matrix with experimental value of starch pastes digestibility

Trial number	Variable						Response digestibility %
	X_1: Pressure, MPa		X_2: Temperature, °C		X_3: Number of passages		
	real	coded	real	coded	real	coded	
1	40.0	1.0	67.5	0.0	15.0	1.0	55.84
2	22.0	0.0	50.0	–1.0	5.0	–1.0	54.52
3	40.0	1.0	85.0	1.0	10.0	0.0	63.41
4	22.0	0.0	67.5	0.0	10.0	0.0	67.98
5	22.0	0.0	67.5	0.0	10.0	0.0	67.62
6	40.0	1.0	67.5	0.0	5.0	–1.0	60.32
7	4.0	–1.0	85.0	1.0	10.0	0.0	58.94
8	22.0	0.0	85.0	1.0	15.0	1.0	61.52
9	4.0	–1.0	67.5	0.0	5.0	–1.0	61.49
10	22.0	0.0	85.0	1.0	5.0	–1.0	71.39
11	22.0	0.0	67.5	0.0	10.0	0.0	67.89
12	22.0	0.0	67.5	0.0	10.0	0.0	67.72
13	4.0	–1.0	50.0	–1.0	10.0	0.0	68.8
14	22.0	0.0	67.5	0.0	10.0	0.0	67.44
15	22.0	0.0	50.0	–1.0	15.0	1.0	69.96
16	40.0	1.0	50.0	–1.0	10.0	0.0	64.42
17	4.0	–1.0	67.5	0.0	15.0	1.0	59.72

Table 3. ANOVA table of starch pastes digestibility

Factor	Sum of squares	df	Mean square	F-value	p
Model	387.922	7	55.417	25.706	< 0.0001
b_1	3.075	1	3.075	1.426	0.2665
b_2	22.161	1	22.161	10.280	0.0125
b_3	17.480	1	17.480	8.108	0.0216
b_{11}	28.206	1	28.206	13.083	0.0068
b_{33}	110.784	1	110.784	51.388	< 0.0001
b_{12}	19.580	1	19.580	9.082	0.0167
b_{23}	207.914	1	207.914	96.444	< 0.0001
Lack of fit	14.812	4	3.703	6.084	0.0541
Pure error	2.434	4	0.608		
Total SS	405.169	15			

Table 4. Reduced quadratic model of response equation in terms of coded factors

Factor	Regression coefficient	Std. err. Pure err.	t	p	–95% conf. limt	+95% conf. limt
b_0	67.2763	0.3185	211.2377	0.0001	66.392	68.1606
b_1	–0.62	0.2759	–2.2479	0.0879	–1.3858	0.1458
b_{11}	–2.8166	0.4138	–6.8079	0.0025	–3.9653	–1.6679
b_2	–1.9665	0.3259	–6.0345	0.0039	–2.8712	–1.0617
b_3	–1.7465	0.3259	–5.3593	0.0059	–2.6512	–0.8417
b_{33}	–5.6844	0.4214	–13.492	0.0002	–6.8542	–4.5147
b_{12}	2.2125	0.3901	5.6722	0.0048	1.1296	3.2955
b_{23}	–9.6504	0.5222	–18.4832	0.0001	–11.100	–8.2007
			Adj-R^2 = 0.9202			

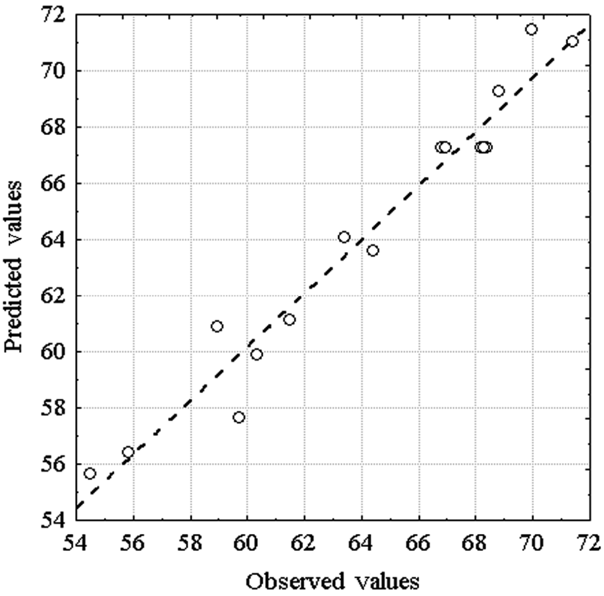

Fig. 1. An factual versus predicted plot enabling correlating inspection of model predictions relative to current data

As shown in Figure 2, the digestibility of starch samples mainly depends on the number of passages (x_3) as it is quadratic effect ($p < 0.0001$) and it is interaction effects ($p < 0.0001$) with homogenization temperature (x_2). Additionally, it was found that the homogenization pressure (x_1) in linear term had no significant net influence ($p = 0.2665$), while in quadratic term was highly significant ($p = 0.0068$), giving an overall curvilinear effect. It can further be seen that the second-order effects for independent variables x_3 and x_1 have negative sign (Table 4), which means that the investigated value increases to reach a maximum and then sharply decreases (Fig. 3, 4). Furthermore,

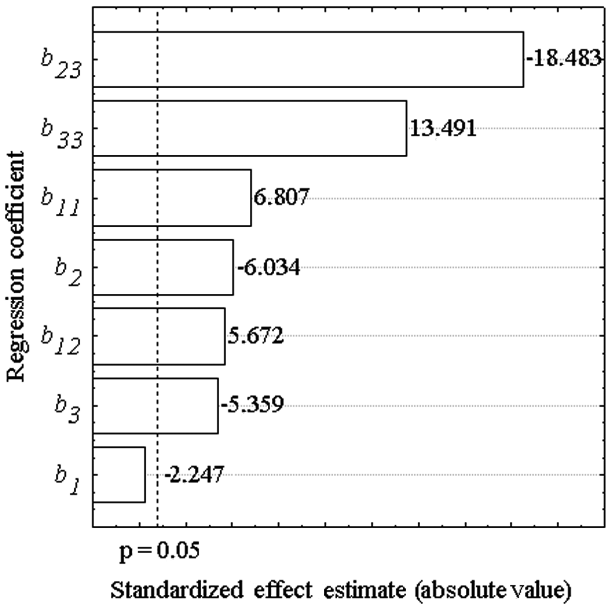

Fig. 2. Pareto Chart of standardized effects

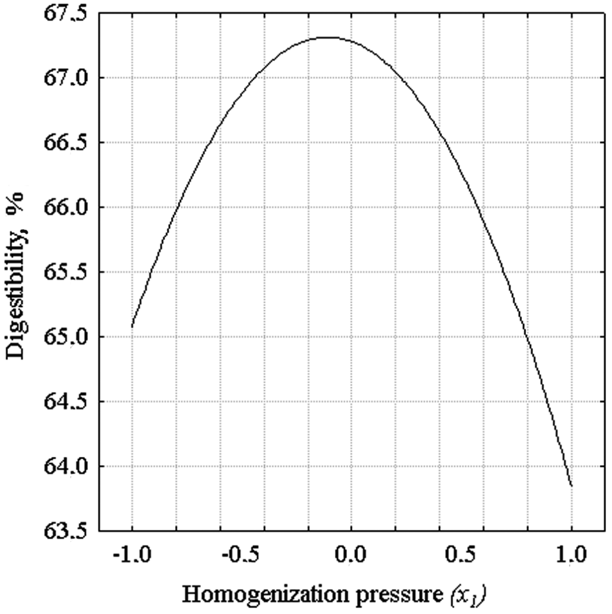

Fig. 3. One factor plot showing variation in digestibility [%] as a function of homogenization pressure (x_1). The remaining factors were fixed at zero coded level

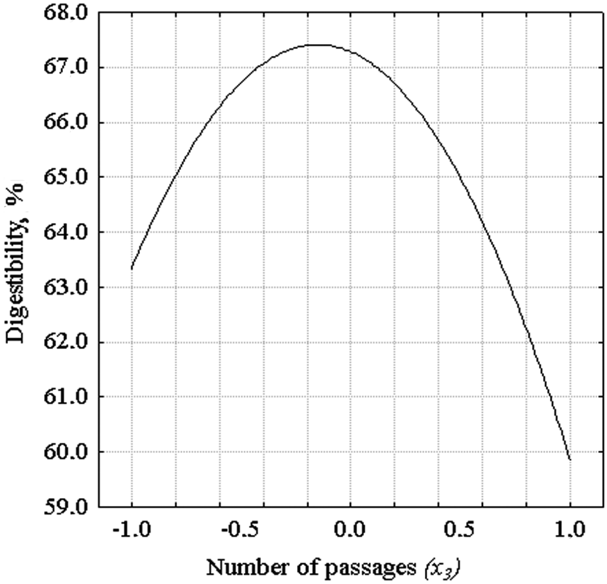

Fig. 4. One factor plot showing variation in digestibility [%] as a function of number of passages (x_3). The remaining factors were fixed at zero coded level

the digestibility was found to be negatively correlated to the homogenization temperature (x_2; $p = 0.0125$), while the second order effect of these independent variable did not significantly influence dependent variable, hence was removed from the model by the backward elimination procedure of ANOVA (Fig. 5). Other factors that significantly contribute to the digestibility of starch samples include the number of passages (x_3) as it is linear effect ($p < 0.025$) and interaction term between pressure (x_1) and homogenization temperature (x_2; $p = 0.0167$).

Figure 6 shows the effects of homogenization pressure (x_1) and temperature (x_2) on the digestibility, while number of passages (x_3) was fixed at its zero coded level. The digestibility decreased gradually from 69.25% to 60.09% as the temperature values increased at a lower level of homogenization pressure. By contrast, at a higher level of homogenization pressure, the digestibility increased from 63.59% to 64.08% when temperature values increased. With the increase in the homogenization pressure, the digestibility curvilinearly decreased from 69.25% to 63.59% at a lower homogenization temperature value.

Figure 7 shows the effects of temperature (x_2) and number of passages (x_3) on the digestibility, while the homogenization pressure (x_1) was fixed at its zero coded level. The digestibility increased gradually from 55.65% to 71.02% as the temperature values increased at the lower limits of number of passages (x_3). By contrast, at the upper limits of number of passages (x_3), the digestibility decreased from 71.46% to 48.22% when temperature values increased. With the increase in the number of passages (x_3), the digestibility sharply decreased from 71.46% to 55.65% at a higher homogenization temperature value. This suggests that increasing the number of passages within the tested range was beneficial to obtain the lower value of digestibility of starch pastes.

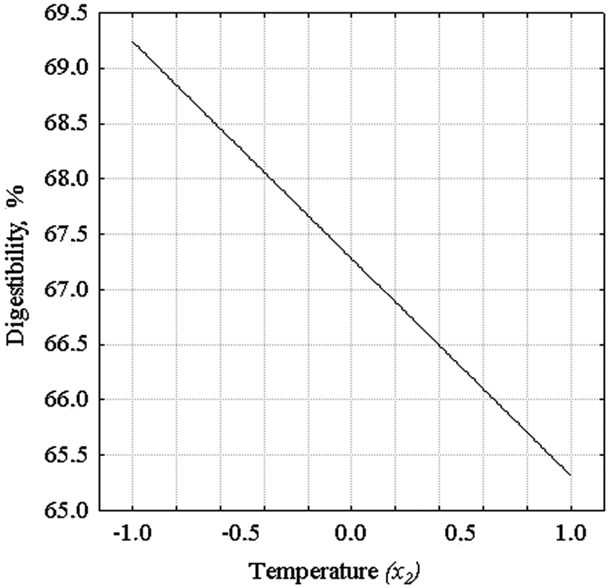

Fig. 5. One factor plot showing variation in digestibility [%] as a function of homogenization temperature (x_2). The remaining factors were fixed at zero coded level

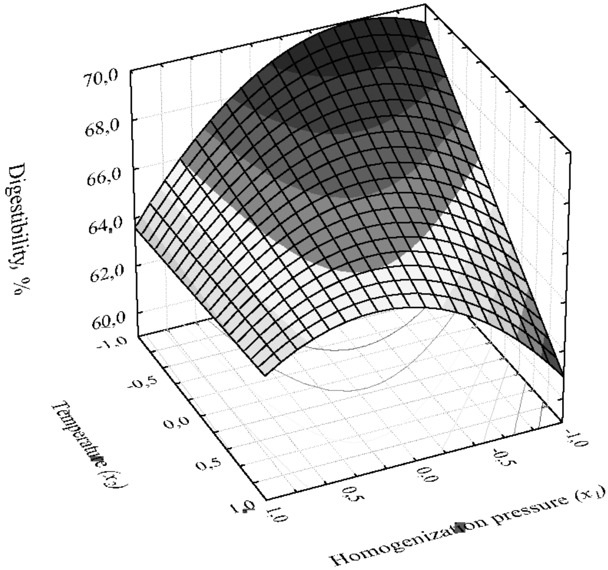

Fig. 6. Response surface plot showing variation in digestibility [%] as a function of homogenization pressure (x_1) and temperature (x_2). The remaining factors were fixed at zero coded level

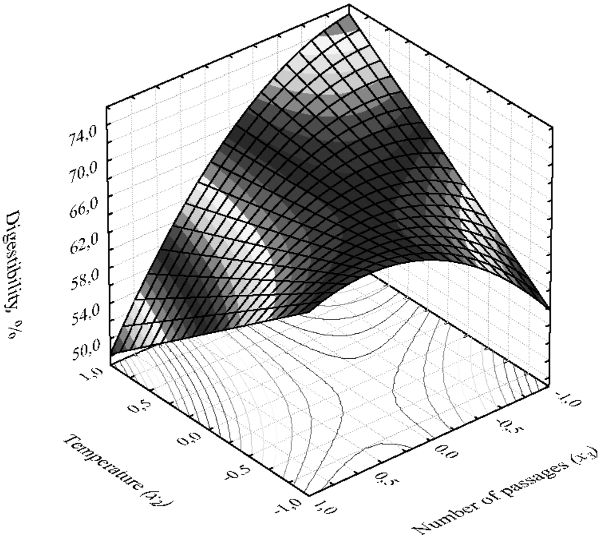

Fig. 7. Response surface plot showing variation in digestibility [%] as a function of homogenization temperature (x_2) and number of passages (x_3). The remaining factors were fixed at zero coded level

Process optimization

A typical problem in a product development is to find a set of conditions of the input variables, that ensures the most desirable product. The aim of this research was to optimize the process parameters of high pressure homogenization of native starch pastes in order to obtain the high yield of resistant starch. In this context, the modified biopolymer should behave like dietary fibre which is excreted without any digestion in the intestine. The procedures used to solve this problem involve several steps: predicting responses to the dependent variable Y by fitting the observed response using an equation based on the levels of the independent variables, and finding the levels of the independent variables that produce the most desirable predicted response to the dependent variable. The relationship between predicted response Y to one or more dependent variables and the desirability of response is called the desirability function [Derringer and Suich 1980]. Presented results clearly indicate that the digestibility of starch samples

Table 5. Optimization results obtained by using desirability function methodology

Solution number	Scale	Independent variable			Predicted solutions				
		pressure	temperature	number of passages	prediction	SE mean	–95% conf. limt	+95% conf. limt	SE pred
1	real	37.067	50.01	5.170	51.964	1.379	48.787	55.142	2.014
	coded	0.83	–1.00	–0.97					
2	real	0.6777	82.52	13.05	54.260	1.356	51.135	57.384	1.999
	coded	-0.84	0.86	0.61					

can be affected by altering all investigated independent variables. For that reason, the following conditions were imposed: the homogenization pressure (x_1), the temperature (x_2) as well as the number of passages (x_3) have coded values from –1.0 to +1.0 and, at the same time, the digestibility value must be kept at the lowest possible level. Before optimization, the response variable Y was converted into the desirability function D that varies from 0 to 1 where, if the response is at its goal or target, then D = 1, and if the response is outside an acceptable region, D = 0. Applying the methodology of the desirability function, two solutions were obtained (Table 5).

CONCLUSIONS

Application of the response surface methodology (RSM) for screening and optimization of the conditions of high pressure homogenization of starch pastes allows a quick identification of important factors and interactions between them. On the basis of the results of statistical analysis, it has been found that the reduced quadratic models were reasonably accurate ($R^2 = 0.92$) and can be used for the prediction within the limits if the factors investigated. Applying of the optimal parameters of high pressure homogenization of starch pastes provides a product of digestibility reduced up to 50%. However, with regards to the complex interactions among various experimental parameters it should be noted that:

- at low temperatures it is necessary to apply high pressure and low number of passages
- at high temperatures it is necessary to apply low pressure and high number of passages
- medium values of all of parameters did not provide low values of digestibility.

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OPTYMALIZACJA PROCESU WYTWARZANIA DIETETYCZNYCH PRODUKTÓW SKROBIOWYCH METODĄ PŁASZCZYZNY ODPOWIEDZI

Wprowadzenie. W ostatnich latach obserwuje się rosnące zainteresowanie żywnością wywierającą pozytywny wpływ na organizm człowieka, wykraczającą poza typowy efekt odżywczy. Wśród tego typu produktów szczególne zainteresowanie budzi skrobia oporna na enzymy amylolityczne. Pomimo iż badania nad skrobiami opornymi na enzymy amylolityczne trwają od początku lat dziewięćdziesiątych ubiegłego stulecia, dotychczas nie opracowano nieskomplikowanych i tanich technologii otrzymywania tego typu produktów. Celem pracy była optymalizacja procesu otrzymywania skrobi modyfikowanej fizycznie w drodze wysokociśnieniowej homogenizacji kleików skrobiowych w aspekcie maksymalnego zmniejszenia strawności produktu.
Materiał i metody. Optymalizację procesu wysokociśnieniowej homogenizacji prowadzono z wykorzystaniem programu STATISTICA. Homogenizację prowadzono dla kleików o stężeniu 5%. Strawność otrzymanego produktu oznaczano jako ilość glukozy wydzielonej po 16-godzinnej inkubacji z α-amylazą trzustkową i glukoamylazą.
Wyniki. Stwierdzono, że zastosowanie optymalnych warunków procesu pozwala na obniżenie strawności o 50%. Zastosowanie średnich wartości parametrów obróbki nie zapewnia zadowalającego zmniejszenia strawności. W niskiej temperaturze jest konieczne zastosowanie wysokiego ciśnienia i małej liczby pasaży; w wysokiej temperaturze konieczne jest zastosowanie niskiego ciśnienia i dużej liczby pasaży.
Wnioski. Zastosowanie metody płaszczyzny odpowiedzi (RSM) w celu otrzymania dietetycznych produktów skrobiowych pozwala na szybkie rozpoznanie warunków prowadzenia procesu (takich, jak temperatura, ciśnienie czy liczba pasaży) oraz wskazuje na wzajemne interakcje pomiędzy badanymi czynnikami.

Słowa kluczowe: modyfikacja skrobi, homogenizacja, strawność, metoda płaszczyzny odpowiedzi (RSM)

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