2011-12-04

Statistical Optimization of Blanching Time and Temperature of Irish York Cabbage Using Desirability Function

Amit Jaiswal
Dublin Institute of Technology, amit.jaiswal@dit.ie

Nissreen Abu-Ghannam
Dublin Institute of Technology, nissreen.abughannam@dit.ie

Shilpi Gupta
Dublin Institute of Technology, shilpi.gupta@dit.ie

Recommended Citation

Gupta, S., Jaiswal, A.K. & Nissreen Abu-Ghannam (2012). Statistical optimization of blanching time and temperature of Irish York Cabbage using desirability function. *Journal of Food Processing & Preservation, doi:10.1111/j.1745-4549.2011.00574.x*

Journal of Food Processing and Preservation ISSN 1745-4549

STATISTICAL OPTIMIZATION OF BLANCHING TIME AND TEMPERATURE OF IRISH YORK CABBAGE USING DESIRABILITY FUNCTION

SHILPI GUPTA, AMIT K. JAISWAL and NISSREEN ABU-GHANNAM[1]

School of Food Science and Environmental Health, College of Sciences and Health, Dublin Institute of Technology, Cathal Brugha Street, Dublin 1, Ireland

[1]Corresponding author. TEL: 353-1-402-7570; FAX: 353-1-878-8978; EMAIL: nissreen.abughannam@dit.ie

Received for Publication January 19, 2011
Accepted for Publication June 21, 2011

doi:10.1111/j.1745-4549.2011.00574.x

ABSTRACT

The effect of different heat treatments, as a means of preprocessing, on the phytochemicals present in Irish York cabbage was studied. A comparison of blanching (by immersing in water) and microwaving (using water as a medium) indicated that microwaving is detrimental to the phytochemicals present in cabbage. To achieve a blanching time and temperature combination that would result in minimal loss of phytochemicals, central composite design that integrates a desirability approach was used. A second-order polynomial equation was developed, indicating the effect of the blanching time and temperature on the total phenol content (TPC), total flavonoid content (TFC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) values. Contour maps generated using the response surface equation showed that the experimental variables significantly affected the response. The optimized factors (85C and 2 min) were used for blanching York cabbage to obtain a TPC, TFC and half maximal effective concentration for DPPH of 31.01 mg gallic acid equivalents/g, 21.2 mg quercetin equivalents/g and 0.93 mg/mL, respectively.

PRACTICAL APPLICATIONS

Processing of cabbage is generally carried out before it can be developed into a product. The present study used statistical software to obtain the time and temperature conditions for preprocessing of cabbage. The statistical software helped in achieving conditions wherein minimal loss of phytochemicals took place. The application of the software for optimizing the conditions helped in cutting down the amount of time and resources for identifying optimum value of different factors.

INTRODUCTION

Cabbage (*Brassica oleracea* Capitata) is a leafy garden plant and is among the most important vegetables consumed worldwide due to its availability in local markets and consumer preference (Kusznierewicz *et al.* 2008). It belongs to the family of Brassicaceae (or Cruciferae) along with collards, brussels sprouts, broccoli, cauliflower and kale. According to the data from the Food and Agriculture Organization of the United Nations (2011) (FAO, http://www.fao.org), the production capacity of Brassicaceae in Ireland was around 45,000 metric tons for the year 2008. Cabbage is rich in phytochemicals such as flavonoids and glucosinolates and their hydrolysis products, and is a good source of health-promoting compounds that show preventive effect against cancer, atherosclerosis, nephritis and diabetes mellitus (Taveira *et al.* 2009). Flavonoids and phenolic acids are the most characterized groups of phenolic compounds in *Brassica*. Flavonoids protect plants against UV radiation, microorganisms and plant-feeding animals. They can act *in vitro* as scavengers of active oxygen species and electrophiles and as chelators of metal ions, and thus may be beneficial *in vivo* to reduce the risk of cardiovascular diseases (Hollman 2001). A number of flavonoids have been identified in cabbage, including myricetin, quercetin, kaempferol, luteolin, delphinidin, cyanidin and pelargonidin (Chu *et al.* 2000; Franke *et al.*

2004). Phenolic acids, such as benzoic, hydroxybenzoic, vanillic and caffeic, have antimicrobial and antifungal properties. Hydroxycinnamic acid derivatives, such as caffeic, chlorogenic, sinapic, ferulic and *p*-coumaric acids, possess strong antioxidant activity because of the inhibition of lipid oxidation and scavenging reactive oxygen species (Sroka and Cisowski 2003). Indole-3-carbinole, sulforaphane and indole from cabbage help in stabilizing the body's antioxidant and detoxification mechanisms that eradicate cancer-producing substances (Brooks *et al.* 2001).

Heat applications are a common practice in the processing of food products in household or in industries in order to render them palatable and microbiologically safe. Typical applications include using heat treatments such as blanching or microwaving. As cabbage would need to undergo some heat treatment prior to usage, it was relevant to assess the effects of heat treatment on the stability of the phytochemicals present in it. Processing can result in reduction of constituents by leaching or due to thermal destruction (Rungapamestry *et al.* 2006). The processing methods might liberate the natural bioactive compounds (Duh *et al.* 2001; Lombard *et al.* 2005; Turkmen *et al.* 2005) or might reduce (Ismail *et al.* 2004) them in comparison with fresh foods. The degree to which phytochemicals change during processing depends on their sensitivity to modifications or degradations and length of exposure to a processing technique (Breena 1994). Puupponen-Pimiä *et al.* (2003) found that blanching reduced the antioxidant capacity by 23% for cauliflower, but increased it by 9% for cabbage. Wu *et al.* (2004) found a reduction of 14% in oxygen radical absorbance capacity values for cooked broccoli and an increase of 41% for cooked red cabbage. Ismail *et al.* (2004) found that thermal treatment decreased the total phenolic content (TPC) in all vegetables such as kale, spinach, cabbage, swamp cabbage and shallots. The evaluation of the influence of food processing is a key factor while establishing technological conditions that enable one to preserve or improve the original activity and bioavailability of naturally occurring compounds in foods (Kusznierewicz *et al.* 2008). Thus, it is important to optimize the processing conditions such that the processed product still retains its health-promoting properties. Proper combination of time and temperature during processing methods such as blanching or microwaving is important in order to minimize quality loss during processing. These methods might cause undesirable changes on the physicochemical properties, such as color, texture or bioactive compounds, on account of heat-induced diffusion or leaching losses. Thus, it is important to optimize the time and temperature of any processing method in order to achieve minimal loss of quality. The "one-at-a-time-approach" can be used to optimize the processing time and temperature in order to obtain conditions that would result in minimal loss of the bioactive compounds. However, this method is extremely time-consuming and disregards the complex interactions among various physicochemical parameters (Abdel-Fattah *et al.* 2005). Response surface methodology (RSM) is a collection of mathematical and statistical techniques for searching optimum conditions of factors for desirable responses and evaluating the relative significance of several affecting factors even in the presence of complex interactions. The design leads to the generation of contour plots by linear or quadratic effects of the key variables, and a model equation is derived that fits the experimental data to calculate the optimal response of the system.

This work is part of an ongoing project to evaluate the potential of Irish York cabbage as a substrate for the development of a fermented product. However, before fermentation, it is important to process the raw material in order to render them free from of contaminating microflora and to make them slightly palatable. At the same time, the processed product should still retain its health-promoting properties. Hence, in the present study, processing was tried with microwaving and blanching. Thereafter, RSM was used to optimize the time and temperature of blanching to obtain conditions that would result in minimal loss of phytochemicals as compared with the raw cabbage. A desirability function was used to simultaneously optimize the responses and to locate the optimal values for the blanching conditions.

MATERIALS AND METHODS

Chemicals

Folin–Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium carbonate, aluminum chloride, sodium nitrate and quercetin were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). All other chemicals used were of analytical grade.

Plant Material

Fresh Irish York cabbage was purchased from a local supermarket in Dublin in December 2009. Immediate outer leaves that get spoiled during transportation were discarded and the layers of leaves after that were chopped into 0.5 × 5- to 6-cm pieces using a vegetable cutting machine.

Processing Treatments

Blanching Treatment. Blanching was carried out by immersing cabbage in hot water in a wire mesh basket (cylindrical; 10 cm in diameter and 15 cm in height). The basket containing 50 g cut cabbage was immersed in a thermostatically controlled water bath (±0.5C) containing 5 L of water. For preliminary experiments, blanching was carried out at 80 and 100C for 4, 8 and 12 min. The blanching time was

TABLE 1. LEVEL AND CODE OF INDEPENDENT VARIABLES, TIME AND TEMPERATURE USED FOR CENTRAL COMPOSITE EXPERIMENTAL DESIGN

Independent variables	Coded symbols	Levels				
		–2	–1	0	+1	+2
Temperature (C)	X_1	83	85	90	95	97
Time (min)	X_2	0.34	2	6	10	12

measured as soon as the vegetables were placed inside the water at respective temperatures. The blanched material was drained, cooled in ice water (1–4C) for 1 min and then allowed to drain for 30 s.

Microwave Treatment. Microwave heating using water as a medium was carried out in a domestic microwave (Sharp, model R 244; Sharp Electronics, U.K.) with a maximum output power of 800 W. A 50 g cabbage sample prepared as described in the Plant Material section was placed in a 500-mL beaker filled with 100 mL of deionized water. These were then placed inside the microwave oven at 400 and 800 W for 4, 8 and 12 min. The microwaving time was noted as soon as the vegetables were placed inside the microwave at the respective power (W). The microwaved material was drained, cooled in ice water (1–4C) for 1 min and then allowed to drain for 30 s.

Experimental Design and Evaluation

RSM was applied to optimize the blanching time and temperature (Table 1) for achieving conditions that result in minimal loss of the phytochemicals present in cabbage (Table 1) using Design Expert (version 5.0.9) software (Stat-Ease Corporation, Minneapolis, MN). A 2^n factorial central composite design (CCD) with two factors and five levels, including five replicates at the center point, was used for fitting a second-order response surface. CCD coupled with a polynomial model is a very powerful combination that usually provides an adequate representation of most continuous response surfaces over a relatively broad factor domain. CCD uses the method of least squares regression to fit the data to a quadratic model. The quadratic model for each response was as follows:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \sum \beta_{ij} X_i X_j \qquad (1)$$

where Y is the predicted response, β_0 is a constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient of variables i and j, and X_i and X_j are independent variables. The software uses this quadratic model to build the response surfaces. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher test value (F value) obtained from the analysis of variance (ANOVA) that was generated by the software. Statistical significance of the model and model parameters were determined at the 5% probability level ($\alpha = 0.05$). Three-dimensional response surface plots and contour plots were generated by keeping one response variable at its optimal level and plotting that against two factors (independent variables). The independent variables selected are shown in Table 1 along with their low, medium and high levels.

Optimization of the Factors

The multiresponse analysis of response surface design using desirability approach was used to optimize the blanching time and temperature. Multiresponse analysis involves first building an appropriate response surface model for each response and then trying to find a set of operating conditions that in some sense optimize all responses or at least keep them in the desired ranges. After fitting the models and residual analysis of all responses, the desirability function was used for optimization of these multiresponses simultaneously. The general approach is to first convert each response into an individual desirability function d_i that varies for the range $0 \leq d_i \leq 1$, wherein if the response is at its goal or target, then $d_i = 1$, and if the response is outside the acceptable region, then $d_i = 0$. Then, the design variables are chosen to maximize the overall desirability:

$$D = (d_1 \times d_2 \times d_3 \times \ldots d_n)^{\frac{1}{n}} \qquad (2)$$

where n is the number of responses.

Preparation of Extracts

The processed sample was submerged in liquid nitrogen and ground to a coarse powder using mortar and pestle. Crushed cabbage (5 g) was extracted using 60% methanol with 1-min nitrogen flushing. Flasks were kept in a shaking incubator (Innova 42; Mason Technology, Dublin, Ireland) at 100 rpm and 40C for 2 h. The infusions were filtered and evaporated to dryness in a multi-evaporator (Syncore Polyvap; Mason Technology) and stored at –20C until used. All extractions were carried out in triplicate.

Phytochemical Analysis

TPC. The amount of total phenolic compounds in the crude methanol extract was determined using the Folin–Ciocalteu phenol reagent (Taga *et al.* 1984). The absorbance of all sample solutions against the blank reagent was determined at 720 nm with a spectrophotometer (Genesys 20; Thermo

TABLE 2. COMPARISON OF TOTAL PHENOL CONTENT AND TOTAL FLAVONOID CONTENT UNDER DIFFERENT MICROWAVING AND BLANCHING CONDITIONS

Time (min)	Microwave (W)				Blanching (C)			
	Total phenol*		Total flavonoid†		Total phenol*		Total flavonoid†	
	400 W	800 W	400 W	800 W	80C	100C	80C	100C
4	20.1 ± 0.6[a]	24.6 ± 1.7[a]	14.4 ± 0.6[a]	13.1 ± 0.6[a]	23.6 ± 0.6	23.6 ± 0.2[a]	17.9 ± 1.9[a]	16.7 ± 0.7[a]
8	17.2 ± 2.6[a]	21.7 ± 0.4[b]	13.8 ± 1.2[a]	10.6 ± 1.6[b]	24.9 ± 0.9	23.5 ± 0.4[a]	17.5 ± 1.2[a]	19.2 ± 1.9[bc]
12	12.3 ± 2.9[b]	15.8 ± 1.3[c]	10 ± 1.2[b]	7.5 ± 1.2[c]	25.4 ± 1.3	33.9 ± 0.4[a]	17.5 ± 1.2[a]	22.1 ± 1.9[c]

* mg GAE/gm dry extract.
† mg QE/gm dry extract.
[a–c] Means within a column are significantly different.
GAE, gallic acid equivalents; QE, quercetin equivalents.

Spectronic, Madison, WI). The TPC of the cabbage was expressed as mg gallic acid equivalents (GAE)/g dry extract.

Total Flavonoid Content (TFC). The TFC was determined by a colorimetric method described by Liu *et al.* (2009). Briefly, 0.25 mL of extract from stock, 1.25 mL of deionized water and 0.075 mL of NaNO2 (5%) solution were mixed in a test tube. After 6 min, 0.15 mL of 10% solution of monohydrate $AlCl_3$ was added and allowed to stand for another 5 min. Finally, 0.5 mL of NaOH (1 M) was added and the volume of reaction mixture was made up to 2.5 mL and mixed well. The absorbance was recorded immediately at 510 nm using the spectrophotometer against the blank. TFC was expressed as mg quercetin equivalents (QE)/g dry extract.

Antioxidant Analysis

DPPH Radical-Scavenging Assay. This assay was carried out as described by Yen and Chen (1995), with some modifications. Briefly, the assay was performed in a 96-well round-bottom microplate with 1:1 ratio of 100 µl of DPPH radical solution (165 µM) and 100 µl of sample. Different concentrations were tested for each sample in order to get half maximal effective concentration (EC_{50}) value. The DPPH solution was freshly prepared for each experiment in methanol. The reaction mixtures were incubated for 30 min at 25C in dark conditions, and the absorbance was measured at 517 nm in a microplate reader (Powerwave; BioTek, Winooski, VT). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging capacity } (\%) = \left(1 - \left[\frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}}}\right]\right) \quad (3)$$

where A_{control} is the absorbance of the control (DPPH solution without sample), A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and $A_{\text{sample blank}}$ is the absorbance of the sample only (sample without any DPPH solution). The calculated EC_{50} values indicate the concentration of sample required to scavenge 50% DPPH radicals. The lower the EC_{50} value of the sample, the higher the antioxidant capacity.

Statistical Analysis

All experiments were carried out in triplicate and replicated at least twice. Results are expressed as mean ± standard deviation. All statistical analyses were carried out using STATGRAPHICS Centurion XV. Statistical differences between extract activities were determined using ANOVA, followed by least significant difference testing. Differences were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Blanching and Microwaving on the Phytochemicals

The values of total phenol and total flavonoid in fresh York cabbage were 33.5 ± 0.7 mg GAE/g dry extract and 21.6 mg QE/g dry extract, respectively. TPC was higher than those reported in other studies (Volden *et al.* 2009a). The values were slightly lower than those reported for red cabbage (Volden *et al.* 2008). The aim of the present study was to find out a preprocessing method that would result in a reduction in the loss of phytochemicals from cabbage. For this, a preliminary study was carried out wherein cabbage leaves were blanched and microwaved. Following heat treatment, the cabbage leaves were extracted with 60% methanol, and TPC and TFC of the extract were studied. In order to determine which heat treatment method will result in a lesser destruction of the phytochemicals as compared with raw cabbage, fresh cabbage was blanched (80 and 100C) and microwaved (400 and 800 W) for 4, 8 and 12 min. It was noted that both heat treatments resulted in a reduction in the TPC and TFC (Table 2), and microwave heating was found to be more destructive. ANOVA showed significant differences

($P < 0.05$) in the TPC and TFC between fresh and heat treated cabbage. Heat processing can induce significant changes in the phenolic content and it could be the result of processes such as thermal degradation (autoxidation or breakdown), diffusion or/and leaching (Lindley 1998; Amin *et al.* 2006). Microwaving at 400 and 800 W resulted in 40–63% and 26.5–53% reduction in TPC, respectively. Blanching at 80C resulted in 24–29% reduction in TPC. When the blanching was carried out at 100C for 4 min, a reduction of 29% was seen, but heating for higher times resulted in almost equivalent TPC as compared with the control samples. Xu and Chang (2008) reported a loss of 40–50% in the phenolic content of green pea, yellow pea and chick pea due to leaching of phenolics into boiling water as compared with raw peas. Zhang and Hamauzu (2004) found a 72% reduction in TPC in broccoli florets boiled for 5 min using a vegetable-to-water ratio of 1:20. This is much larger than the losses found in our study for both blanched (vegetable-to-water ratio of 1:100) and microwaved (vegetable-to-water ratio of 1:10) samples.

In the case of TFC, similar results were seen wherein microwaving resulted in a 33.5–65.9% reduction in TFC for all the combinations studied. Blanching resulted in a reduction of 11–23% in TFC and almost similar TFC was obtained as compared with control when blanching was carried out at 80C for 12 min. Thermal processing might have resulted in a loss of phytochemicals from the cabbage to the treatment water. The results showed microwaving to be more destructive of phytochemicals. Similar results have been found by other researchers. Ismail *et al.* (2004) found that thermal treatment decreased the TPC in all vegetables, such as kale, spinach, cabbage, swamp cabbage and shallots, and antioxidant activity in some of them. Puupponen-Pimiä *et al.* (2003) reported a 13% reduction in TPC upon blanching and 10–21% reduction was reported by Volden *et al.* (2009b). Sultana *et al.* (2008) reported that different cooking methods affect the antioxidant properties of vegetables, with microwave cooking showing the most deleterious effect than other methods. Ceretani *et al.* (2009) also reported a loss of phenolic content in olive oil after microwave heating.

However, in spite of all the reductions, blanching at 100C for 12 min resulted in 44 and 31% increase in TPC and TFC, respectively. Although the TPC and TFC were reduced as compared with raw cabbage, however, the content was found to increase as the blanching time was increased. Generally, phenolic compounds in fruits and vegetables are bonded to dietary fiber, proteins or to sugars in plants to form complex structures. It could be possible that higher temperature resulted in the breakdown of these complexes, thus increasing their extractability (Gawlik-Dziki 2008). Roy *et al.* (2009) also reported that an increase in phenolic and flavonoid content in broccoli due to heat could be a result of the disruption of cell wall and cell membranes, which ultimately release phytochemicals from the insoluble portions. As blanching resulted in better retention of phytochemicals than microwaving, optimization using RSM was applied to the blanching process with respect to time and temperature.

Statistical Analysis of Results Obtained by Experimental Design

The aim of this work was to focus on optimizing preprocessing conditions of cabbage in preparation for further product development. As cabbage is rich in many antioxidant compounds, it is important for the processed product to have bioactives comparable with those present in the raw vegetable. Time and temperature combination used for the processing of vegetables is very crucial if the aim is to optimize the concentration of bioactive compounds. To perform such work using conventional techniques such as the "one-factor-at-a-time" method is extremely laborious and time-consuming; moreover, such methods do not guarantee the determination of optimal conditions and are unable to detect synergistic interactions, if any, between these two factors. Thus, RSM was used for the optimization of these parameters.

The 13 experiments proposed by the RSM with two factors and five levels (Table 3), including five replicates at the center point, were used for fitting a second-order response surface model. The five center point runs provided a measure of process stability and inherent variability. The variation in the bioactives under different conditions is shown in Table 3.

Effect of Process Variables on TPC. Experimental results for TPC were fitted to a full quadratic second-order polynomial equation by applying multiple regression analysis (Eq. 4), and the regression coefficients obtained to predict the polynomial model for TPC are as follows:

$$TPC = 25.47 + 0.53X_1 - 1.27X_2 - 0.81X_1^2 + 3.04X_2^2 + 1.95X_1X_2 \quad (4)$$

When the values of X_1–X_2 were substituted in the above equation, the predicted TPC (Y) was obtained. A comparison of predicted and experimentally obtained values can be seen in Table 3.

In order to determine whether or not the quadratic model is significant in simulating the experimental results, ANOVA was conducted. The P values were used as a tool to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the P values are, the higher the significance of the corresponding coefficient (Murthy *et al.* 2000). The corresponding P values suggest that among the test variables used in this study, $(X_2)^2$ (time × time) is a significant model term with P values of less than 0.05 (Table 4). Thus, time is the major limiting factor for TPC and a small variation can alter the content. The goodness

TABLE 3. CENTRAL COMPOSITE DESIGN WITH EXPERIMENTAL AND PREDICTED VALUES OF TOTAL PHENOL CONTENT, TOTAL FLAVONOID CONTENT AND DPPH

		TPC (mg GAE/g [DE])		TFC (mg QE/g [DE])		DPPH (mg/mL)	
Temp (C)	Time (min)	Exp[a]	Pred[b]	Exp[a]	Pred[b]	Exp[a]	Pred[b]
90	6	27	25.47	16.04	15.53	1.1	1.15
90	0.34	33.48	33.34	20.21	20.7	0.90	0.94
83	6	24.2	23.11	17.6	17.6	1.06	1.13
90	6	24.7	25.47	15	15.53	1.12	1.29
85	10	25.07	24.85	18.33	18.17	1.33	12.9
97	6	21.74	24.6	16.46	17.47	1.08	1.02
85	2	28.26	29.49	20.42	20.28	0.98	0.91
90	6	25.65	25.47	15	15.53	1.18	1.15
90	6	24	25.47	15.62	15.53	1,240	1.15
95	2	30	28.45	18.75	17.9	1,024.6	1.05
95	10	31.01	28	21.25	20.37	934.5	0.99
90	6	26	25.47	16	15.53	1,098.7	1.15
90	12	27.83	29.74	20.42	20.95	1,197.2	1.17

[a] Experimental values.
[b] Predicted values.
DPPH, 2,2-diphenyl-1-picrylhydrazyl; DE, dry extract; GAE, gallic acid equivalents; QE, quercetin equivalents; TFC, total flavonoid content; TPC, total phenol content.

of fit of the model was examined by *F*-test and the determination coefficient R^2. The greater the *F* value is from unity, the more certain it is that the factors explain adequately the variation in the data around its mean, and the estimated factor effects are real. The ANOVA (Table 5) showed that this regression model was highly significant ($P < 0.01$), as is evident from the Fisher, *F*-test (F_{model}, the ratio of mean square regression to mean square residual is 4.18), and has a low probability value ($[P_{model} > F] = 0.0445$). The value of 0.0521 for lack of fit implies that it is not significant as compared with the pure error, and that the model equation was adequate for predicting the TPC. The fitness of the model was further con-

TABLE 4. COEFFICIENT ESTIMATE, STANDARD ERROR AND *P* VALUES FOR TOTAL PHENOL CONTENT, TOTAL FLAVONOID CONTENT AND DPPH

	Total phenol content			Total flavonoid content			DPPH		
	Estimate	SE*	*P* value	Estimate	SE*	*P* value	Estimate	SE*	*P* value
Intercept	25.47	0.95	–	15.53			1.15	0.032	
A†	0.53	0.75	0.5063	–0.045	–0.17	0.8724	–0.039	0.026	0.1685
B‡	–1.27	0.75	0.1338	0.088	0.33	0.7546	0.084	0.026	0.0136
A^2	–0.81	0.8	0.349	1.00	3.44	0.0109	–0.038	0.027	0.21
B^2	3.04	0.8	0.0069	2.64	9.07	<0.0001	–0.048	0.027	0.1244
AB	1.05	1.06	0.355	1.15	2.08	0.0204	–0.11	0.036	0.0195

* SE: standard error.
† A: temperature.
‡ B: time.
DPPH, 2,2-diphenyl-1-picrylhydrazyl.

TABLE 5. ANALYSIS OF VARIANCE AND REGRESSION ANALYSIS FOR TOTAL PHENOL CONTENT, TOTAL FLAVONOID CONTENT AND DPPH

	Total phenol content					Total flavonoid content					DPPH				
Source	SS[a]	DF[b]	MS[c]	*F* value	Prob > *F*	SS[a]	DF[b]	MS[c]	*F* value	Prob > *F*	SS[a]	DF[b]	MS[c]	*F* value	Prob > *F*
Model	93.89	5	18.78	4.18	0.0445	57.06	5	11.41	19.31	0.0006	0.14	5	0.028	5.31	0.0247
Residual	31.48	7	4.5			4.14	7	0.59			0.036	7	0.005		
Lack of fit	26.07	3	8.69	6.43	0.0521	3.09	3	1.03	3.91	0.1103	0.022	3	0.007	2	0.257
Pure error	5.41	4	1.35			1.05	4	0.26			0.015	4	0.003		
Total	125.37	12				61.20	12				0.17	12			

[a] Sum of squares.
[b] Degrees of freedom.
[c] Mean square.
DPPH, 2,2-diphenyl-1-picrylhydrazyl.

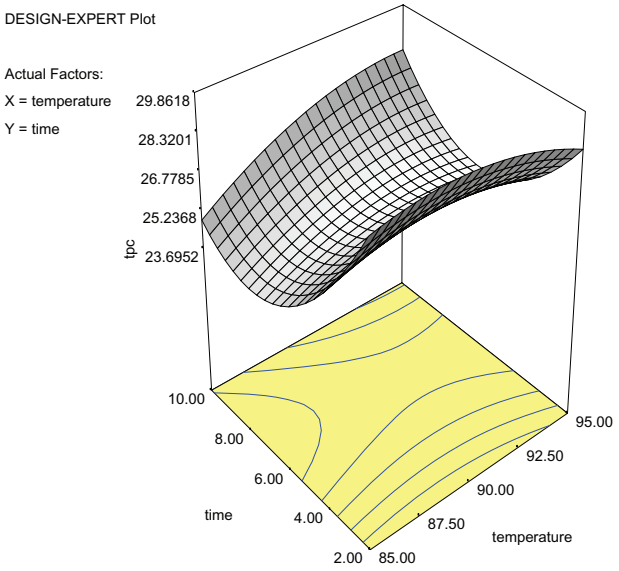


FIG. 1. SURFACE PLOT OF THE TOTAL PHENOLIC COMPOUNDS AS A FUNCTION OF BLANCHING TIME AND TEMPERATURE

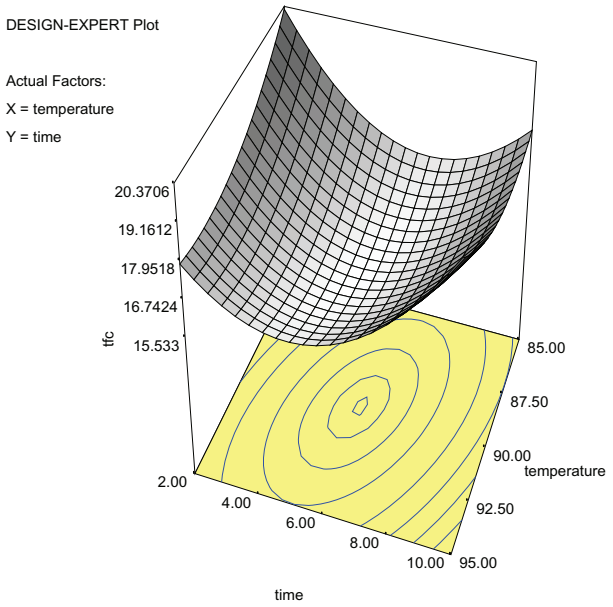


FIG. 2. SURFACE PLOT OF THE TOTAL FLAVONOID COMPOUNDS AS A FUNCTION OF BLANCHING TIME AND TEMPERATURE

firmed by a satisfactory value of determination coefficient, which was calculated to be 0.7489, indicating that 74.89% of the variability in the response could be predicted by the model. Furthermore, the predicted TPC by the final quadratic model, along with the corresponding values observed, is given in Table 3. The agreement between the log colony-forming units (cfu)/mL predicted by the model and the experimental data is good, as shown by a fine value of correlation coefficient, R (0.8654).

The three-dimensional response surfaces were generated to study the interaction among the two factors tested and to visualize the combined effects of factors on the TPC (Fig. 1). The interactions between the variables can be inferred from the shapes of the contour plots (Yu *et al.* 2008). Circular contour plots indicate that the interactions between the variables are negligible. In contrast, elliptical- or saddle-shaped contours indicate the evidence of the interactions (Fig. 1) (Liu *et al.* 2010). As shown in Fig. 1, an increase in blanching time reduces the TPC up to 5 min. Similar values were obtained for different temperatures at similar blanching times. However, from 6 to 10 min, a reverse trend was obtained, wherein an increase in blanching time resulted in an increase in TPC.

Effect of Process Variables on TFC. Experimental results for TFC were fitted to a full quadratic second-order polynomial equation by applying multiple regression analysis (Eq. 5), and the regression coefficients obtained to predict the polynomial model for TFC are as follows:

$$TFC = +15.53 - 0.045X_1 + 0.088X_2 + 1.00X_1^2 + 2.64X_2^2 + 1.15X_1X_2 \quad (5)$$

When the values of X_1–X_2 were substituted in the above equation, the predicted TFC (Y) was obtained. A comparison of predicted and experimentally obtained values can be seen in Table 3. The corresponding P values suggest that among the test variables used in this study, $(X_1)^2$ (temperature × temperature), $(X_2)^2$ (time × time) and $(X_1 \times X_2)$ (temperature × time) are the significant model terms with P values of less than 0.05 (Table 4). The ANOVA (Table 5) showed that this regression model was highly significant ($P < 0.01$), as is evident from the Fisher, F-test (F_{model} is 19.33), and has a low probability value ($[P_{model} > F] = 0.0006$). The value of 0.1103 for lack of fit implies that it is not significant in comparison with the pure error, and that the model equation was adequate for predicting the TFC. The fitness of the model was further confirmed by a satisfactory value of determination coefficient, which was calculated to be 0.9324, indicating that 93.24% of the variability in the response could be predicted by the model. Furthermore, the predicted and experimental TFC values are given in Table 3. The agreement between the TFC predicted by the model and the experimental data is very strong, as shown by a high value of correlation coefficient, R (0.9658).

As shown in Fig. 2, the symmetrical sunken shape contour with the maximum response at the central contour indicates that there was a significant interaction between temperature and time for TFC. The response surface of TFC gradually

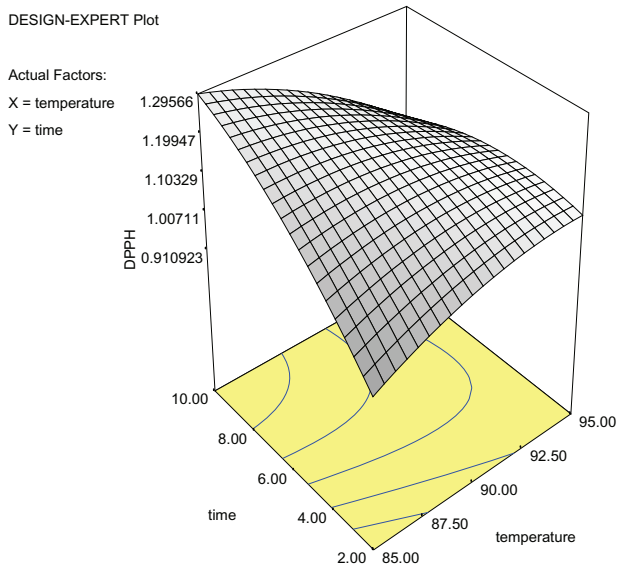


FIG. 3. SURFACE PLOT OF 2,2-DIPHENYL-1-PICRYLHYDRAZYL AS A FUNCTION OF BLANCHING TIME AND TEMPERATURE

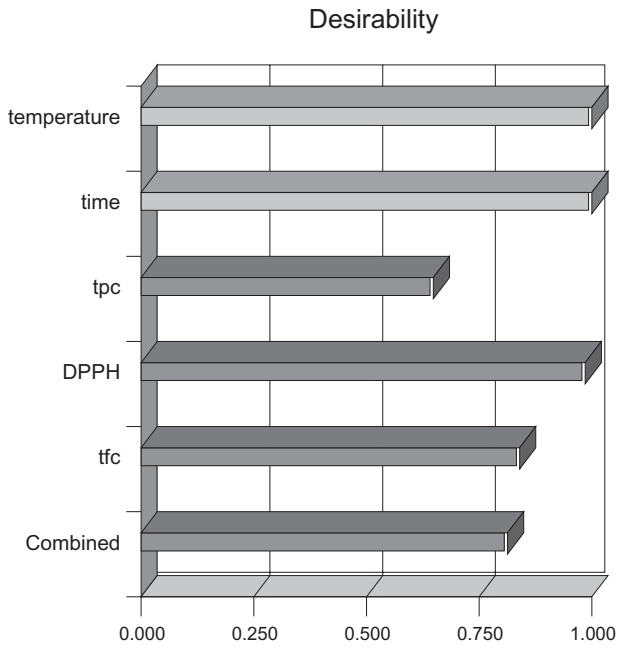


FIG. 4. BAR GRAPH REPRESENTING INDIVIDUAL DESIRABILITY OF ALL RESPONSES (d_i) IN CORRESPONDENCE WITH COMBINED DESIRABILITY (d)

decreased with increasing temperature from 85 up to 90C for any value of time and again gradually increased after 95C.

Effect of Process Variables on DPPH. A quadratic second-order polynomial equation was obtained for DPPH by applying multiple regression analysis (Eq. 6) and predicted the polynomial model for DPPH as follows:

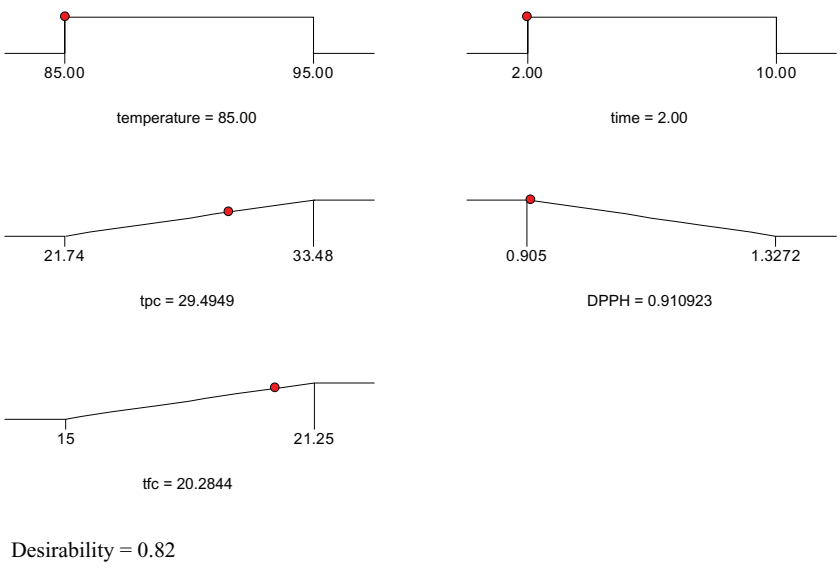
$$DPPH = +1.15 - 0.039X_1 + 0.084X_2 - 0.038X_1^2 - 0.048X_2^2 - 0.11X_1X_2 \quad (6)$$

A comparison of predicted and experimentally obtained values can be seen in Table 3. The corresponding P values suggest that among the test variables used in this study, (X_2) (time) and ($X_1 \times X_2$) (temperature × time) are the significant model terms with P values of less than 0.05 (Table 4). The ANOVA (Table 5) showed that this regression model was highly significant ($P < 0.01$), as is evident from the Fisher, F-test (F_{model} is 5.31), and has a low probability value ($[P_{model} > F] = 0.0247$). The value of 0.2570 for lack of fit was not significant compared with the pure error. The value of determination coefficient (0.7914) was satisfactory along with a good value of correlation coefficient ($R = 0.8896$).

Figure 3 shows the effect of time and temperature on the DPPH levels. Time and temperature have inverse effects on the DPPH levels. The EC_{50} values were reduced as the temperature increased and as the time decreased. A lower value of EC_{50} (concentration of sample required to scavenge 50% free radical) indicates a higher antioxidant capacity. EC_{50} value is negatively related to the antioxidant activity as it expresses the amount of antioxidant required to reduce the radical concentration by 50%. Thus, high temperature and shorter time seem to be favorable for a high antioxidant capacity. This could be the result of the formation of Maillard reaction products, which have been shown to have potent antioxidant activities (Turkmen *et al.* 2005). Similar to the trends obtained in the present study, Amin *et al.* (2006) also reported that the antioxidant activity of blanched spinach was reduced as the blanching time increased. Chu *et al.* (2000) reported that blanching for less than 1 min would retain the high antioxidant activity in the green leaves of sweet potatoes.

Optimization of the Blanching Conditions

The above experiments showed the effect of different blanching combinations of blanching time and temperature on the phytochemicals in cabbage. Thus, in order to achieve a combination wherein minimal loss of phytochemicals was occurring, optimization was carried out using Numerical option of the Design expert software. The values of responses were converted into a desirability function. The desirability values of the minimum and maximum yields were configured as 0 and 1, respectively, and then all the other response yields could be converted into desirability values between 0 and 1. The desired goal was selected by adjusting the weight or importance that might alter the characteristics of a goal. The goal fields for response have five options: none, maximum, minimum, target and within range. For each goal, the importance can be varied



Colour online, B&W in print

FIG. 5. DESIRABILITY RAMP FOR NUMERICAL OPTIMIZATION FOR DIFFERENT FACTORS AND RESPONSES

from 1 (less importance) to 5 (maximum importance). As the aim was to achieve higher concentration of TPC and TFC, the goal was set to "maximize" with importance "5." For DPPH, the goal was set to "minimize" as lesser DPPH value means higher antioxidant capability. The individual desirability function (d_i) for each of the responses and the calculated geometric mean as maximum over all desirability ($D = 0.82$) are represented in Fig. 4. Applying the desirability function with all the preselected goals for each factor gave the specific value for all responses, which are presented in Fig. 5. The software optimized 29.5 mg GAE/gm TPC, 20.3 mg QE/gm TFC and EC_{50} of 0.91 mg/mL with the optimized factors of 2 min blanching time at 85C. Finally, for their validation, duplicate confirmatory experiments were conducted using the optimized parameters. The experimentally obtained values of TPC, TFC and DPPH were 31.01 mg GAE/gm, 21.2 mg QE/g and 0.93 mg/mL, respectively. The results are closely related to the data obtained from optimization analysis, resulting in a very good agreement. The difference between the experimental and model predicted values is less than 5% for all the three responses. This affirms that the models developed are precisely adequate for predicting the responses. Therefore, CCD along with the desirability functions could be effectively used to optimize the blanching time and temperature conditions for maximizing the phytochemical content in processed vegetables. The utilization of the software was very helpful in achieving a combination of temperature and time that could result in almost similar phytochemical content as compared with fresh cabbage (TPC: 33.5 mg GAE/g; TFC = 21.6 mg QE/g; and DPPH: 0.827 mg/mL).

CONCLUSION

The present study investigated the effect of two heat processing methods (microwaving and blanching) on the phytochemical content of York cabbage as a pretreatment method prior to its further utilization and applications. Microwaving was seen to have more deleterious effect on the phytochemicals as compared with blanching. Blanching time and temperature were optimized by RSM in order to achieve conditions that result in minimal loss in phytochemicals. An empirical model was developed to simulate TPC, TFC and DPPH in terms of blanching time and temperature (factors) by RSM, and an ANOVA test was performed, which showed a good fitting of the model. The application of RSM in optimization of blanching time and temperature helps in cutting down the amount time and resources for identifying optimum value of different factors and allows better understanding of the interaction between the variables.

ACKNOWLEDGMENT

The authors would like to acknowledge funding from the Irish Government under the Technological Sector Research Scheme (Strand III) of the National Development Plan.

REFERENCES

ABDEL-FATTAH, Y.R., SAEED, H.M., GOHAR, Y.M. and EL-BAZ, M.A. 2005. Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs. Proc. Biochem. *40*, 1707–1714.

AMIN, I., NORAZAIDAH, Y. and EMMY HAINIDA, K.I. 2006. Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. Food Chem. *94*, 47–52.

BREENA, W.M. 1994. Healthfulness and nutritional quality of fresh versus processed fruits and vegetables: A review. J. Foodserv. Syst. *8*, 1–45.

BROOKS, J.D., PATON, V.G. and VIDANES, G. 2001. Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. Cancer Epidemiol. Biomarkers Prev. *10*, 949–954.

CERRETANI, L., BENDINI, A., RODRIGUEZ-ESTRADA, M.T., VITTADINI, E. and CHIAVARO, E. 2009. Microwave heating of different commercial categories of olive oil: Part I. Effect on chemical oxidative stability indices and phenolic compounds. Food Chem. *115*, 1381–1388.

CHU, Y.H., CHANG, C.L. and HSU, H.F. 2000. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric. *80*, 561–566.

DUH, P.D., YEN, G.C., YEN, W.J. and CHANG, L.W. 2001. Antioxidant effects of water extracts from Barley (*Hordeum vulgare* L.) prepared under different roasting temperatures. J. Agric. Food Chem. *49*, 1455–1463.

FRANKE, A.A., CUSTER, L.J., ARAKAKI, C. and MURPHY, F.P. 2004. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. J. Food Compost. Anal. *17*, 1–35.

GAWLIK-DZIKI, U. 2008. Effect of hydrothermal treatment on the antioxidant properties of broccoli (*Brassica oleracea* var. *botrytis italica*) florets. Food Chem. *109*, 393–401.

HOLLMAN, P.C.H. 2001. Evidence for health benefits of plant phenols: Local or systemic effects? J. Sci. Food Agric. *81*, 842–852.

ISMAIL, A., MARJAN, Z.M. and FOONG, C.W. 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chem. *87*, 581–586.

KUSZNIEREWICZ, B., BARTOSZEK, A., WOLSKA, L., DRZEWIECKI, J., GORINSTEIN, S. and NAMIESNIK, J. 2008. Partial characterization of white cabbages (*Brassica oleracea var. capitata f. alba*) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins. LWT – Food Sci. Technol. *41*, 1–9.

LINDLEY, M.G. 1998. The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. Trends Food Sci. Technol. *9*, 336–340.

LIU, S.C., LIN, J.T., WANG, C.K., CHEN, H.Y. and YANG, D.J. 2009. Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. Food Chem. *114*, 577–581.

LIU, J., LUO, J., YE, H., SUN, Y., LU, Z. and ZENG, X. 2010. Medium optimization and structural characterization of exopolysaccharides from endophytic bacterium *Paenibacillus polymyxa* EJS-3. Carbohydr. Polym. *79*, 206–213.

LOMBARD, K., PEFFLEY, E., GEOFFRIAU, E., THOMPSON, L. and HERRING, A. 2005. Quercetin in onion (*Allium cepa* L.) after heat-treatment simulating home preparation. J. Food Compost. Anal. *18*, 571–581.

MURTHY, M.S.R., SWAMINATHAN, T., RAKSHIT, S.K. and KOSUGI, Y. 2000. Statistical optimization of lipase catalyzed hydrolysis of methyloleate by response surface methodology. Bioprocess Eng. *22*, 35–39.

PUUPPONEN-PIMIÄ, R., HÄKKINEN, S.T., AARNI, M., SUORTTI, T., LAMPI, A.M., EUROLA, M., PIIRONEN, V., NUUTILA, A.M. and OKSMAN-CALDENTEY, K.M. 2003. Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. J. Sci. Food Agric. *83*, 1389–1402.

ROY, M.K., JUNEJA, L.R., ISOBE, S. and TSUSHIDA, T. 2009. Steam processed broccoli (*Brassica oleracea*) has higher antioxidant activity in chemical and cellular assay systems. Food Chem. *114*, 263–269.

RUNGAPAMESTRY, V., DUNCAN, A.J., FULLER, Z. and RATCLIFFE, B. 2006. Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (*Brassica oleracea* var. *capitata*) cooked for different durations. J. Agric. Food Chem. *54*, 7628–7634.

SROKA, Z. and CISOWSKI, W. 2003. Hydrogen peroxide scavenging antioxidant and anti-radical activity of some phenolic acids. Food Chem. Toxicol. *41*, 753–758.

SULTANA, B., ANWAR, F. and IQBAL, S. 2008. Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan. Int. J. Food Sci. Technol. *43*, 560–567.

TAGA, M.S., MILLER, E.E. and PRATT, D.E. 1984. Chia seeds as a source of natural lipid antioxidants. J. Am. Oil Chem. Soc. *61*, 928–931.

TAVEIRA, M., PEREIRA, D.M., SOUSA, C., FERRERES, F., ANDRADE, P.B., MARTINS, A., PEREIRA, J.A. and VALENTÃO, P. 2009. *In vitro* cultures of *Brassica oleracea L. var. costata* DC: Potential plant bioreactor for antioxidant phenolic compounds. J. Agric. Food Chem. *57*, 1247–1252.

TURKMEN, N., SARI, F. and VELIOGLU, Y.S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem. *93*, 713–718.

VOLDEN, J., BORGE, G.I.A., BENGTSSON, G.B., HANSEN, M., THYGESEN, I.E. and WICKLUND, T. 2008. Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (*Brassica oleracea* L. ssp. *capitata* f. *rubra*). Food Chem. *109*, 595–605.

VOLDEN, J., BENGTSSON, G.B. and WICKLUND, T. 2009a. Glucosinolates, L-ascorbic acid, total phenols, anthocyanins, antioxidant capacities and colour in cauliflower (*Brassica oleracea* L. ssp. *botrytis*); effects of long-term freezer storage. Food Chem. *112*, 967–976.

VOLDEN, J., BORGE, G.I.A., HANSEN, M., WICKLUND, T. and BENGTSSO, G.B. 2009b. Processing (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (*Brassica oleracea* L. ssp. *botrytis*). LWT – Food Sci. Technol. *42*, 63–73.

Food and Agriculture Organization of the United Nations 2011. http://faostat.fao.org/site/339/default.aspx (accessed January 15, 2011).

XU, B. and CHANG, S.K.C. 2008. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chem. *110*, 1–13.

YEN, G.C. and CHEN, H.Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenecity. J. Agric. Food Chem. *43*, 27–37.

YU, L., LEI, T., REN, X., PEI, X. and FENG, Y. 2008. Response surface optimization of l-(+)-lactic acid production using corn steep liquor as an alternative nitrogen source by *Lactobacillus rhamnosus* CGMCC 1466. Biochem. Eng. J. *39*, 496–502.

ZHANG, D.L. and HAMAUZU, Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem. *88*, 503–509.