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Convergence Study on the Optimization for Suppression of Starch Hydrolysis using Rutin, Quercetin and Dietary Fiber Mixture Design

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루틴, 퀘르세틴, 식이섬유 혼합물 설계를 이용한 전분소화 지연 효과의 최적화에 대한 융합 연구

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Abstract This study was conducted to develop the efficient system for starch hydrolysis suppression using rutin, quercetin and dietary fiber through the statistical mixture design. The three components were replaced with wheat flour at the level of 10% and the mixed gel with three components was characterized by in vitro starch digestion. The mixture design was applied by simplex-centroid experimental model. The quadratic model (R^2=0.86) was well fitted and the obtained regression equation indicated that the significant positive effects was observed in the quercetin and fiber mixture. Based on the statistical results, the best mixing ratio of quercetin and fiber was 72: 28 that led to the lowest predicted glycemic index (pGI). Their interactions on the pGI of starch digestibility were clearly visualized in the 3D surface plot. These results suggested that the mixture of quercetin and fiber interact strongly with wheat flour, consequently retarding starch hydrolysis by 15%.

Key Words : Mixture design, Glycemic index, *In vitro* digestibility, Rutin, Quercetin, Fiber

요 약 본 연구는 루틴, 퀘르세틴, 식이섬유가 전분 소화율에 미치는 영향을 통계적 혼합물 설계방법을 이용하여 확인하고자 하였다. 세 가지 성분들은 모두 농도가 증가함에 따라 전분소화 지연 효과가 나타났으며, 그 중에서도 퀘르세틴과 식이섬유가 함께 섞였을 때 가장 높은 지연 효과를 보였다. 이 혼합물 설계는 simplex-centroid 실험설계법을 이용하였고, 최적 모델은 quadratic 모델에서 나타났다. 이때 얻어진 회귀방정식을 통하여 유의한 상승효과를 확인할 수 있었다. 최적화 통계 방법을 사용하여 혼합비를 분석한 결과 퀘르세틴과 식이섬유가 72: 28 비율에서 전분 소화 지연 효과가 최대로 나타나는 것을 확인하였다. 전분소화에 대한 영향을 3차원 표면 도표로 시각화하여 나타내었으며, 이 결과로 퀘르세틴과 식이섬유의 상호작용으로 인하여 전분 소화를 15% 이상 지연시키는 것을 확인할 수 있었다.

주제어 : 루틴, 퀘르세틴, 식이섬유, 전분 소화, 혼합물 설계

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1. Introduction

Many researches about phytochemicals have been conducted in relation to the mechanisms with reduced risk of chronic diseases such as cancer, obesity, hyperlipidemia, and type II diabetes and the increased consumption in food industry have been attributed to their bioactivities as functional materials [1, 2]. The use of phytochemical in starch digestibility has recently been paid attention due to the retarding effect of starch hydrolysis. The slowing down the rate of starch digestion led to retard blood glucose response, to reduce insulin requirements, and to cause satiety by reducing the stress on regulatory systems related to energy metabolism [3]. Various phytochemicals could decrease *in vitro* starch digestibility by inhibiting digestive enzyme and interacting with starch. The interaction between phytochemical and starch could alter its rheological properties, gelatinization, retrogradation, and gelling properties as well as inhibit the digestive enzyme [4, 5]. Barros et al. [6] represented the effect complex of tannin and starch in starch digestibility and Chai et al. [7] investigated the interaction between maize starch and tea polyphenol.

Rutin and quercetin are widely distributed in edible plants, and as powerful phytochemicals, they contain various bioactivities such as antioxidation [8], anti-inflammation [9], and anti-carcinogenicity [10]. Also rutin and quercetin in buckwheat was well known to act as α-amyloglucosidase inhibitors [11] and the conformation of complex between quercetin and starch resulted in alternation of the granule morphology and thermal properties and resulted in the dramatically reduction of digestion rate [12].

Dietary fiber is also known as one of the important factors that can affect starch hydrolysis. There are many studies that dietary fiber could be suppressed the starch digestibility. In according to the Brennan [13], dietary fiber has shown to be effective in delaying starch digestion, and they have also resulted in slow starch degradation by reducing enzyme accessibility or enclosing starch molecules. And the previous study reported that the ratio of soluble and insoluble dietary fiber could be affected the starch digestibility in food model [14]. However, the optimization using a statistical treatment among phytochemicals, dietary fiber, and starch on starch digestion have not been insufficiently studied even though a single effect based on the concentration of extract or ingredient was investigated.

Therefore, the purpose of this study was to investigate the combined effects and optimal levels of the three components (rutin, quercetin and dietary fiber) that delay the starch digestibility by using a statistical method using simplex-centroid design.

2. Materials and Methods

2.1 Materials

The dietary fiber (95% purity), rutin (95% purity), and quercetin (88% purity) from Tartary buckwheat (*Fagopyrum tartaricum*) were used in this study (Hunan Huacheng Biotech, Inc., China). Salivary amylase (A0521, EC 3.2.1.1), pancreatin from porcine pancreas (P7545), bile extract (B8631), and amyloglucosidase from Aspergillus niger (A9931, EC 3.2.1.3) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A total starch assay kit (K-TSTA) and a glucose oxidase-peroxidase assay kit (K-GLUC) were obtained from Megazyme International Ireland Ltd. (Bray, Ireland). The wheat flour was bought from CJ Co. (Seoul, Korea).

2.2 *In vitro* starch digestibility and predicted glycemic index

In vitro starch digestion of wheat flour gel

(10.71%) was conducted based on the method of Minekus et al. [15]. with slight modification. Gel sample (5 g) was prepared by gelatinization by heating in a boiling water bath (20 min). For oral digestion, a salivary fluid stock solution (3.5 mL) and a salivary α-amylase solution (0.5 mL) were added in the gel sample (37°C, 2 min). And the gastric fluid stock solution (7.5 mL) and a porcine pepsin stock solution (1.6 mL) were mixed (37°C, 2 h) and the gastric phase digestion was adjusted at pH 3.0 with 1 M HCl. After then, the intestinal fluid stock solution (11 mL), a pancreatin-bile solution (7.5 mL), and amyloglucosidase (0.2 mL/gram starch) were reacted for 3 h and the intestinal phase digestion was adjusted to pH 6.0. Hydrolyzed starch was collected at 0, 30, 60, 90, 120, and 180 min. The amount of glucose released during digestion were analyzed using GOPOD kit. The hydrolysis index (HI) was evaluated as the total glucose amount released from the sample relative to that from white bread. The pGI (predicted glycemic index) was estimated using the equation: pGI = 39.71 + 0.549HI, described by Goñi [16].

2.3 Statistical experimental design

The mixture design of the experiment was used to obtain the optimal mixture composition of three components, which were included dietary fiber, rutin and quercetin and selected based on literature research as independent variables, for retarding the starch hydrolysis *in vitro* starch digestion model system. The experimental design of mixed flour gels was performed using a Design expert software (DX10, Stat-Ease, Inc., MN, USA). Augmented simplex-centroid design was applied and the independent components were fiber, rutin and quercetin, coded as A, B and C, respectively. And each component was applied in five level (0, 2.5, 3.3, 5, and 10%). Simplex-centroid design was used and their experimental data are summarized in Table 1(A). Linear, quadratic, special cubic, cubic, special quartic and quartic interaction in terms of the polynomial models were fitted to investigate the effects of each component and their interactions on the response.

2.4 Statistical analysis

The experimental measurements were conducted in triplicate. The data were analyzed by one-way analysis of variance, and the means were compared by Duncan's multiple range test at $p<0.05$ by using a SPSS software (version 25, SPSS Inc., Chicago, IL, USA). The mixture design analysis and ternary plot were generated by Minitab software (version 14, Minitab Co. Ltd., USA).

3. Results and Discussion

The design of experiment is a statistical method to investigate the interaction of the rutin, quercetin and dietary fiber *in vitro* starch digestibility and also to determine their optimal levels. Table 1 shows the results of the experimental data using simplex-centroid design. The lowest pGI value was observed in quercetin (10%) sample (No. 3), followed by the fiber (5 and 2.5%)-quercetin (5%) samples (No. 5 and 10). However, the samples with fiber (10%) and fiber (5%)-rutin (5%) showed a slightly decrease in pGI suppression. To further analyze the interaction between three components and the optimal level, statistical analysis of the response was performed and represented in Table 2.

The fitness and goodness of the fitted models were evaluated by the significance of lack-of-fit, and adjusted and predicted R^2 values. Linear, cubic and special cubic models were excluded from the fitting due to their significance of lack-of-fit values. Quadratic mixture model was well fitted for all variations (R^2=0.86) compared to other mixture models as a function of the significant interaction effects between the levels. The results of ANOVA for the response (pGI) of

Table 1. Mixture design for 10 trials with three components and their responses

No.	Flour	Fiber	Rutin	Quercetin	Sum	Observed GI	Predicted GI
1	90	10	0	0	100	85.69	85.08
2	90	0	10	0	100	80.70	78.83
3	90	0	0	10	100	74.33	76.31
4	90	5	5	0	100	88.36	85.89
5	90	5	0	5	100	79.56	83.21
6	90	0	5	5	100	80.71	83.38
7	90	3.3	3.3	3.3	100	83.40	83.58
8	90	5	2.5	2.5	100	82.45	84.62
9	90	2.5	5	2.5	100	81.85	85.54
10	90	2.5	2.5	5	100	79.03	84.25

Table 2. List of summary statistics of the fitted models for the response of predicted glycemic index

Model	Lack-of-fit	Standard deviation	R^2	Adjusted R^2	Predicted R^2
Linear	0.0017	2.9	0.5987	0.5564	0.4367
Quadratic	0.4345	1.61	0.89567	0.8631	0.7518
Special Cubic	0.3274	1.65	0.8974	0.8564	0.71956
Cubic	0.1266	1.71	0.9047	0.8461	0.6998
Special Quartic	0.1266	1.71	0.9047	0.8461	0.6998
Quartic	N/A	N/A	N/A	N/A	N/A

N/A: not analyzed

the fitted quadratic mixture model are summarized in Table 3, which was demonstrated that the pGI values experimentally obtained were close to those predicted by the fitted quadratic mixture model. The fitted model for the prediction of the response can be expressed as follow:

$$pGI = 85.07A + 80.50B + 75.41C + 22.73AB - 22.04AC + 11.21BC$$

The negative quadratic terms (AC) of the fitted regression equation indicated the antagonistic effects *in vitro* starch digestibility. According to the fitted regression equation, the fiber and quercetin mixture (AC) is the most effective to lower the glycemic index. But AB and BC were not observed in suppression effects.

The effects of ternary mixture components and their interactions on the response (pGI) were projected onto the two-dimensional ternary contour and three-dimensional surface plots as shown in Fig. 1(a) and (b). As can be seen in the Fig. 1(a), the effects of all individual components and binary interaction of fiber/quercetin had positive effects, while the binary interactions of fiber/rutin and rutin/quercetin synergistically gave negative effects on the pGI *in vitro* starch digestibility.

Furthermore, the effects of individual components and their interactions on the pGI of starch digestibility were clearly visualized in the 3D surface plot (Fig. 1(b)). A steep cliff indicated that the fiber/quercetin mixture had significantly lower effects on *in vitro* starch digestibility than the other components. Moreover, when the synergistic effects of the three components of fiber, rutin and quercetin were analyzed using the optimization tool, it was found that the most effective mixing ratio of fiber and quercetin was 28:72, that was effective by 15% lowering the glycemic index.

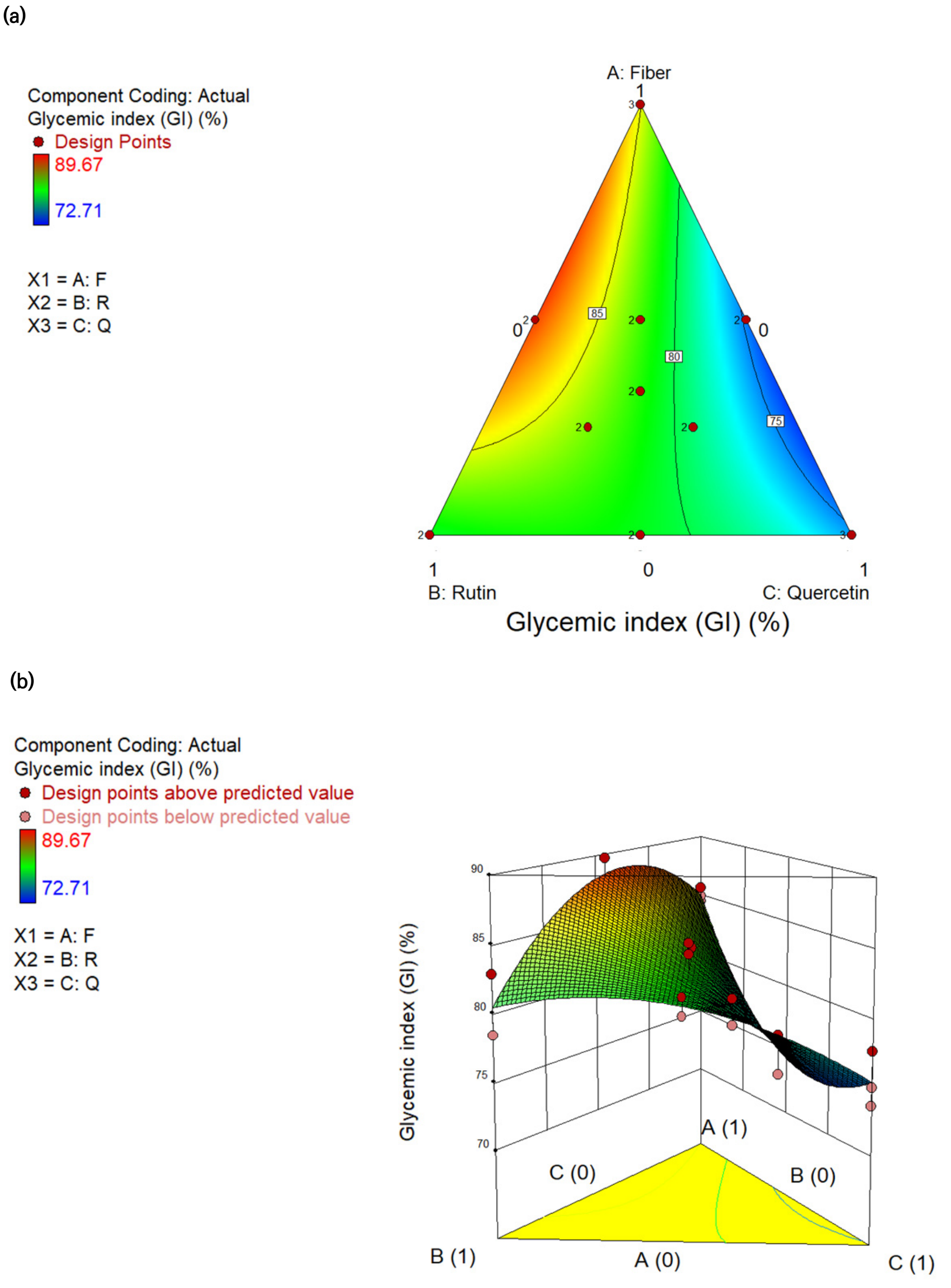

Fig. 1. Schematic plots of (a) ternary contour and (b) 3D surface plots for the response of predicted glycemic index of the fitted quadratic mixture model

Table 3. Analysis of variance (ANOVA) of the fitted quadratic mixture model for the response of predicted glycemic index

Source	Sum of squares	Degree of freedom	Mean Square	p-value
Model	355.6	5	71.11	〈 0.0001
Linear Mixture	237.7	2	118.83	〈 0.0001
AB	59.7	1	59.65	0.0002
AC	59.7	1	59.72	0.0002
BC	14.5	1	14.5	0.0309
Residual	41.4	16	2.59	
Lack of Fit	10.5	4	2.63	0.4345
Pure Error	30.9	12	2.57	0.1131

The beneficial effect of quercetin is due to partial inhibition of amylase and α-glucosidase enzyme and delay the absoption of glucose, that could ultimately control the blood glucose level [17]. The beneficial effect of dietary fiber is usually attributed to delays starch degradation in the accordance with physical mechanisms including restriction of water and entrapping starch granule [14]. Based on these properties, the synergistic effect of quercetin and dietary fiber *in vitro* starch digestibility could be explained in this study. Indeed, further study on physico- chemical charcteristics of their interaction is needed to provided to explain accurately this effect.

4. Conclusion

The objective of this work was to investigate the combined influences of rutin, quercetin and dietary fiber *in vitro* starch digestibility. That is, three components were substituted with wheat flour at the 5 levels that designed by statistical method and then their effects *in vitro* starch digestibility were analyzed. The quercetin and fiber mixture was the most effective in retarding starch hydrolysis and their ratio was obtained 72:28 by optimization tool. The various statistical models were analyzed to find valid experimental model and the regression equation which was represented synergic effect on the suppression of starch hydrolysis was obtained. Although more study is needed to investigate the interaction with quercetin and dietary fiber, its combination may be useful for lowering starch digestibility.

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