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## Response Surface Methodology Approach for the Optimisation of LAB Fermentation using Vegetable Based Substrate

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### ABSTRACT

In the present studies, an attempt was made to optimize the conditions for the lactic acid bacteria (LAB) fermentation using Irish York cabbage (*Brassica oleracea* var. capitata alba subvar. conica) as a substrate. Prior to fermentation with *Lactobacillus plantarum*, York cabbage was blanched at 95°C for 12 min to inactivate surface microflora. To achieve an optimal fermentation condition which would result in higher release of phytochemicals and antioxidant capacity in the broth, Box-Behnken design integrating a desirability approach was used. The optimized factors (fermentation time 36h, solid/liquid ratio 0.25 g/ml and agitation rate 100 rpm) were used for fermenting York cabbage. Results showed that there was  $\approx 5$  log cfu/ml increment in bacterial growth after fermentation; whereas lactic acid production reached up to 4.97 mg/ml. Fermentation retains 95-98% and 90-95% of total phenolic content (TPC) and antioxidant (AO) capacity, respectively. On contrary, none of the glucosinolates were observed in the York cabbage broth while Isothiocyanates content almost doubled after fermentation.

### INTRODUCTION

Fruit and vegetables constitute an important part of a healthy human diet. A high intake of fruit and vegetables in the diet is positively associated with the prevention of cardiovascular diseases, cancer, aging, diabetes, hypertension, and stroke (Blasa et al., 2010). Cabbage is one of the most commonly consumed vegetables in the world, which is rich in several bioactive compounds such as polyphenols, flavonoids and glucosinolates. These compounds are well-known for their various biological activities such as antimicrobial, antioxidant, anticarcinogenic properties. Fermentation is widely used in the food industry to improve the sensory characteristics of a product as well as to eliminate certain undesirable constituents. It also formulates nutrients more accessible while preserving and even improving the nutritional properties. A few reports have emphasized the fermentation of Brassica vegetables and mainly focused on spontaneous fermentation of white cabbage and their antioxidant activity (Kusznierewicz et al., 2008; Sun et al., 2009). Recently, Kusznierewicz et al. (2008) observed that the fermentation process increased the initial antioxidant activity of cabbage, which could be combines effects of wounding and chemical processes incurred by lactic acid bacteria (LAB).

The aim of the work was optimize the parameters for the lactic acid bacteria fermentation using York cabbage as a substrate. Response surface methodology was applied, and a nonlinear response surface model was proposed with high LAB, lactic acid (LA) content, TPC, total flavonoid content (TFC), and AO capacity. Finally, effect of LAB fermentation on glucosinolates and isothiocyanates were evaluated.

## MATERIALS AND METHODS

### Plant materials and their preparation

Fresh samples were purchased from a local supermarket in Dublin in January 2011. York cabbage (20-22 kg) were randomly selected and trimmed off their outer leaves and stem. The heads were then divided into four segments, and the central core was removed. The segments were chopped into 0.5 × 5-6 cm pieces, using a vegetable cutting machine. A pooled batch of about 15 kg cabbage was stored under dark refrigerated conditions (4 °C) as the raw material.

### Culture and Inoculum preparation

*L. plantarum* ATCC 8014 was purchased from Medical Supply Company, Dublin, Ireland. For the preparation of the inoculum, 25 ml of sterile MRS broth was inoculated with 1 ml of stock culture and incubated at 37°C for 12-14h. This was then serially diluted 100 times to obtain working culture containing 5-6 log CFU/ml cells as determined by plate counts.

### Fermentation based on Box-Behnken design (BBD)

In order to statistically optimize the York cabbage fermentation and evaluate main effects, interaction effects and quadratic effects of the three factors (S/L ratio, agitation rate and fermentation time) on the growth of *L. plantarum*, a design with three factors and three levels including five replicates at the centre point was used (Table 1). To optimize the fermentation, appropriate amount of cabbage was mixed with double distilled water as per the nutrient illustration (Table 1) in order to achieve the required solid to liquid (S/L) ratio. The flasks containing York cabbage and water (YCB) were blanched and inoculated with 5% inoculum (5ml) upon cooling. Uninoculated flask was kept as control for the respective batch of experiments. The flasks were incubated at 37°C at their respective agitation rate (0, 100 or 200 rpm). Three flasks were harvested at the times specified by the software and supernatant was used for all the analysis.

Table 1 Process variables and level in BBD experimental design

Independent variables	Coded variable level		
	-1	0	+1
Solid to liquid ratio (w/v)	0.05	0.15	0.25
Fermentation time (h)	8	22	36
Agitation rate (rpm)	0	100	200

### Viable cell counts

Viable cell counts in the YCB (log CFU/ml) were determined by the standard plate method with MRS medium. Dilution of 1 ml broth was carried out in 9 ml MRD to plate the suitable dilution. The plates were incubated at 37°C for 36-48h for cell enumeration.

### Analytical procedure

TPC and TFC analysis was carried out according to Jaiswal et al. (2012). Individual glucosinolates were identified using high-performance capillary electrophoresis at 230 nm (Bjergegaard et al., 1995). Total isothiocyanates was estimated according to the method of Zhang et al. (1992).

Total sugars in the test samples were estimated by the phenol-sulphuric acid method (duBois et al., 1956). Analysis of organic acid and individual sugars were done on an Alliance HPLC (Waters, e2695 Separation module). Respective standards were used to identify and quantify sugars and organic acids contents in the samples.

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging capacity (DPPH RSC) and Ferric reducing AO potential (FRAP) assay were used for the estimation of total AO capacity and analysis was carried out as reported elsewhere (Rajauria et al., 2010).

### Statistical analysis

Results are expressed as mean values  $\pm$  standard deviation (SD). Data from the BBD were subjected to a second-order multiple regression analysis using least-squares regression to obtain the parameter estimated for the mathematical model. The regression analysis and analysis of variance (ANOVA) were carried out using the STATGRAPHICS Centurion XV. Values of  $P < 0.05$  were considered as statistically significant.

## RESULTS AND DISCUSSION

Development of a new functional food requires several important factors to be considered; one of the important factors is bioactive components and its properties such as antioxidant capacity. For a fermented vegetable-based product, the desired parameters would be the final viable cell population, LA content, phytochemicals and its AO properties.

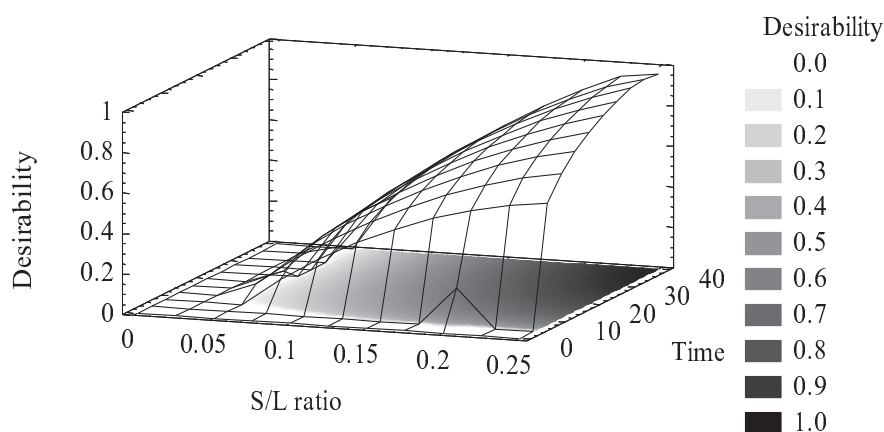


Figure 1. Response surface plots representing the effect of S/L ratio (w/v), fermentation time (h) and agitation rate (rpm) on overall desirability (high bacterial growth, LA production, TPC, TFC, DPPH scavenging capacity and FRAP). Agitation is constant at 100 rpm.

As the aim was to achieve higher bacterial growth, LA production, phytochemical content and AO capacity, the goal was set to 'maximize' with importance '5'. The values of responses were converted to a desirability function. The desirability values of the minimum and maximum yields were configured as 0 and 1, respectively. Applying the desirability function with all pre-selected goals for each factor, gave the specific value for all responses are presented in Figure 1.

The optimized factors were: fermentation time 36h, S/L ratio 0.25 g/ml and agitation rate 100 rpm. Software optimized predicted desirability and experimentally obtained value from

the confirmatory experiments which was carried in order to validate the design are presented in Table (2). The results are closely related with the data obtained from optimization analysis resulting in a very good agreement. The difference between the experimental and model predicted values was less than 5% for all the six responses. This affirms that the models developed are adequate for predicting the responses. Therefore, BBD along with the desirability function could be effectively used to optimize the S/L ratio, fermentation time and agitation rate for maximizing the targeted responses.

Results showed that both S/L ratio and fermentation time had significant effects on bacterial growth and LA production. There was  $\approx 5$  log cfu/ml increment in bacterial growth after fermentation. The growth of LAB in the YCB was comparable with the results obtained from previous workers in cabbage juice and other vegetables. Yoon et al. (2006) reported a maximum growth of *L. plantarum* of  $10^9$  CFU/ml in cabbage juice. It was also evident that some other organic acids were also produced during the fermentation such as propionic acid and citric acid while sugar content reduced continuously as the bacterial population increased with fermentation time. Fermentation resulted in a slight reduction in the TPC, TFC and AO capacity but the change was not significant.

Table 2. Software optimized predicted desirability and experimentally obtained value

Response	Predicted optimum desirability	Observed optimum desirability
<b>Bacterial counts (cfu/ml)</b>	9.98	10.31
<b>DPPH (<math>\mu\text{g AscE/ml}</math>)</b>	256.05	232.3
<b>FRAP (<math>\mu\text{g TE/ml}</math>)</b>	9.08	8.96
<b>LA production (mg/ml)</b>	4.97	5.21
<b>TFC (<math>\mu\text{g QE/ml}</math>)</b>	180.51	180.7
<b>TPC (<math>\mu\text{g GAE/ml}</math>)</b>	200.50	209.5

Finally, the effect of fermentation on glucosinolates and isothiocyanates were evaluated. Results showed that unfermented sample showed the presence of several glucosinolates such as Sinigrin, Gluconapin, Glucoiberin, Glucoraphanin, Glucotropaeolin (IS), Glucobrassicin, Neoglucobrassicin, 4-Hydroxyglucobrassicin, 4-Metoxylucobrassicin. It was observed that lactic acid bacteria affected the degradation of glucosinolates and the formation of breakdown products in fermented cabbage. Results showed that concentration of glucosinolates degradation product was increased almost double after fermentation while there was no glucosinolates observed in the broth after fermentation. It was concluded that *L. plantarum* may possess some enzymatic activity which hydrolyze glucosinolates into isothiocyanates.

## CONCLUSIONS

Application of RSM in optimization of LA fermentation helps in cutting down on time and resources for identifying the optimum value for different factors. Slight reduction in TPC, TFC and AO capacity were observed after fermentation while there was complete degradation of Glucosinolates. Isothiocyanates content almost doubled after fermentation.

The results of this study present an indication of the potential of fermentation of York cabbage using LAB with a possibility for the development of a range of functional foods.

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